

THE NEED TO IDENTIFY *RHIZOBIUM* STRAINS

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Introduction

The aim of the Biological Dinitrogen Fixation (BNF) project is to increase protein production, in order to reduce the use of N fertilizers and to improve soil fertility. Enrichment of the soil with nitrogen will only occur with fully effective *Rhizobium*-Legume associations, which will be capable of fixing the maximum amounts of N₂ from the atmosphere. The *Rhizobium*, a group of bacteria, will grow vigorously and fix N₂ if they have functioning nodules, and this depends on how well the plant (host) roots encounter an appropriate *Rhizobium* strain in the soil.

So, there are differences among *Rhizobium* strains. Some cannot infect the particular legume being grown whilst others invade vigorously, producing effective nodules that continuously supply nitrogenous compounds to the plant.

The identification of *Rhizobia* spp.

The identification of *Rhizobium* strains that occupy nodules is essential in studies designed to evaluate the competitiveness and effect of specific inoculant strains on the growth of leguminous plants. Strain identification is a major problem in field experimentation with strains of *Rhizobium*.

Marked strains are widely used to identify microorganisms introduced to the environment under study. With the application of molecular biology, the taxonomy and classification of *Rhizobia* is continuously evolving.

However, When large numbers of bacterial strains are involved, DNA and RNA techniques are too tedious and too expensive. Microbiologists are therefore inclined to rely more on the structure of protein molecules for strain identification.

SDS polyacrylamide gel electrophoresis (SDS-PAGE) is one of the most popular electrophoretic separation techniques employed in bacterial taxonomy.

SDS-PAGE as a method of strain identification

Polyacrylamide gel electrophoresis of the total cellular proteins is a powerful tool to screen large numbers of strains; it can unravel very fine taxonomic relationships and even allow their identification to the species and subspecies level. This technique has already been used for *Rhizobia* in several taxonomic and ecological studies.

Basic principles of electrophoresis

The term electrophoresis is used to describe the migration of charged particles under the influence of an electric field. The relationship between different bacterial strains is determined by comparing the protein fingerprints they produce.

The migration rate of a molecule is proportional to the strength of the electric field and the charge on the molecule, but it is inversely proportional to the molecular size and the pore sizes of the migration field. Comparing small numbers of bacteria can be done directly by usual comparison of the strained gels or indirectly by comparing gel photographs on densitometric tracing.

Polyacrylamide gel as a sieving matrix

Electrophoresis in polyacrylamide gel (PAGE) was historically the first form of electrophoresis to be used widely. The gel is formed by the vinyl polymerization of acrylamide monomers, $\text{CH}_2\text{-CH-CO-NH}_2$ into long polyacrylamide chains and the cross linking the chains by the inclusion of an appropriate bifunctional commoners, usually N, N ethylene- bis Acrylamide $\text{CH}_2\text{-CH-CO-NH-CH}_2\text{-NH-CO-CH-CH}_2$. This results in a three dimensional network. The major advantage of PAGE is the ease of changing sieve size by simply changing the concentration of acrylamide and 'Bis'. With the fixed 'Bis' concentration, the pore size is inversely varied approximately linearly to the total monomer concentration.

Samples are stacked in to a thin layer in the stacking gel and actual separation occurs in the resolving gel. Shaper bands can be obtained even with very low concentrated samples.

Detection of protein banding pattern

Separated constituents are revealed in the great majority of cases by staining or by examination for the presence of a fluorescent or radioactive label.

Analysis of protein banding pattern

For a few strains visual observation is possible, but objective methods are needed to compare large numbers of strains. Computer assisted numerical analysis of the entire bands is the widely accepted method by several authors.

Advantages of PAGE in strain identification

1. Prior knowledge of the composition of the indigenous population is not needed. This provides an easy access to identify strains of *Rhizobia* that nodulate thousands of legumes.
2. Possible to identify those strains which do not react with antiserum.
3. Protein patterns match with serological groups and strains within a serological group can be identified.
4. The surface charge of bacterial cells may not be affected.
5. A large number of strains can be compared by this relatively inexpensive technique.
6. Using microbial analysis of protein banding patterns, the genetic 'relatedness' can be ascertained.
7. It allows a quick decision on whether two colonies in a culture are contaminants or variants.

Some limitations of SDS-PAGE

- High degree of standardization is needed to obtain reproducible results.
- It is not possible to identify a single colony in a few hours.
- Need a large number of experimental steps each introducing its particular experimental error.

Other methods of *Rhizobium* strain identification

Serological markers

The serological marker is the antibody found in the serum of an animal injected with a foreign substance (an antigen). *Rhizobium* strain possesses a high degree of antigenic heterogeneity and can be separated into many antigenically distinct types. Therefore, serological markers are important for strain identification and have indeed been used widely.

Antibiotic resistant markers

Antibiotics (AB) are organic products produced by living organisms, mainly fungi and actinomycities. They kill or inhibit the growth of specific groups of microorganisms. *Rhizobia* are naturally exposed to ABs and have, to a certain extent, a natural resistance against them. A small number of cells might be resistant to a very high concentration of a particular AB, and this AB therefore provides an useful marker in distinguishing that *Rhizobium* strain from other strains.

The development and use of AB resistant markers is relatively inexpensive and easy.