MI-AAU-709 21

Rhizobium and Legume Germ-Plasm : Collections and Evaluation

Report on The First Workshop on Nitrogen Fixation

December, 1980

Co-operative Research Project on Food Production and Preservation

Organization for Economic Co-operation and Development

Compiled by H.H. Keyser and R.T.J. Clarke

CONTENTS

Page

I. WORKSHOP PLANNING AND DEVELOPMENT 3

II. PARTICIPANTS 7

III. ABSTRACTS OF ISSUE PAPERS AND SUMMARIES OF PLENARY DISCUSSIONS 8

IV. PROPOSALS FOR OECD-SPONSORED RESEARCH FELLOWSHIPS 22

V. ADVISORY COMMITTEE MEETING ON SELECTION OF PROPOSALS FOR FELLOWSHIPS 24

VI. ADDRESSES OF MAIN CONTRIBUTORS, COMMITTEE AND NATIONAL CORRESPONDENTS 26

I. WORKSHOP PLANNING AND DEVELOPMENT

As part of its nitrogen fixation programme the OECD Co-operative Research Project on Food Production and Preservation held a workshop on "*Rhizobium* and Legume Germ-Plasm : Collections and Evaluation" in December 1980, in Australia.

The purpose of the workshop was to allow experts interested in legume and rhizobial germ-plasm to discuss problems and exchange ideas associated with collection and evaluation, with the specific aim of identifying topics suitable for OECD-Sponsored Fellowship research. The subject was one not covered in recent scientific meetings, and was chosen following a report* prepared by H.H. Keyser as a project consultant.

The workshop was organised by H.H. Keyser (USA) under the general direction of R.T.J. Clarke (New Zealand), Nitrogen Fixation Project Co-ordinator. By scheduling the workshop to follow immediately the IVth International Symposium on Nitrogen Fixation in Canberra, Australia, we were able to attract qualified participants from around the world. An attempt was made to obtain participants from all OECD countries contributing to the project, and 10 of the 14 countries that were members in 1980 were represented. The project paid *per diem* expenses to 19 participants from contributing countries, and six persons received travel assistance.

Invited participants were able to have an input to the planning of the workshop programme by responding to an initial announcement of the workshop and suggested topics. Response was excellent, and the format finally chosen was that of having brief issue-papers presented on the selected topics, followed by plenary discussion of the subject. Discussions were to occupy at least three-quarters of each session. Topic chairmen and discussion leaders were chosen by the Organiser.

The Workshop was held December 6 to 8, 1980, at the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organisation in Canberra. Dr Alan Gibson of that Division kindly took charge of local arrangements. Most of the Workshop participants were housed in the dormitory halls of the Australian National University, where they had been for the Symposium.

Details of the workshop, its format and programme, are given in the copy of the final announcement of the Workshop that follows.

---

* An Evaluation of *Rhizobium* and Legume Germ-Plasm Collections. 1980.

FIRST OECD WORKSHOP ON NITROGEN FIXATION

FINAL ANNOUNCEMENT

September 1980

PLACE

The Workshop will be held in the CSIRO Division of Plant Industry Lecture Room, Canberra, Australia.

DATE

December, 6, 7 and 8, 1980, following the IVth International Symposium on Nitrogen Fixation.

SUBJECT

Rhizobium and Legume Germ-Plasm : Collections and Evaluation.

SPONSOR

The Workshop is sponsored by the Organisation for Economic Co-operation and Development's Nitrogen Fixation Project, Dr Richard T.J. Clarke, Co-ordinator.

PURPOSE

The Workshop is being convened to address and discuss important practical aspects of the evaluation and collections of the germ-plasm of rhizobia and legumes. It will provide a forum where the state of the art and science of these areas can be discussed with open participation, with an aim towards identifying problem areas worthy of increased research effort. Many of the subjects that need to be discussed are not usually included in most meetings on nitrogen fixation. The Workshop will provide the basis for selecting topics for OECD-sponsored research Fellowships.

FORMAT

Each topic will have a Topic Chairman and a Discussion Leader. The Chairman will introduce contributors, moderate discussions and provide summary comments. The Discussion Leader will open the topic with a general issue paper, to be followed by other contributors. Finally, there will be open discussion of the subjects within the topic. Open discussion is often limited at meetings, and the Workshop format will provide for this worthwhile exchange.

PROGRAM

The program has been built around participants' comments and suggestions to the first announcement. The Taxonomy of *Rhizobium* topic is not scheduled because it is to be dealt with separately during the week of the Symposium, though the Workshop may hear a brief report of that meeting. If there remains sufficient interest on this topic and time permits, an impromptu session can possibly be arranged during the Workshop.

SATURDAY, DECEMBER 6

TOPIC 1. Collections of *Rhizobium* and Legume Germ-Plasm : An overview of major collections, contents and associated activities.

Topic Chairman: L. Frederick, USA
Discussion Leaders: R. Pullen, Australia
D. Date, Australia

TOPIC 2. Adequacy of the Germ-Plasm Base : The need to collect material from their centres of origin and diversity, and the value of strains from wild and primitive legumes.

Topic Chairman: K.W. Clark, Canada
Discussion Leader: T.A. Lie, Netherlands

TOPIC 3. Isolation, Maintenance, Preservation and Documentation of *Rhizobium* : Methods, media, intrastrain variation and useful documentation.

Topic Chairman: Tomas Ruiz-Argueso, Spain
Discussion Leader: J. Brockwell, Australia

SUNDAY, DECEMBER 7

TOPIC 4. Evaluation of the Symbiosis for Effectiveness : Laboratory, greenhouse and field methodologies, cultivar-strain interactions, and factors influencing host preference of strains.

Topic Chairman: D.G. Jones, Wales
Discussion Leader: D. Weber, USA

AFTERNOON - Free for rest from a week of meetings.

MONDAY, DECEMBER 8

TOPIC 5. Evaluation of the Symbiosis Under Special Ecological Conditions : Characterization of performance under stress conditions of acidity, salinity, alkalinity, aridity, temperature and other environmental parameters.

Topic Chairman: R. Roughley, Australia
Discussion Leader: H. Keyser, USA

TOPIC 6. Field Inoculation of Legumes : The kinds and uses of high quality inocula, and the problem of indigenous rhizobia.

Topic Chairman: W. Broughton, West Germany
Discussion Leader: J. Burton, USA

ORGANIZER

Harold H. Keyser, USDA SEA AR CCNFL Rm 116 Bld 011A BARC-W Beltsville, Md, 20705, USA

ADVISORY COMMITTEE

John Beringer, England
A. Quispel, Netherlands
Pat Murphy, Ireland

PARTICIPANTS

Up to 30 invited participants

FINANCE

OECD will pay per diem accommodation costs for December 6 through 8 for participants from OECD-member countries participating in the Agricultural Research Project: Canada, USA, New Zealand, Belgium, West Germany, Ireland, Netherlands, United Kingdom, Finland, Spain, Sweden, Switzerland, Yugoslavia and Turkey. Payment will be made at the beginning of the Workshop.

ACCOMMODATION

Rooms for Workshop participants are being reserved at Bruce Hall on the campus of the Australian National University for December 6-9. Please notify the Organizer if you are making other arrangements.

II. PARTICIPANTS

B. Ahmed (Trinidad)
J. Beringer (United Kingdom)
P. Bishop (USA)
L. Bordeleau (Canada)
J. Brockwell (Australia)
W.J. Broughton (West Germany)
J.C. Burton (USA)
K.W. Clark (Canada)
R.T.J. Clarke (New Zealand)
P. Dart (India)
R.A. Date (Australia)
Sir O. Frankel (Australia)
L. Frederick (USA)
D. Gareth Jones (United Kingdom)
A. Gibson (Australia)
R. Howell (USA)
B.D.W. Jarvis (New Zealand)
H.H. Keyser (USA)
T.A. Lie (Netherlands)
K. Lindstrom (Finland)
F. Minchin (United Kingdom)
P. Murphy (Ireland)
L. Mytton (United Kingdom)
P. Nutman (United Kingdom)
F. O'Gara (Ireland)
C.A. Parker (Australia)
R. Pullen (Australia)
A. Quispel (Netherlands)
V. Radulovic (Yugoslavia)
C. Rodriguez-Barrueco (Spain)
B. Rolfe (Australia)
R. Roughley (Australia)
T. Ruiz-Argueso (Spain)
D.B. Scott (New Zealand)
H. Van Bushby (Australia)
J.M. Vincent (Australia)
D.F. Weber (USA)

III. Abstracts of Issue Papers and Summaries of Plenary Discussions

At the opening of the Workshop Harold Keyser welcomed the participants and gave details of programme and format. Dick Clarke gave a brief talk on the OECD Project's interests and goals in the area of nitrogen fixation.

At the commencement of each session the Chairman spoke briefly on the topic, and introduced the Discussion Leader who presented an issue paper, outlining the important issues of the particular topic, to provide the basis for the subsequent discussion. The discussions were not structured and were very informal, and no time limits were put on individual sessions. Discussions flowed freely at all sessions: they were very stimulating and in them was the true value and success of the Workshop.

Abstracts of the issue papers and a summary of the discussion highlights are presented in order of the topic presentations. Because of its practical nature the paper of Joe Burton on inoculant techniques is presented in full. A detailed report of the discussions would have required recording and transcription; this might be considered for future workshops.

TOPIC 1(a) Collections of Legume Germ-Plasm : An Overview of Major Collections, Contents and Associated Activities (Abstract)

R. Pullen

There are approximately 690 genera of legumes, although only some 55 are used to any significant extent in world agriculture. Following a meeting of the FAO/IBP Council in 1968, an International Board on Plant Genetic Resources (IBPGR) was formed to identify plant germ-plasm collection centres and decide on relevant activities to assure preservation of valuable material, among other duties. The IBPGR group has made good progress to date in this area. The Consultative Group on International Agricultural Research sponsors legume germ-plasm collection and research at the international centres: The International Center for Tropical Agriculture (CIAT) in Columbia has a mandate to collect and research *Phaseolus* (beans) germ-plasm, The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in India has a mandate for mung bean and peanuts, and The International Institute for Tropical Agriculture (IITA) in Nigeria for cowpeas.

Throughout the world there are many collections of legumes, both large and small. Efforts by IBPGR have been made to designate Base Collections of plant germ-plasm for particular species, and back-up collections. Also, they have identified priorities for the collection of plant material from their centres of diversity. They

have also set guidelines for testing, storage and documentation of material. A particular problem in this regard is the concomitant collection of the rhizobia-harbouring nodules along with plant seeds, and their subsequent evaluation together. On collection expeditions, as well as in legume evaluation the co-operation of the rhizobiologists is valuable and should be made a common practice.

Discussion

Several participants asked about quarantine regulations and problems in transferring seed material. This is subject to different regulations in each country.

Regarding questions on specific lisitings of legume collections, it was pointed out that IBPGR puts out a "Plant Genetic Resources Newsletter" which often identifies collections and their holdings. Conditions relating to storage and maintenance were discussed. A publication, "Food Legume Germ-Plasm Collections" is available from: Publications, Executive Secretary IBPGR, FAO, Rome, 00100, Italy.

One recommendation discussed was that collection centres should not overburden themselves with too much material, but should better evaluate that which they already have.

The Chairman in his opening remarks pointed out that collections of rhizobia are often less well organized and recognized than plant collections, and that they often become extinct when their curator leaves that position. He emphasized the need to consider the intimate relationship of legumes and rhizobia, and that their collection and evaluation should not be considered separately.

TOPIC 1(b) Collections of *Rhizobium* Germ-Plasm : An Overview of Major Collections, Contents and Associated Activities (Abstract)

R. Date

Two attempts to catalogue strains of *Rhizobium* have been made in recent years. The first of these was the IBP World Catalogue of *Rhizobium* Collections. The second was by the World Data Centre for Micro-organisms, which used the IBP Catalogue as a starting point.

The IBP Catalogue lists 59 collections and the World Data Centre some 48 collections. Some collections are recorded in both lists but there are at least 15 important collections not named in either of the above catalogues. In all, some 125 collections of value can be identified. Of these only about 30 are major genetic resource banks (greater than 200 strains) but all contain useful cultures worthy of preservation. A catalogue of collections and details of working cultures would be a useful document for curators and many research groups.

In very general terms strains of rhizobia for clovers, medics, peas, trefoil, beans and soybeans constitute the majority of strains in most European and North American collections. In recent years

several national and international research groups have specialized in isolating and maintaining rhizobia for specific groups of legumes. For example, ICRISAT have a large proportion of their collection devoted to strains for pigeon pea, groundnuts and chickpea, IITA to cowpeas and CIAT to beans and tropical forage legumes of the genera *Stylosanthes*, *Centrosema*, *Desmondium* and *Leucaena*. The CSIRO collection in Brisbane is devoted to the tropical forage and grain legumes plus a range of native Australian legumes, whereas the CSIRO Canberra collection concentrates on the more temperate forage species as clovers, medics and lotus, but includes also rhizobia for some grain legumes.

Knowledge of the composition of these collections is not readily available. As few as 6 collections have published catalogues (so far as the author is aware). Several have catalogue lists that are made available on a very restricted basis. There is no generally available document which lists culture collections and their contents.

The World Data Centre (Prof. V. Skerman, University of Queensland, Brisbane) is prepared to produce such a catalogue if collection and strain information is submitted (see Appendix). It is suggested the OECD could support a project to encourage culture collection curators to submit information for at least those strains which have known characteristics either as useful research material or as potential inoculum strains.

Discussion

A large number of the strains in *Rhizobium* collections are probably not very important, as extensive evaluation has often been made for only a few.

However, strains which are considered 'poor' because of their relative ineffectiveness on a designated host may be worth keeping in collections because we are finding out how to breed effectiveness into fast growing rhizobia, and the 'poor' strains may have other valuable characteristics.

A discussion ensued on the need to have more listings or catalogues of rhizobia available. One suggestion was to have collection curators list in the Rhizobium Newsletter legume species for which they have rhizobia available.

In publishing catalogues of rhizobia, curators should perhaps list only those well characterized strains that they are willing to service, and that would include serological type strains.

In testing rhizobia for host-effectiveness, it was pointed out that variation in the host genotype may be large, even within supposedly pure lines.

TOPIC 2 Adequacy of the Germ-Plasm Base : The Need To Collect Material from Their Centres of Origin and Diversity, and the Value of Strains from Wild and Primitive Legumes (Abstract)

T.A. Lie

Vavilov demonstrated that certain regions of the earth are particularly rich in genetic variants of plants, related to, or even ancestral forms of, our cultivated crop plants. These regions, originally called 'centres of origin' by Vavilov are now known as 'centres of diversity' in view of the abundance of wild or primitive genotypes in a restricted area.

The value of these centres as a source for disease resistance in plants is well established. We demonstrated that these centres are also an important source for genetic variation of host plants and *Rhizobium* for symbiotic nitrogen fixation. The cultivated pea genotypes show little variation in nodulation and nitrogen fixation when in symbiosis with *Rhizobium* strains isolated from cultivated peas from Europe (European strains). Two non-nodulating exceptions are primitive pea genotypes from Iran and Afghanistan, one of the centres of diversity of peas. When more primitive and wild pea genotypes were analysed, symbiotic variability was shown to be very large in the primitive genotypes, and the 'non-nodulation' trait widespread in peas of Afghanistan. There is another noteworthy observation: peas belonging to the ecotypes "abyssinicum" and "fulvum", occurring respectively in Ethiopia and Israel, form nodules with no or low nitrogenase activity. Further experiments showed that these symbiotic defects are due to the use of non-compatible European *Rhizobium* strains. These primitive and wild genotypes require specific strains occurring only in their centres of diversity as shown for the pea cv. Afghanistan.

Evaluation of *Rhizobium* strains from wild and primitive legumes has hardly started, but from the little data available the impression is that these bacteria, similar to wild plants, have a greater genetic variability than *Rhizobium* strains from cultivated legumes. Perhaps they deserve more attention, and selection within this group is more rewarding than in the specialized *Rhizobium* strains from cultivated crops. The establishment of a gene pool to conserve this germ plasm should be recognized as being potentially very valuable.

Discussion

Dr Lie often obtained "more primitive" rhizobia from the Middle East by collection of soil samples, with subsequent isolation back in his laboratory. This matter of collecting just soil as a source of rhizobia was discussed, including quarantine problems, soil storage banks for such purposes, and the longevity of strains stored in soil.

It was mentioned that for certain legumes it would be a lot easier to collect nodules from a healthy green plant and let the plant do the selection.

However, the most effective rhizobia strains for *Stylosanthes* accessions adapted to alkaline environments were indeed isolated from alkaline soils in areas of diversity.

It was pointed out that *Rhizobium* strains from areas of diversity are likely to have other valuable characteristics besides effectiveness.

TOPIC 3 Isolation, Maintenance, Preservation and Documentation of *Rhizobium* : Methods, Media, Intrastrain Variation and Useful Documentation (Abstract)

J. Brockwell

The symbiotic association represented by the nodulated legume may be considered an under-exploited resource. Full exploitation of this resource will depend largely on the improvement of the legume itself, but ecological and genetic manipulations of the rhizobial partner will also contribute. Nevertheless, at all stages of improvement programmes, the legume scientist, be he plant introduction worker, breeder, or agronomist, must retain an awareness that it is the symbiotic capacity of these plants which is of primary importance. The continuing, collaborative function of the legume bacteriologist in such programmes is to act as a supplier of appropriate inoculum strains and as a monitor to detect and deal with any symbiotic aberrancies which may develop. The raw material for these objectives is a diverse collection of *Rhizobium* strains of known performance, which are maintained in good condition, which remain true to type, and which are comprehensively documented.

Some aspects of strain isolation, culture maintenance and preservation, and documentation of a collection are dealt with very briefly. The significance of culture media is a common theme running through this issue paper. Numerous reports exist of the harmful effects of various medium components, including energy sources, vitamins, amino acids, mineral salts, and hydrogen ions, on growth of rhizobia and its stability in culture. Descriptions of media designed for preservation of strains in a stable state, on the other hand, are very few in number. The whole subject of the role of specific media in *Rhizobium* strain acquisition and maintenance should be a subject for fruitful discussion.

Intrastrain variation is a matter of great concern if only because of the requirement in symbiotic, ecological, and genetic work with rhizobia for a stable strain to act as a yardstick against which to measure the performance of other strains and mutants. The incidence of this variation is not known, but the frequency with which it appears in strains which have been studied over a period of some years suggests that it may be more widespread than is commonly realized. There appear to be three categories of intrastrain variation:

i) Rapid deterioration following isolation.
ii) Symbiotically undesirable mutation.
iii) Minor, but significant, shifts in effectiveness.

Some discussion of them should be worthwhile.

A potential source of confusion and unproductive overlapping of research effort lies in the use by different laboratories of different strain numbers to designate the same strain. (Some strains are known

to have as many as five synonyms). It would be profitable if it were possible to initiate some measures designed to rationalize and standardize the allocation of strain numbers.

Discussion

The difficulty in isolation for some strains of *Rhizobium* may lie in the media, as with Date's finding of low pH-requiring strains.

John Brockwell demonstrated a nodule sterilizer to facilitate *Rhizobium* isolation that has the capacity to sterilize 1200 nodules per day. Blue prints for it are available from him.

Media composition was discussed: the medium best for growing high numbers of cells is not necessarily best for preservation in a genetically stable condition. Also, the very high amounts of nutrients in media might 'train' or adapt strains to these luxurious conditions.

It was mentioned that rhizobia from *Astragalus* can have a gradual and fairly complete decay in effectiveness and viability once isolated and cultured.

The issue of variation in effectiveness and colony types within strains was thoroughly discussed. It seems that the more we examine this area the more variation we are likely to uncover. Could this be related to media?

Regarding preservation, it was generally agreed that lyophilization was best, although we don't really know what selective pressure this exerts on a culture, or what effect it may have on plasmids. Preservation in glycerol, stored at -20°C, was discussed.

On documentation it was noted that computerized information systems are the best where it is available (an example print-out is attached), and the computer can print a catalogue. Although strain history, date and location of isolation are sometimes difficult to track down, every effort should be made to keep this for records.

It was suggested that some system uniform between laboratories be applied to the numbering of cultures, and that synonyms be carefully retained.

Descriptors for documentation of rhizobia was discussed as to minimal necessary information. It was noted that the form for the World Data Centre (attached) has about 40 descriptors.

A plea was made to include short statements on strain histories in publications.

On the effect of media on growth rates, it was asked if perhaps slow growers might be able to grow faster on different media. Is their metabolism slow even when associated with a plant?

TOPIC 4 Evaluation of the Symbiosis for Effectiveness : Laboratory, Greenhouse and Field Methodologies, Cultivar-Strain Interactions, and Factors Influencing Host Preference of Strains (Abstract)

D. Weber

Testing of strains of *Rhizobium* obtained for a culture collection is of primary importance. Unless the strains nodulate important legume varieties and fix nitrogen there is little justification for maintaining them in collections (exceptions would be for studies on genetic maniuplation where several traits might be transferred). New isolates are routinely sought from domestic and international sources. These are screened for purity and confirmation as *Rhizobium* by routine procedures in the laboratory, further screened on several legume varieties grown in nitrogen-free media in the growth chamber or glasshouse. Isolates which perform best are tested further in field experiments, generally where no background interference from soil rhizobia for the test crop is present.

Methodologies for routine testing for *Rhizobium* and the symbiosis in the laboratory, greenhouse and field, although aimed at similar objectives, exhibit considerable diversity of measured parameters among laboratories.

Laboratory procedures are probably more standardized because of the utilization of J.M. Vincent's Manual for the Practical Study of Root-Nodule Bacteria in most laboratories. Routinely, isolates are cultured on yeast extract mannitol agar (YEMA) with and without pH indicators, examined for growth rate, examined for morphology under the microscope, and checked for purity. The nodulation ability which confirms their status as *Rhizobium* is usually conducted in artificial media first.

Field trials are quite varied ranging from single to multiple row plots for grain legumes to solid block seeding for forages. Measured parameters under both glasshouse and field conditions often include number, mass, location on root, size, appearance and color of nodules. Agronomic characteristics include plant height, weight (fresh and dry), seed yield, etc. The amount of nitrogen fixed is determined by total plant N, acetylene reduction, N^{15} isotopes, and seed protein converted to nitrogen. Differences between controls and test strains are usually indirect for most measurements with the exception of N^{15} methods. Many laboratories do not measure nodule numbers or mass, but only differences in plant growth (there is a positive correlation between plant growth and total plant nitrogen). The author is of the opinion that any methodology that ascertains the objectives sought in a reliable way is sound.

Host-strain interactions for soybeans and clovers are alluded to and the soybean examples were shown.

Discussion

In testing effectiveness in the field, the effect of the environment is probably the least understood factor.

Cultivar-strain interactions are a source of incredible variation, as seen in the soybean-*japonicum* and clover-*trifolii* systems.

The variation in aspects of testing for effectiveness may be as great as the natural variation in the germ plasm itself.

The fact that the terminology used in describing ineffectiveness and effectiveness remains confusing was discussed briefly.

In field inoculation trials it is important that we know the population level of competing rhizobia. Several participants agreed that field inoculation trials should only be conducted with marked strains (capable of being identified upon recovery).

There is a need for more field inoculation trials, especially in lesser developed countries. Large yield responses are not necessary to make an economic difference to farmers - a small to medium response may well justify the investment in the inoculum.

Some discussion was held on the merits of using low amounts of nitrogen in test media in the growth chamber and glasshouse tests.

TOPIC 5 Evaluation of the Symbiosis Under Special Ecological Conditions : Characterization of Performance Under Stress Conditions of Acidity, Salinity, Alkalinity, Aridity, Temperature and Other Environmental Parameters

H.H. Keyser

Since agricultural legumes are adapted to a wide range of growing conditions around the world, the assessment of the fixation potentials under these various conditions would be valuable in determining strategies for agricultural improvement.

Vast areas throughout the world where legumes have potential are affected by a particular environmental stress. In most cases it is too costly, if not physically impossible, to ameliorate the environment. The natural alternative is the selection and use of adapted germ plasm, both for the legume and the rhizobia.

Findings in the past two decades have indicated it is possible to select plant species and varieties, as well as strains of *Rhizobium*, which give relatively better yields under certain specific environmental conditions than do other symbiotic associations.

Essentially two sources of germ plasm are available to examine for adaptation to specific ecological conditions: material found in existing collections (whose adaptation is unknown) and material collected from areas where known environmental stresses exist.

Alan Gibson has put forth 4 criteria that must be met in the selection and use of environmentally adapted symbioses:

i) We must understand the response of the symbionts to the specific environmental conditions;
ii) We must know which stage of the symbiosis is most severely affected by the particular stress;
iii) We must determine the natural genetic variation to these stresses in the available germ-plasm;
iv) We must understand the environment in which we are working.

I will particularly address this third criterion, exploring the variation in the germ-plasm to stress adaptation, and reviewing examples of how this has been evaluated for the major limiting stresses. These include soil acidity problems, salinity, alkalinity, aridity, temperature and available nitrogen.

Selection and utilization of specifically adapted symbioses has recently received much attention, and initial findings are quite promising. Field evaluation of the selected material will undoubtedly increase.

Discussion

Tests performed on the stress tolerance of legumes are often conducted with combined nitrogen. Without having the rhizobial component present, the tests may vastly overestimate the stress tolerance of a legume.

Which are the parameters of tolerance curators/researchers would like to see identified in strains? Knowledge of tolerance to major environmental limitations would surely be useful.

The vast majority of strains in existing collections have not been evaluated for such tolerances and it is possible they represent the existing range of adaptations. However, the value of collecting rhizobia from soils of natural stress adaptation was pointed out as in Date and Halliday's findings with acid-tolerant rhizobia.

Although rhizobia as a group are quite salt tolerant (compared to the host plants) there is some evidence that strain selection for salt tolerance in some crops can be useful.

TOPIC 6 Field Inoculation of Legumes : The Kinds and Uses of High Quality Inocula, and the Problem of Indigenous Rhizobia

J.C. Burton

1. Introduction

The cardinal objective of most research on symbiotic N_2 fixation is to increase world production of nutritious, body-building legume food crops. Some milestones of progress are:

a) To find or develop more effective nitrogen-fixing strains of rhizobia for all important food and feed leguminous crops.

b) To find or develop good N_2-fixing strains of rhizobia which are also capable of working effectively under various stress conditions, e.g. low pH, high aluminium, alkalinity, high temperature, low moisture.

c) To evolve an effective technology for using these superior strains of rhizobia to assure effective nodulation of the target legumes in the environment where they will be grown.

The major part of all the work on symbiotic nitrogen fixation by legumes will be lost unless we can achieve these objectives.

2. Qualities of the "Super Inoculum"

a) The "Super Inoculum" must contain highly effective N_2-gathering rhizobia for its designated host plant.

b) The strain or strains of rhizobia must be competitive in the presence of other highly infective rhizobia. The strain or strains must be acceptable to their designated host and the rhizobia must be able to survive and multiply even in an adverse environment.

c) The strain or strains of rhizobia must be adapted to the environmental stress whatever it is -- high acidity, high aluminium or manganese, excessive salt concentration, drought, high temperature. Sufficient progress has been made in this general area to justify optimism. (Personal communication D.N. Munns, University of California, Davis).

Acid-tolerant strains have been found for the cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), peanut (*Arachis hypogaea*), pigeonwings (*Clitoria ternatea*), and the soybean (*Glycine max*). Further, the laboratory procedure of testing has been validated by field experiments. Laboratory tests have shown that high aluminium affects rhizobial growth in acid soils and that rhizobial strains differ markedly in their tolerance of low pH and aluminium concentration. The acid/aluminium tolerance is apparently a stable characteristic of rhizobial strains.

It has also been shown that cultivars or varieties of cowpea differ greatly in their tolerance to high aluminium concentrations. Aluminium-tolerant cultivars of the host must be used in conjunction with the aluminium-tolerant rhizobia if success is to be attained. Apparently all soybeans studied to date are aluminium sensitive. Likewise, varieties of chickpea (*Cicer arietinum*) studied have demonstrated a high degree of sensitivity to salt concentration.

d) The inoculum must supply ample numbers of vigorous, aggressive, effective, selected rhizobia. There is safety in numbers, particularly when the soil is heavily

infested with highly infective rhizobia which fix little or no nitrogen. The numbers needed will vary with legume and environmental conditions, but are likely to be in the range of 10^5 to 10^7 per seed.

e) The inoculum must be accessible, easy to use and not excessively expensive. Improved technology in the manufacture of inoculants will be required. While some improvements in methods of inoculating legume seeds have been introduced, it appears evident that technology in this area must be expanded greatly if we expect to nodulate our legumes with these new "super strains" of rhizobia, particularly in soils which have grown the particular legume for years.

3. Inoculation Methods Currently Used -- Advantages and Disadvantages

a) Dust or Dry Method: Inoculum powder (peat, charcoal or a mixture) is stirred with dry seed in a drill hopper without using any water or liquid. This method is commonly used for small-seeded forage legumes as well as for soybeans, peas, and other grain legumes, particularly in temperate climates. It is quick and easy, but ineffective.

Disadvantages: Most of the inoculum is lost; it does not adhere well to seed. Much of it never reaches the soil. The amount is too small to be metered out with the common drill or seeder.

b) Sprinkle Method: The seed is moistened with water (14 or 15 ml of H_2O to 1 kg seed) and then the inoculum powder is mixed with the moistened seed. This method is reasonably easy, requires little extra work, can be done in the drill seed hopper, and appears to cover the seed fairly uniformly immediately after mixing.

Disadvantages: Since the water is added first, the seed quickly absorbs the water and little free water is available to really stick the inoculum to the seed. The inoculant falls from the seed when it dries and the loose inoculant is lost to the wind. This sytem is, at best, only moderately effective under good planting conditions.

c) Slurry Method: The inoculum powder is first mixed with water, milk, or some other non-noxious liquid to form a slurry (22 to 23 ml of liquid and 4 g of inoculum for 1 kg seed). The slurry is mixed with the seed until it is thoroughly covered with the slurry. The inoculum adheres tightly. Twenty to thirty minutes of drying in the shade is recommended to facilitate smooth, uniform seeding. This method is fairly effective providing the inoculum is fresh and virile and environmental conditions are favourable at sowing time.

Disadvantages: Slurry inoculation requires more time and labour. Careless operators may make the seed too wet and

difficult to drill. This method is not effective when seed has a chemical fungicide or insecticide dressing. Seed not planted within 24 hours should be reinoculated.

d) Lime Coating (Brockwell, 1977): The inoculum is first mixed with a gum solution (Cellophos-A, Methocel or gum arabic) to form a slurry (8.5 g inoculum to 75 ml of gum solution for 1 kg of seed). This is poured onto the seed in a rotary drum mixer and while the seed is still very wet, finely pulverized lime or some other inert powder (386 g per kilogram of seed) is added and the seed rolled in the drum until thoroughly coated. Screening after the seed is dry is recommended. This method is advantageous when seed is to be mixed with acid fertilizers before sowing in highly acid soils. The lime provides a protective coating in the micro-environment while the seed is germinating. It is advisable to increase the size of the inoculum for greater assurance of success. The larger inoculum will not interfere with seeding because the lime covers and dries the seed.

Disadvantages: This system is very laborious, expensive, requires special mixing equipment and almost doubles the weight of seed. The coated seed are highly abrasive and can cause serious damage to drills. In many cases the seeds must be transported to a processor for custom inoculation. This increases the expense.

e) Seed Coating With Peat Inoculum (Burton, 1975): A gum solution (gum arabic, carboxy-methyl-cellulose and sucrose (10.5 %, 2.1 %, and 10.5 % respectively) is used to prepare an inoculant slurry (17 g inoculant in 45 ml of solution for 1 kg of seed). While the seed is wet with the inoculum slurry, additional peat inoculant (33 g/kg) is added and mixed with the seed to dry it. Seed should be allowed to dry in the shade for 20 to 30 minutes before sowing. The advantages of this method are: It provides a very large inoculum which adheres well to the seed. Many rhizobia survive and they multiply rapidly when the seed is sown in moist soils; good results are usually obtained even in hot, dry soils providing rains come within 2 to 3 weeks. No special equipment is needed and the inoculating can be done on the farm.

Disadvantages: This method is laborious and the inoculum is expensive.

f) Solid or Granular Soil Inoculum (Burton, 1979): An inoculum is prepared using granules of peat (16-50 mesh) to give a moisture content of 33 to 36%. The inoculum is drilled into the seed furrow at time of planting with a granular applicator designed for uniform distribution of small amounts of inoculum (6 to 15 kg per hectare) in the seed furrow. This method is easy and dependable

even when legume seeds are sown under adverse conditions. The inoculum can be placed at a soil depth favourable for survival. Large amounts of inoculum can be applied without interference with drilling. The inoculum is not affected by chemical fungicides or insecticides on the seed.

Disadvantages: This method is expensive, the inoculum is heavy to transport, and the drills must be equipped with granular applicators.

g) Slurry Liquid Soil Inoculation (Brockwell and Gault 1979): Peat-base inoculum made with finely pulverized peat is suspended in water and sprayed into the furrow with the seed. Drills must be equipped with a large reservoir, a gear pump, a pressure regulator, and a hose and nozzle for each unit of the planter. This system is dependable, easy to operate, and requires only minor modification of the drill. It allows rate adjustment. The inoculum is not affected by chemical dressings applied to seed for control of fungi and insects. Also, the regular seed inoculum can be used; the inoculum is light in weight, easy to transport, and thus not overly expensive.

Disadvantages: The drill has to be modified. Plugging of the nozzles can occur with wet soil and if the peat inoculum contains any large particles. The slurry inoculum must be used on the day of preparation unless refrigeration facilities are available to handle the large volume of inoculant suspension required. A large water supply and auxiliary equipment is needed.

4. Competition With Indigenous Rhizobia

It is generally recognized that inoculation of leguminous seeds is necessary and desirable when a legume is planted for the first time in a field which has not grown that species or another species nodulated by the same kind of rhizobia. However, once a field has successfully grown a leguminous crop, the problem of introducing new strains of rhizobia is exceedingly difficult. Only the methods described above under e), f) and g) could be considered, and even then it is very likely that the size of the inoculum would have to be greatly increased. As Nutman (1975) pointed out, seed inoculation at the recommended rate introduces a maximum of 10^9 *R. trifolii* or 10^8 *R. japonicum* per hectare when the seeds are inoculated. In contrast, an ordinary good soil after growth of a legume contains up to 10^{20} rhizobia per hectare to a depth of 10 cm. Working with soybeans in an old field, Ham (1978), by increasing the size of the inoculum 1,000-fold, was able to get as many as 50% of the nodules produced by the inoculum strain. There was however, no increase in yield.

5. Introduction of Rhizobia Into Soils With Various Stresses

Some success has been attained in introducing rhizobia into acid soils by coating the inoculated seeds with finely pulverized limestone but our technology on overcoming acid and various other soil and

environmental stresses is still in its infancy. The establishment of selected rhizobial strains in rhizobia-free soils with stresses might well prove to be easier than establishing them in old fields which have grown the particular legume previously. There is a good possibility, however, that the inoculum will have to provide large numbers of viable rhizobia. We will have to develop effective methods of culturing these rhizobia in mass, and of maintaining their desired characteristics, and an improved technology for application to the seed or soil to assure rapid multiplication and early nodulation of the roots. The task will be difficult and challenging.

References

Brockwell, J. (1977). Application of Legume Seed Inoculants, pp 277-310 In A Treatise on Dinitrogen Fixation, Sec. IV Agronomy. R.W.F. Hardy and A.H. Gibson (eds), John Wiley and Sons, New York.

Brockwell, J., R.R. Gault and F.W. Hely (1979). Field experiments with solid and liquid inoculants introduced directly into the seed bed. Proc. Australian *Rhizobium* Conference, Perth.

Burton, J.C. (1976). Methods of inoculating seeds and their effect on survival of rhizobia. Chap. 15, pp 170-175 In Symbiotic Nitrogen Fixation in Plants. P.S. Nutman (ed.), Cambridge University Press, Cambridge.

Burton, J.C. (1979). New developments in inoculating legumes, pp 380-405 In Recent Advances in Biological Nitrogen Fixation. N.S. Subba Rao (ed.), Oxford and IBH Publishing, New Delhi.

Burton, J.C. (1979). *Rhizobium* inoculation and soybean production, pp 89-100 In World Soybean Research Conference II. Westview Press, Boulder, Colorado.

Ham, G.E. (1978). Recent developments in Soybean Inoculation. Proc. of Eighth Soybean Research Conference.

Nutman, P.S. (1975). *Rhizobium* in the Soil. Chap. 6, pp 111-132 In Soil Microbiology, N. Walker (ed.), Halsted Press, John Wiley and Sons, New York.

Discussion

The subject of soil replacement of inoculant versus indigenous strains was discussed. Nodule occupancy percentages can be shifted against a competitive background, but often there is not a resultant yield increase.

Even when competing rhizobia are present in very low numbers they can sometimes interfere with successful nodulation of an introduced strain.

The described approach of selecting nodules in the field from the exceptional green plant in a general population of ineffective but very competitive strains, and subsequently producing a competitive inoculant from these isolates, was well received.

IV. PROPOSALS FOR OECD-SPONSORED RESEARCH FELLOWSHIPS

At the conclusion of the scheduled programme the Organizer extended his personal, as well as OECD's, appreciation to Alan Gibson for his arrangement of Workshop facilities at CSIRO, to the Topic Chairmen and Discussion Leaders for their duties and fine presentations, and to all participants for critical, stimulating and useful discussions.

The final session of the Workshop was a discussion by participants of ideas for the fellowships, based on the issues and problems previously discussed and on the requirements of OECD.

Aside from the fellowships, two recommendations arising from general discussion were put forth:

1. In response to the question on whether or not a new edition of Skerman's catalogue of rhizobia collections was deemed useful or necessary, there was a generally favourable reaction to the suggestion that Rhizobium Newsletter publish the holdings of collections by strain numbers for each host. The two of three editors of the Newsletter present (Date and Gibson) agreed that they would be prepared to publish this if Curators forward the information.

2. A recommendation was made that a few of the very experienced *Rhizobium* Curators present should consider getting together to exchange detailed notes on the matter of strain duplication/pedigree information, so as to keep duplicated efforts to a minimum in the future.

Following discussion the following proposals regarding Fellowship topics were made.

1. J. Beringer proposed a Fellowship to study the DNA homology of serological type strains to determine the relatedness of groups and how many species of rhizobia are represented in collections.

2. W. Broughton proposed a Fellowship in his laboratory to study the relationship of plasmids to the competitive ability of *R. leguminosarum* strains on *Pisum*.

3. B. Jarvis proposed a study in his laboratory on the DNA homology and plasmid complement of rhizobia from centres of diversity to assist determination of the origins of genetic diversity and rationalisation of germ-plasm collections.

4. C. Parker proposed a general study on the survival of rhizobia on seed dealing with the biochemical and

physiological aspects of initial shock, death, and training.

5. L. Bordeleau proposed a Fellowship in his laboratory on the study of factors affecting the survival of rhizobia on legume seed, but made a plea for more DNA homology studies.

6. P. Bishop proposed a general undertaking for a co-operative, uniform inoculation trial with several soybean genotypes and a single marked strain of *B. japonicum* which would be evaluated over a number of years at different sites around the world.

7. K. Lindstrom proposed a general form of OECD assistance for further development of the N-fixation research programme in Helsinki.

8. T.A. Lie made a plea for general assistance in further studies on the relationships among primitive legumes and rhizobia.

Other suggestions related to germ-plasm for some of the more unusual legumes (Scott); studies on host genotypes (Murphy); studies on rhizobia/legume interactions and variation in existing gene pools (Gibson); studies on strain competition and preference with marker strains (Jones); effect of monoculture in tropical agriculture on persistence, etc. (Nutman).

V. Advisory Committee Meeting On Selection of Proposals for Fellowships

The members of the committee met following the Workshop to discuss and select two or three proposals that could be funded for 10 month periods from May 1981. The members were John Beringer, Anton Quispel, Pat Murphy, Harold Keyser and Dick Clarke. The committee discussed the merits of the proposals and also considered the minimum time in which some reasonable accomplishment could be expected. Two research topics were chosen to be recommended for support. The announcement of the Fellowships relating to these topics is shown below. This was circulated to all national correspondents immediately following the Workshop. A preliminary announcement of the intended Fellowships had been distributed some months earlier.

OECD Co-operative Research Project on Food Production and Preservation

Research Fellowships - Nitrogen Fixation

Two Fellowships are available for 10 month periods from May 1981 for research on *Rhizobium*. For each Fellowship one return air fare (economy) from the appointee's home is available, in addition to a monthly renumeration of 5000 French francs. Applicants should, preferably, have post-doctoral experience. Details of the Fellowships are given below.

1. DNA Homology of *Rhizobium* strains

The aim is to examine "type" strains of *Rhizobium* using DNA hybridization techniques to determine how many "species" or related groups are represented among "type" strains, how well these groups are represented in collections and in inoculant strains, and how these groups represent the known immunological groupings.

Applicants should have experience in *Rhizobium* microbiology and immunology : instruction can be given in DNA hybridization techniques. The position is tenable with Dr B.D.W. Jarvis, Senior Lecturer in Microbiology and Genetics, Massey University, Palmerston North, New Zealand.

2. Factors Affecting Survival of *Rhizobium* on Seeds

The aim is to elucidate factors that affect survival of rhizobia on legume seed. Type strains will be used to study parameters such as the biochemical activities of resting cells, changes in the cell

wall and in cell reserves, and the effect of "training" on survival.

Applicants should have experience in *Rhizobium* microbiology with a strong background in biochemistry and instrumentation. The position is tenable with Dr L.M. Bordeleau, Canada Agriculture, 2560 Hochelaga Boulevard, St Foy, Quebec G1V 2J3, Canada.

Applications

Applications with full details of the applicant's training and experience should be sent by 31 January 1981 to

Dr R.T.J. Clarke,
Applied Biochemistry Division,
Department of Scientific & Industrial Research,
Private Bag,
Palmerston North,
New Zealand.

VI. ADDRESSES OF MAIN CONTRIBUTORS, COMMITTEE AND NATIONAL CORRESPONDENTS

WORKSHOP CHAIRMEN AND DISCUSSION LEADERS

J. BROCKWELL, Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, ACT 2601, Australia.

W.J. BROUGHTON, Max-Planck Institut fur Zuchtungsforschung, B5000, Koln 30, West Germany.

J.C. BURTON, 4930 N. Elkhart Avenue, Milwaukee, Wisconsin 53217, United States of America.

K.W. CLARK, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada.

L.R. FREDERICK, Agency for International Development, DS/AGR, 420 SA-18, Washington, DC, 20523, United States of America.

D.G. JONES, Department of Agricultural Botany, University College of Wales, Aberystwyth, Dyfed SY23 3DD, Wales, United Kingdom.

H.H. KEYSER, Cell Culture and Nitrogen Fixation Laboratory, USDA, BARC-W, Beltsville, Maryland 21029, United States of America.

T.A. LIE, Microbiology Laboratory, Hesselink van Suchtelenweg 4, Wageningen, 6700JE, Netherlands.

R. PULLEN, Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, ACT 2601, Australia.

R.J. ROUGHLEY, Horticultural Research Station, P.O. Box 720, Gosford, New South Wales 2250, Australia.

T. RUIZ-ARGUESO, Microbiologia, Escuela Tecnica Superior de Ingenieros Agronomos, Ciudad University, Madrid 3, Spain.

D. WEBER, Cell Culture and Nitrogen Fixation Laboratory, USDA, BARC-W, Beltsville, Maryland 20705, United States of America.

ADVISORY AND ORGANIZING COMMITTEE FOR WORKSHOP

J. BERINGER, Department of Soil Microbiology, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JA, England, United Kingdom.

R.T.J. CLARKE, Applied Biochemistry Division, Department of Scientific & Industrial Research, Private Bag, Palmerston North, New Zealand.

H.H. KEYSER, Cell Culture and Nitrogen Fixation Laboratory, USDA, BARC-W, Beltsville, Maryland 21029, United States of America.

P. MURPHY, Agricultural Institute, Johnstown Castle Research Centre, Wexford, Ireland.

A. QUISPEL, Botanical Laboratory, University of Leiden, Nonnensteeg 3, 2311 VJ Leiden, Netherlands.

NATIONAL CORRESPONDENTS FOR PROJECT (1981)

Belgium	Prof. C. BONNIER, Recteur de la Faculté des Sciences agronomiques, Impasse des déportés, 5800 - GEMBLOUX.
Canada	Dr L. BORDELEAU, Agriculture Canada, Research Station, 2560 Hochelaga Boulevard, Sainte Foy, QUEBEC G1V 2J3.
Finland	Professor Eero VARIS, Department of Plant Husbandry, University of Helsinki, Viikki, 00710 HELSINKI 71.
France	M. Jean DENARIÉ, Maître de recherches à l'I.N.R.A., Station de Pathologie végétale, Centre national de Recherches agronomiques, Route de Saint-Cyr, 78000 VERSAILLES.
Germany	Prof. Dr M. DAMBROTH, Bundesforschungsanstalt für Landwirtschaft, Braunschweigh-Vokenrode, Bundesallee 50, D-3300 BRAUNSCHWEIG.
Ireland	Mr C. MASTERTON, Soil Biology Department, Johnstown Castle Research Centre, WEXFORD.
Netherlands	Prof. Dr A. VAN KAMMEN, Department of Molecular Biology, Agricultural University, De Dreijen 11, 6703 BC WAGENINGEN.
New Zealand	Dr R.T.J. CLARKE, Applied Biochemistry Division, Department of Scientific & Industrial Research, Private Bag, PALMERSTON NORTH.
Sweden	Professor Börje NOREN, Department of Microbiology, The Swedish University of Agricultural Sciences, 750 07 UPPSALA 7.

Spain — Dr Manuel BIJERIEGO, Département de Physiologie, I.N.I.Z. Apartado 8.111, MADRID 3.

Switzerland — Dr W. JÄGGI, Station de recherches agronomiques de Zurich-Reckenholz, Reckenholzstrasse 191/211, 8046 ZURICH.

Turkey — Dr N. ULGEN, Toprak ve Gubre Arastirma Enstitusu, P.K. 54, Yenimahalle, ANKARA.

United Kingdom — Dr J. BERINGER, Soil Microbiology Department, Rothamsted Experimental Station, Harpenden, HERTS., AL5 2JA.

United States — Dr P. BISHOP, SEA, USDA, Department of Microbiology, North Carolina State University, Raleigh, N. CAROLINA 27650.

Yugoslavia — Dr Zivota VOJINOVIC, Institut des sols, 7, rue Teodora Drajzera, 11000 BEOGRAD.

PROJECT CO-ORDINATOR, NITROGEN FIXATION

Dr R.T.J. CLARKE, Applied Biochemistry Division, Department of Scientific & Industrial Research, Private Bag, Palmerston North, New Zealand.

Provided by John Brockwell CSIRO Canberra, Australia

RHIZOBIUM TRIFOLII

CANBERRA NO. TA1 SYNONYMS CC246,NA14-1,SU659

LAST TESTED FOR VIABILITY 1.4.80 INFECTIVITY 1.4.80 EFFECTIVENESS 1.4.80

ISOLATED FROM TRIFOLIUM SUBTERRANEUM

COLLECTING SITE BRIDPORT,TASMANIA ALTITUDE 20 M

SOIL INFORMATION LOAMY SAND DERIVED FROM WIND-BLOWN SAND

ENVIRONMENTAL INFORMATION GRASSLAND,UNDULATING,FREELY-DRAINING SOIL

RAINFALL INFORMATION 975 MM. AUTUMN,WINTER,SPRING INCIDENCE.MARITIME CLIMATE

FULLY EFFECTIVE FOR TRIFOLIUM PRATENSE,T.FRAGIFERUM,T.CHERLERI,T.REPENS, T.SUBTERRANEUM,T.HIRTUM

PARTLY EFFECTIVE FOR TRIFOLIUM INCARNATUM

INEFFECTIVE FOR TRIFOLIUM AMBIGUUM

GENERAL REMARKS ONE-TIME AUSTRALIAN INOCULANT STRAIN. POOR NODULATION OF WOOGENELLUP SUBTERRANEAN CLOVER IN THE FIELD.

RELEVANT LITERATURE

GIBSON,AH. AUST.J.BIOL.SCI.19,499-515,1966.
GIBSON,AH. AUST.J.AGRIC.RES.19,907-18,1968.
GIBSON,AH. AUST.J.AGRIC.RES.19,891-905,1968.
BROCKWELL,J. J.AUST.INST.AGRIC.SCI.34,224-7,1968.
DATE,RA. J.AUST.INST.AGRIC.SCI.35,27-37,1969.
DUDMAN,WF. AUST.J.AGRIC.RES.19,739-47,1968.
MARQUES PINTO,C. AUST.J.AGRIC.RES.25,317-29,1974.
CHATEL,DL. SOIL.BIOL.BIOCHEM.5,443-50,1973.

Form for the World Data Centre for Microorganisms

Provided by Dick Date, CSIRO St. Lucia, Australia

NOMENCLATURE DATA

Genus	Species, subspecies, infrasubspecies	Type
RHIZOBIUM		B

*100 Author(s) of the name (with date(s)) (e.g. *Rhizobium phaseoli* Dangeard 1926)

*110 World Directory Collection Number	Collection Acronym	Collection Accession Number

HISTORY

*120 Received as (Genus, species, subspecies, infrasubspecies)

*125 Date received / / Day Month Year

*130 Received from (Name and address of person or collection) As *140 Strain designation

*150 Who received it from (Name and address of person or collection) As *160 Strain designation

*170 Who received it from (Name and address of person or collection) As *180 Strain designation

*185 Any previous history (include accession number)

*190 Also held in following permanent collections (Include acronym and accession number, e.g. ATCC 12345)

ORIGIN

*200 Isolated from

*201 Genus _____ *202 Species _____ *203 Cultivar _____

*210 Part of plant (Tick applicable block)

☐ Root nodule ☐ Other (specify) _____

*220 Original collection site *221 _____ Country

*222 _____ State/Province *223 _____ Nearest town *224 _____ Distance and direction from town

*225 _____ Altitude *226 _____ ° ' " Latitude (indicate North or South) *227 _____ ° ' " Longitude (indicate East or West)

Collection site soil properties

*350 Soil family _____ *358 Phosphorous (ppm) _____

*352 pH (water) _____ *360 P-extraction solution used _____

*354 pH (N KCl) _____ *362 Salinity (m moles cm) _____

*356 pH (.01 M $CaCl_2$) _____ *364 Base saturation (%) _____

*230 Isolated by (Name and address) As *235 Strain designation *240 Date / / Day Month Year

*250 Identified by *260 Date / / Day Month Year

MAINTENANCE

WFCC Form SCC-4(R)

*270 Method of preservation (Please tick whichever is applicable) and *280 Temperature of storage

Original source material	°C	Agar culture	°C
Lyophilised culture	°C	Liquid nitrogen	°C
In soil	°C	Under oil	°C
Dessicated on beads	°C	Other (Specify) _____	°C

*290 Preservation suspending medium

*300 Growth medium (Name and reference, or give details on separate page) *310 Temperature

*320 Growth conditions (e.g. aerobic, anaerobic, special gas mixture, light)

*322 Incubation time (Tick applicable block)

Slow (> 5 days) ☐ Fast (< 5 days) ☐ *327 Period between subcultures

SPECIAL FEATURES AND USAGE (e.g. for citric acid production, Type strain etc.)

*370 Other legumes nodulated	*373 Effectiveness (E, PE, I)	*375 Suitability as inoculant (Excellent, good, fair, poor)

Tolerance to stress: Indicate either (a) range or (b) high, medium, low tolerance

*380 As free-living organisms:

pH _____, Al^{+++} _____, Mn^{++} _____, NaCl _____, Temp. _____ °C

*390 As symbionts in host nodules:

pH _____, Al^{+++} _____, Mn^{++} _____, NaCl _____, Temp. _____ °C

*330 Other special features (CHO metabolism, etc.) *335 Reaction in standard YMA ☐ Acidic ☐ Basic

REFERENCES (Journal, volume, pages, year)

*340

If space is inadequate to reply to any question, please submit additional information on separate pages identified by appropriate reference number (e.g. *310)

E = Effective PE = Partially effective I = Ineffective

30