THE SURVIVAL OF RHIZOBIUM BACTERIA IN SOIL

S. Saubert von Hausen and N. O. van Glyswyk

ABSTRACT

Experiments carried out during the past three years have indicated that South African strains of Rhizobium bacteria are better able to survive in the soil under our climatic conditions than certain foreign strains tested. This is an important reason for recommending the use of local strains rather than foreign strains of Rhizobium in South Africa.

INTRODUCTION

Various investigators have found that Rhizobium bacteria are often capable of surviving for long periods in the soil in the absence of the host plant; but under certain conditions the number of bacteria diminishes. Factors that have been suggested as causes of the decrease in the rhizobial population include light, soil fertility, and antagonistic micro-organisms. Yet the only factor that has received much attention is the presence of antagonistic micro-organisms such as Streptomycetes and sporulating bacteria.

As part of the Plant Physiological Research Institute’s programme of research on nitrogen fixation by legumes of importance in South Africa, the authors have compared the behaviour of foreign strains of Rhizobium with that of strains isolated from South African soils. The authors have found that the foreign strains which they have tested are very often inferior to local strains with respect to nitrogen fixation under our conditions. Another important factor to consider in evaluating the properties of a strain of bacteria is its ability to survive in the soil, particularly under dry, hot conditions such as are often likely to be encountered in Southern Africa. Experiments were accordingly carried out in which the behaviour of foreign and local strains of Rhizobium under such conditions was compared.

METHODS, MATERIALS AND RESULTS

Experiment 1: In this experiment, the ability of a Finnish, an American and a local strain of Rhizobium trifolii to survive in four different media under dry, hot conditions or cool, moist conditions was tested. The media used were a soil rich in organic matter, a soil deficient in organic matter, river sand and vermiculite. The media were placed in glass dishes of approximately 160 ml. capacity. Suspensions of the different strains of bacteria were added to the dishes. Each dish received an inorganic nutrient solution. To allow development of the bacteria, the dishes were kept at a constant temperature of 27°C for two months, during which period water was regularly added.

After this period, one group of dishes was exposed to the sun under a perspex shelter outside and allowed to dry out, and another group was placed under shade outside and kept moist. These two treatments were also duplicated in a greenhouse maintained at 27°C during the day and 21°C during the night, and in a greenhouse maintained at a constant temperature of 21°C. The dishes were exposed to these conditions for two months.

At the end of the two month period, Hiltner’s nitrogen-free nutrient solution was added to each dish and seeds of Trifolium pratense were sown. In each treatment, control dishes were sown with Trifolium pratense seed inoculated with the respective strain of Rhizobium trifolii. All the dishes were kept outside and water was added at regular intervals.

One month after sowing the seeds, the plants were carefully lifted and the nodules counted. The results obtained with the dishes which had originally been kept outside are presented in Table 1. Essentially similar results were obtained with dishes originally kept in the two greenhouses.

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TABLE I

Survival of *Rhizobium trifolii* strains under cool, moist and under hot, dry conditions, as measured by the number of nodules subsequently produced on 60 *Trifolium* plants contained in 4 dishes per treatment.

<table>
<thead>
<tr>
<th>Source of <em>Rhizobium</em> strain</th>
<th>Treatment of Seeds</th>
<th>Average number of nodules per plant in</th>
<th>Soil rich in organic matter kept</th>
<th>Soil deficient in organic matter kept</th>
<th>River sand kept</th>
<th>Vermiculite kept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hot and dry</td>
<td>Cool and moist</td>
<td>Hot and dry</td>
<td>Cool and moist</td>
<td>Hot and dry</td>
</tr>
<tr>
<td>American strain isolated from &quot;Hugonogerm&quot;</td>
<td>Uninoculated Inoculated</td>
<td>2 28</td>
<td>40 38</td>
<td>37 22</td>
<td>18 20</td>
<td>24 30</td>
</tr>
<tr>
<td>Finnish strain &quot;A. 10&quot;</td>
<td>Uninoculated Inoculated</td>
<td>22 52</td>
<td>39 21</td>
<td>17 23</td>
<td>19 21</td>
<td>20 21</td>
</tr>
<tr>
<td>Local strain &quot;Trif. 6&quot; isolated from Potchefstroom soil</td>
<td>Uninoculated Inoculated</td>
<td>33 30</td>
<td>31 24</td>
<td>15 15</td>
<td>18 20</td>
<td></td>
</tr>
</tbody>
</table>

Where the bacteria had been exposed to cool, moist conditions, there were very small differences in the number of nodules produced on plants from inoculated control seed and those from uninoculated seed, with all three strains of bacteria in all four media. However, when the bacteria were exposed to hot, dry conditions, the survival of the Finnish and American strains was poor and very few nodules were produced on plants from uninoculated seed. On the other hand, the survival of the South African strain under hot, dry conditions was excellent.

This experiment was repeated three times with essentially similar results.

**Experiment 2:** In this experiment, a study was made of the ability of an American, a Dutch, a Finnish and a South African strain of *Rhizobium* nodulating well with "Greenfeast" peas to survive in a red loam soil and in a grey-brown sandy soil under hot, dry conditions.

Sterilized soil was placed in plastic dishes of 150 ml. capacity. A suspension containing about 2.5 x 10^{11} bacteria of the required strain was added to each dish. The dishes were covered with lids and kept at room temperature for one week.

After one week, the covers were removed from the dishes. One group was placed outside in direct sunlight and removed to shelter in rainy weather. The other was placed under shade outside and protected from rain. There were three dishes for each strain of bacteria in each soil in each group, i.e. a total of 48 dishes. No further water was added to the dishes. The dishes were exposed to these conditions for 86 days.

The method employed to measure the survival of the bacteria was based on that used by Wilson in estimating the number of rhizobia in soil. At the end of the 86 day period, the contents of the three dishes in each treatment were mixed. A suspension was made by adding sterile water to a 10 g. sample of the soil from each treatment. An aliquot of the suspension corresponding to 0.1 g. soil was added to each of six sterile water cultures containing Hiltner's nitrogen-free nutrient solution. Five "Greenfeast" pea plants were planted in each container. The plants were kept in a greenhouse maintained at approximately 21°C during the day and 15°C at night. After 16 days, the plants were harvested and the number of nodules counted.

The results of this experiment are presented in table II.
TABLE II

Survival of *Rhizobium leguminosarum* under various conditions, as measured by the number of nodules subsequently produced on 30 *Pisum sativum* var. *Greenfeast* plants.

<table>
<thead>
<tr>
<th>Source of <em>Rhizobium</em> strain</th>
<th>Average number of nodules per plant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhizobium in red loam, kept in</td>
<td>Rhizobium in grey-brown sandy soil, kept in</td>
</tr>
<tr>
<td></td>
<td>Sun</td>
<td>Shade</td>
</tr>
<tr>
<td>Local strain isolated from Vaal-Hartz soil</td>
<td>61</td>
<td>103</td>
</tr>
<tr>
<td>American strain isolated from Plantogerm</td>
<td>49</td>
<td>81</td>
</tr>
<tr>
<td>Dutch strain A.2</td>
<td>49</td>
<td>107</td>
</tr>
<tr>
<td>Finnish strain H.43</td>
<td>12</td>
<td>69</td>
</tr>
</tbody>
</table>

In the grey-brown, sandy soil, the local strain of *Rhizobium* was far better able to withstand exposure to direct sunlight than the three foreign strains; the Finnish strain was least able to withstand these conditions. In the red loam, the local, American and Dutch strains withstood exposure to direct sun about equally well and again, the Finnish strain fared very poorly. The Dutch strain survived better in the red loam than in the grey-brown, sandy soil, while the other strains survived better in the latter soil.

DISCUSSION

In these experiments, the foreign strains of *Rhizobium* bacteria were never superior to the local strains tested in their ability to survive hot, dry conditions. Many of the foreign strains, in fact, showed a very poor rate of survival. For this reason, and because the authors have found that the local strains of *Rhizobium* that they have tested are often superior to foreign strains in their ability to fix nitrogen under our conditions, it is recommended that local strains of *Rhizobium* be used in South Africa wherever possible.

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**TO CONTRIBUTORS**

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