supplying 0–1 phage, and of Dr. J. B. Shrivastava, National Salmonella Centre, Kasauli, Punjab, for his help, and Dr. F. Kauffmann, International Salmonella and Escherichia Centre, Copenhagen, for final confirmation of the new species, and to Sri C. V. G. Choudary, principal of the Veterinary College, Mathura, for providing the necessary facilities.

V. K. Sharma
C. M. Singh

Department of Pathology and Bacteriology,
U.P. College of Veterinary Science and Animal Husbandry,
Mathura, India.

[1] Smith, H. W., and Buxton, A., *Brit. Med. J.*, i, 1478 (1951).
[2] Edwards, P. R., Bruner, D. W., and Moran, A. B., *J. Infect. Dis.*, **83**, 220 (1948).
[3] Cherry, W. B., Davis, B. R., Edwards, P. R., and Hogan, R. B., *J. Lab. Clin. Med.*, **44**, 51 (1954).

Transfer of Virulence in *Rhizobium trifolii*

So far, very few reports concerning transformations in *Rhizobium* have appeared. Krasilnikov[1] reported as early as 1941 that non-infective strains turned infective when they were grown for several months in a filtrate of a highly infective strain. By ultrasonic treatment and with preparations of deoxyribonucleic acid (DNA) Balassa[2,3] has obtained transformations concerning several properties, for example, infective ability under formation of new host specificity, increase or decrease in nitrogen-fixing ability, variation in colony types, and changes in antigenic structure.

I happened to meet with a case of transformation in *Rhizobium trifolii*. As part of a study of the infection process a polysaccharide was prepared from an infective strain[4]. As it had been observed (unpublished results) that inoculated plants formed nodules 1–2 days earlier when this preparation was added, the polysaccharide was added to plants inoculated with a non-infective strain to see whether it might help the avirulent bacteria to overcome the infection barrier.

The clover strain *A* used in the experiment described here has been studied thoroughly by Nutman[5,6]. The original strain lost its infection ability in 1940 (called Bart *A*), but a virulent line (*A* 1) was contained in an isolate from a nodule produced by the original strain. *A* 11 is an avirulent and *A* 121111 a virulent line, isolated from *A* 1. 226, the other clover strain, is a Swedish strain isolated by Bjälfve and so far as known not related to the *A* strain.

A polysaccharide from strain 226 was prepared[7] from a 2-wk.-old culture (Dorn's A_5-medium[8]) by precipitating the whole culture in alcohol, dissolving the precipitate in water and separating the cells by centrifugation. The clear solution was deproteinized according to Sevag[9], precipitated again, and dialysed against distilled water. After another precipitation, the preparation was dried *in vacuo* and a white water-soluble material was obtained.

Seeds of *Trifolium repens* (var. Morsö, Svalöf, Sweden) were germinated aseptically in tubes containing slopes of Jensen's seedling agar. After 2 days, they were inoculated with a few drops of a suspension of *Rhizobium* (Table 1, series 1–8). Immediately after inoculation, 2 mgm. of the 226 polysaccharide in water solution was added. The

Table 1. Plants of *Trifolium repens* inoculated with Avirulent (Bart *A* and *A* 11) and Virulent (*A* 121111 and 226) Clover Bacteria without (−) and with (+) Polysaccharide Preparation from Strain 226 (2 mgm./tube). Each Tube contained 10 ml. of Jensen's Agar. The Number of Nodules are Mean Values of Five Replicants

Series No.	Bacterial strain	Poly-saccharide addition	Appearance of the first nodule in days after inoculation	Mean number of nodules per tube after 2 wk.	3 wk.	4 wk.
1	Bart *A*	−	−	−	−	−
2	*A* 11	−	−	−	−	−
3	*A* 121111	−	10	3·3	8·5	12·5
4	226	−	9	2·7	7·0	10·0
5	Bart *A*	+	9	2·5	7·3	9·3
6	*A* 11	+	9	3·5	9·0	12·0
7	*A* 121111	+	7	6·7	12·0	15·0
8	226	+	7	5·8	8·5	10·3
9	Bart *A*	−	−	−		
10	Bart *A*·2	−	8	4·2		
11	Bart *A*	+	8	6·4		
12	Bart *A*·2	+	6	7·4		

appearance of the first nodule in days after inoculation and the number of nodules after 2, 3 and 4 wk. are recorded in Table 1.

Bacteria from the nodules produced by the formerly avirulent Bart *A* in the presence of the polysaccharide were isolated (Bart *A*·2). The colonies had the smooth appearance of 226, not rough like Bart *A*.

New seedlings were inoculated with Bart *A* and Bart *A*·2 with and without polysaccharide 226 (Table 1, series 9–12). From this experiment it is evident that Bart *A* has become infective. A serological test showed that Bart *A*·2 did not react with antiserum prepared from the *A* strain (*A* 121111), indicating that the new strain has not recovered the antigenic properties lost by the *A* strain, but has gained something new, most likely from strain 226. Investigations into the nutritional requirements of the involved strains showed that strain 226 and Bart *A*·2 are greatly stimulated by pantothenic acid, but that Bart *A* is not.

The most likely explanation of the above results is that a transformation induced by the polysaccharide preparation (which might contain DNA) has taken place. This evidence and the earlier report[4] point to the surface material of the *Rhizobium* cells as playing an important part in their ability to infect the root hairs of the appropriate host.

Detailed work on the importance of the capsular material for the infection process is in progress and will be published elsewhere.

I wish to express my indebtedness to Dr. P. S. Nutman for the opportunity to work at his laboratory. My stay at Rothamsted was financed by a grant from Magnus Bergvalls Stiftelse.

Hans Ljunggren

Soil Microbiology Department,
Rothamsted Experimental Station,
Harpenden, Herts.
and
Institute of Microbiology,
Royal Agricultural College,
Uppsala 7.

[1] Krasilnikov, N. A., *C.R. Acad. Sci., U.R.S.S.*, **31**, 75 (1941).
[2] Balassa, R., *Acta Mikrobiologica* (Hungary), **2**, 51 (1954).
[3] Balassa, R., *Nature*, **188**, 246 (1960).
[4] Ljunggren, H., and Fåhraeus, G., *Nature*, **184**, 1578 (1959).
[5] Nutman, P. S., *J. Bact.*, **51**, 411 (1946).
[6] Nutman, P. S., *Heredity*, **8**, 35 (1954).
[7] Wiley, B. B., and Scherp, H. W., *Canad. J. Microbiol.*, **4**, 505 (1958).
[8] Dorn, M., *Zbl. Bact.*, II Abt. **109**, 120 (1956).
[9] Sevag, M. G., *Biochem. Z.*, **273**, 419 (1934).