A Contribution to the Serological Typization of the Rhizobia

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The application of the root-nodule bacteria for the inoculation of legumes makes it necessary to assess the virulence and efficiency of the strain used. For this purpose, identification of the re-isolates from the root-nodules of legumes must be carried out, which can also serve to increase the accuracy of investigating the effect of passage of rhizobia through the plant. No biochemical and morphological features can be used for the identification of the strains as these features are not sufficient to distinguish even between rhizobial species. Serology alone makes it possible to carry out at least an approximate identification of the strains.

Serological studies of the rhizobia were made by a number of authors, who, however, frequently disagree in their conclusions. Thus, for example, Thornton and Kleczkowski (1950) tested 161 strains of \textit{Rhizobium trifolii}, using 6 antisera, the agglutinin reactions having exhibited no antigen common to all the strains tested. They are of the opinion that the agglutination technique is applicable to the differentiation between rhizobial strains. Bushnell and Sarles (1939) investigated the serological relationships in a group of rhizobia specific for soybean, lupine and cow-pea. Agglutinin tests have shown that a number of serological types exist among the rhizobial strains of this group. The strains of one serotype have common antigens which were positive in all antiserum modifications. There is no relationship between the serotype and the appurtenance to a certain group according to their relation with respect to the plant.

The antigenic structure of different strains of a single species is markedly varied. According to Drožańska (1959), serological relationship exists between the rhizobia specific for clover, pea and vetch. Rhizobia specific for lucerne represent an independent group as would correspond to the findings of Thornton and Kleczkowski (1950) and of Vincent (1943), who, on the basis of the antigenic structure, divided rhizobia into two groups. The first comprises rhizobia specific for lucerne, the other, rhizobia specific for clover and pea. The antisera against \textit{Rhizobium meliloti} were quite strain-specific. The specificity of somatic and flagellar antigens in the rhizobia was investigated by Read (1953), Vincent (1943) and Vincent and Waters (1953). The last-named authors are of the opinion that the strains might be identified through a combination of antigens. A very thorough investigation of the antigenic structure of rhizobia was carried out by Shtern (1948, 1953). The rhizobia can be divided basically into two groups according to their antigen structure. Serotype I—rhizobia specific for alfalfa, sweet clover and vetch, serotype II—rhizobia specific for broad bean, garden bean and pea. This division is very different from that by Thornton and Kleczkowski (1950) and by Vincent and Waters (1953). Shtern is of the opinion that both groups have a common array of heat-stable antigens which is located deeper underneath the cell surface. Individual rhizobial strains possess some of these heat-stable antigens in different combinations and
amounts, whereby the different reactions of the strains with different antisera may be explained. Pochon (1958) and Drožańska (1959) maintain that the serological character of the rhizobia is stable and does not change with the substrate or with passage through a different plant. On the other hand, Vincent (1943), although admitting the stability of antigenic properties on changing the nutrient substrate, assumes that the character of the antigen structure may be changed by transfers through a plant. The above authors all agree on the following points:

2. There is no correlation between the antigen structure, resistance to phage, fixation of molecular nitrogen and other physiological properties.
3. There appears to be no relationship between the ability of the strains to infect individual plant species and their serological type.
4. No common antigens were found with respect to the individual rhizobial species.
5. The substrate is without effect on the character of the serological reactions in the rhizobia.

On the other hand, the following are the views in which the above authors disagree:

1. The specificity of the prepared antisera and thus the applicability of serological methods to the identification of cultures.
2. The division into the fundamental serological types and the effect of transfers on the change of the serological type and on the specificity of flagellar and somatic antigens.

MATERIALS AND METHODS


Media. Pea agar: broth of 50 g. pea in 1000 ml. water, glucose 10 g., K$_2$HPO$_4$ 1 g., agar 15 g., pH 7.0. Yeast agar: yeast extract 500 ml. (Stárka, 1952), water 500 ml., agar 15 g., K$_2$HPO$_4$ 1 g., pH 7.0.

Immunization. After 48 hr. incubation on a solid medium at 30°C, the cultures were washed down with 0.5% NaCl and diluted to the corresponding density. Increasing doses were used for immunizing rabbits (into the vena marginalis or i.p.). The amount of suspension used and the immunization intervals were as follows: (1) Four times, at five-day intervals, i.v., 1.0—2.0—3.0—3.5 ml. suspension; the rabbit was bled one week after the last injection (Bushnell & Sarles, 1939). (2) Four consecutive days, i.v., 1.0—2.0—3.0—3.5 ml. doses of suspension; the rabbit was bled two weeks after the last injection (Manil & Bonier, 1950). (3) With mucous cultures, the immunization was carried out on four consecutive days. i. p. 4.0—5.0—5.0—5.0 ml. doses; the rabbit was bled two weeks after the last injection.

Suspensions of cells with full antigenic apparatus, and of cells heated for two hours on a water bath at 100°C (deprived of the heat-labile antigens), and finally of cells heated as above and washed three times with hot physiological saline, free