Strain Variations of *Rhizobium* sp. (Cowpea Group) from Root Nodules of Healthy and Yellow Mosaic Virus (YMV) infected *Phaseolus aureus* Plants

By

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*With one figure*

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**Introduction**

Earlier work on virus infection of plants in relation to root nodulation has shown that the number of nodules was significantly reduced in clover (*Trifolium repens*), soybean (*Glycine max*) and field bean (*Dolichos lab lab*) by clover phyllody virus, soybean mosaic virus and Dolichos enation mosaic virus, respectively (Joshi and Carr 1967, Tu, Ford and Quiniones 1970, Tu, Ford and Grau 1970, Rajagopalan and Raju 1972). In clover, the virus seemed to induce a change in the *Rhizobium* as a result of which the root nodule bacteria isolated from virus infected plants produced mainly small nodules on reinoculation to healthy cuttings (Joshi, Carr and Jones 1967). Vanderveken (1964), on the other hand, noted that bacteria isolated from virus plants regained their full effectiveness on reinoculation to healthy plants. However, no study has been undertaken to find out the strain variations, if any, among *Rhizobium* isolates from nodules of healthy and virus infected plants. The present report embodies some findings in this direction with reference to Yellow Mosaic Virus (YMV) infection of Baisaki Mung (*Phaseolus aureus* L.), an important grain-yielding protein-rich pulse crop of India.

**Experimental**

Healthy and YMV infected plants of Baisaki mung were collected from the fields of Indian Agricultural Research Institute, New Delhi. The number of leaves, lateral roots and nodules and the size of nodules were determined.
Several isolates of bacteria were obtained from nodules and agrobacteria were excluded by the ketolactose test (Bernaerts and De Ley 1963). Two strains of Rhizobium, H1 and H2 from nodules of healthy plants and four strains, Y2, Y3, Y4 and Y9 from YMV-infected plants were tested for nodulation on agar slopes in test tubes in a growth room and taken up for a detailed study. The bacteria were maintained on yeast extract mannitol (YEM) agar slants. The ability of these bacteria to react with litmus milk and to utilize mannitol, mannose, glucose, lactose, sucrose, and raffinose was studied according to the method of Norris (1965).

Three strains, H1 from healthy plants and Y2 and Y9 from YMV infected plants were used for studying their antigenic properties. The methods described in IBP hand book (Vincent 1970) were followed in the preparation of bacterial cells and their antigens. Two rabbits were immunized separately for each one of the three strains. The immunization of rabbits and collection of sera were done as described by Vincent and Humphrey (1970). The agglutination test was carried out in tubes as suggested in IBP hand book (Vincent 1970).

Results

Observations under field conditions

The number of lateral roots were not altered by YMV infection whereas the number of leaves and nodules increased significantly due to virus infection (table 1). Visual observations revealed that the nodules were bigger in healthy plants than in virus infected ones.

\[
\text{Table 1}
\]
Number of lateral roots, leaves and nodules in healthy and YMV-infected mung (P. aureus) plants (mean of 30 plants)

<table>
<thead>
<tr>
<th></th>
<th>Lateral roots</th>
<th>Leaves</th>
<th>Nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>18</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>YMV-infected</td>
<td>14</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>t value</td>
<td>0.43</td>
<td>6.28*</td>
<td>5.80*</td>
</tr>
</tbody>
</table>

* Significant.

Characterization of true rhizobia

Of the forty strains of nodule bacteria, 34 strains turned out to be non-nodulating strains which were also positive to ketolactose test confirming their identity as agrobacteria (table 2). The remaining six strains which proved to be negative to ketolactose test were found to nodulate mung plants grown in test tubes on agar slopes under aseptic conditions in a growth room. Hence for subsequent studies, these six Rhizobium strains were taken up.

Growth in litmus milk

All the six strains did not form a serum zone indicating their identity as Rhizobium sp. (Cowpea group) (Fred, Baldwin and McCoy 1932). The fact that all the strains showed an alkaline reaction indicated that they behaved similarly with regard to this biochemical criterion.
Strain Variations of *Rhizobium* sp. (Cowpea Group)

**Table 2**
Ketolactose test and nodulation tests in aseptic conditions with nodule bacteria

<table>
<thead>
<tr>
<th>Plant type</th>
<th>No. of nodules plated</th>
<th>No. of isolates made</th>
<th>No. of agrobacteria found as contaminants by ketolactose test</th>
<th>No. of <em>Rhizobium</em> as revealed by nodulation tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>30</td>
<td>18</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>YMV-infected</td>
<td>30</td>
<td>22</td>
<td>18</td>
<td>4</td>
</tr>
</tbody>
</table>

**Utilization of different carbon substrates**

The results are given in table 3. All the six strains responded differently to different sugars and utilized the carbon sources except the fact that the reaction of the medium at the end of the growth phase was either acidic or basic depending upon the carbon source.

**Table 3**
Growth and reactions of strains of *Rhizobium* sp. (Cowpea group) grown on yeast extract agar medium containing different carbohydrates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Control</th>
<th>Mannose</th>
<th>Mannitol</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Maltose</th>
<th>Raffinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>-</td>
<td>++a</td>
<td>++a</td>
<td>+++a</td>
<td>+a+b</td>
<td>--</td>
<td>--</td>
<td>+b</td>
</tr>
<tr>
<td>H2</td>
<td>-</td>
<td>+++a</td>
<td>+++a</td>
<td>++++a</td>
<td>+a+b</td>
<td>--</td>
<td>+a</td>
<td>--</td>
</tr>
<tr>
<td>Y2</td>
<td>-</td>
<td>++a</td>
<td>+++a</td>
<td>++++a</td>
<td>+a+b</td>
<td>--</td>
<td>+b</td>
<td>--</td>
</tr>
<tr>
<td>Y3</td>
<td>-</td>
<td>+b</td>
<td>++a</td>
<td>+++a</td>
<td>+a+b</td>
<td>--</td>
<td>++b</td>
<td>+b</td>
</tr>
<tr>
<td>Y4</td>
<td>-</td>
<td>++a</td>
<td>+++a</td>
<td>++++a</td>
<td>+a+b</td>
<td>--</td>
<td>+b</td>
<td>+b</td>
</tr>
<tr>
<td>Y9</td>
<td>-</td>
<td>++a</td>
<td>+++a</td>
<td>++++a</td>
<td>+a+a</td>
<td>+a</td>
<td>+a</td>
<td>--</td>
</tr>
</tbody>
</table>

R: Reaction. a Acidic; b basic; — no change.
G: Growth. ++++ Very good growth; +++ good growth; ++ moderate growth; + slight growth; — no growth.

**Antigenic properties of H1, Y2 and Y9 strains**

The antigenic properties were tested by tube agglutination test. The antigens of three strains reacted with their own sera (homologous) diluted up to 1/1600 to 1/3200. Antigen of H1 strain did not agglutinate with y9 serum, but did agglutinate with y2 serum. Antigen of Y2 strain reacted with h1 and y9 strains sera at lower dilution (1/400). The reaction of Y9 antigen with y2 and h1 was similar to that of Y2 antigen. The antigen of Y2 and Y9 was
similar in nature but was different from that of H1. In this way, a differentiation can be made into two serum groups — H1 strain belonging to one and Y2 and Y9 to the other serum group (fig.).

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Antisera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h1</td>
</tr>
<tr>
<td>H1</td>
<td><img src="image" alt="Agglutination with antiserum dilution upto 1/3200" /></td>
</tr>
<tr>
<td>Y2</td>
<td><img src="image" alt="Agglutination with antiserum dilution upto 1/400" /></td>
</tr>
<tr>
<td>Y9</td>
<td><img src="image" alt="Agglutination with antiserum dilution upto 1/400" /></td>
</tr>
</tbody>
</table>

Agglutination reactions of the strains of *Rhizobium* sp. (Cowpea group)

**Discussion**

YMV infection significantly increased the number of leaves in mung plants confirming the earlier observation that stimulation of axillary buds due to virus infection was one of the factors leading to higher leaf numbers (Diener 1963).

Since Müller and Stapp (1925) showed that various cross inoculation groups of *rhizobia* behaved differently towards various carbon compounds, this technique was used to some extent in the subdivision of species of root nodule bacteria (Fred, Baldwin and McCoy 1932). However, its value as a diagnostic feature was limited due to contradictory results (Ishizawa 1953, Vyas and Prasad 1959). Graham (1964), however, again used carbohydrate utilization as a diagnostic feature in the classification of *Rhizobium* and *Agrobacterium*.

The results of the present study demonstrated that the ability to utilize carbon sources and fermentative reaction did not provide any clue for differences between *Rhizobium* strains from healthy and virus infected plants. Nevertheless, further study on this aspect with other leguminous crops is warranted and will be taken up in the near future.
Immunoserological methods have been used by earlier workers to differentiate strains of *Rhizobium* into several serogroups. (Vincent 1941, 1942, Purchase, Vincent and Ward 1951, Skrdleta 1965, 1969, Damirgi, Frederick and Anderson 1967, Vincent and Humphrey 1970). In the present study, somatic antigens were used to distinguish serogroups among strains of *Rhizobium* isolated from virus infected and healthy plants. The results, however, do not throw any light on intrinsic differences in serological characteristics between strains obtained from nodules of the two sets of plants.

**Summary**

*Phaseolus aureus* L. (Baisaki mung) infected by Yellow Mosaic Virus (YMV) produced mainly small white nodules. Several isolates of nodule bacteria were obtained from infected and healthy plants. Of those, six strains of *Rhizobium*, two from nodules of healthy plants and four from nodules of infected plants were studied for some cultural biochemical and antigenic properties. The results did not show any critical difference between *Rhizobium* strains in relation to virus infection of the host.

**Literature**


Fred, E. B., J. L. Baldwin, and E. McCoy, 1932: Root nodule bacteria and leguminous plants. Univ. Wisconsin, Madison, Wisconsin, USA.


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