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TECHNICAL ISSUES RELATING TO AGRICULTURAL MICROBIAL GENETIC RESOURCES (AMIGRS), INCLUDING THEIR CHARACTERISTICS, UTILIZATION, PRESERVATION AND DISTRIBUTION

A DRAFTINFORMATION PAPER PREPARED FOR THE GENETIC RESOURCES POLICY COMMITTEE (GRPC) OF THE CGIAR

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EXECUTIVE SUMMARY

PART 1

[ES.1] Plants and animals can not grow optimally without microbes, and 90 percent of flowering plants form some association with microbes to enhance their growth. Biological Nitrogen Fixation (BNF), for example, is one of the most important biological processes on the planet, turning inert nitrogen gas from the air into a form that plants and animals can use to make protein.

[ES.2] Agricultural Microbial Genetic Resources (AMiGRs) may be defined as microbes that assist the production of plants or animals, either directly or indirectly, in agricultural settings. AMiGRs can be differentiated from Microbial Genetic Resources (MGRs) utilized in food, medicine and industry, but for many this can only be upon the basis of their functionality (or end-use), as species overlap both categories. AMiGRs have been preserved in a series of *ex situ* repositories associated with institutions or individuals around the globe, in more modern times as freeze dried or frozen (-80°C) cultures.

[ES.3] After root nodule bacteria (RNB), the most preserved microbes appear to be pathogenic fungi and bacteria that are used as type specimens in breeding efforts. Germplasm repositories for bacteria, in particular, have embraced lyophilization as the preferred storage method.

[ES.4] There is evidence that germplasm collections are discarded as the key curator retires, particularly if the germplasm is not freeze dried. Only about half the germplasm repositories surveyed seem to have an accessible electronic database.

[ES.5] The development of a series of *in situ* plant repositories coordinated by ICARDA in West Asia provides an opportunity for associated preservation of AMiGRs for plants and insects, but perhaps not for animal microbes. The AMiGRs most likely to be successful are those that are endophytic (i.e. they invade host tissue) because organisms that only colonize the surface of the target are often non-competitive against microbes already well adapted to that environment

[ES.6] AMiGRs have been used since antiquity, but they have only been properly scientifically described since the late 19th and early 20th centuries, and this process is ongoing

[ES.7] Many currently exploited AMiGRs evolved in developing countries, but were transported to alien shores by accident through contamination of plants, animals or fodder, or in jet-streams. AMiGRs are considered in some quarters to be the ‘bio-prospecting’ entities of tomorrow, as plants are today. International coordination of genetic resources has yet to focus upon, or manage, AMiGRs. There is uncertainty whether AMiGRs utilized today can be reliably traced to their origins, even with the genetic techniques now available.

[ES.8] RNB seem to have been de-emphasized in the CGIAR system in the last decade, perhaps because many consider the work with them to have been completed. However, there appears to be continuing advances with RNB in other agricultural economies. It seems incongruous that many projects are built around microbial germplasm repositories that are uniformly poorly resourced. Some countries, such as China, India and the former Soviet Union, have a cultural history of utilizing AMiGRs, and this is becoming reflected in the nature of the AMiGRs held by some CGIAR centres.

PART 2

[ES.9] It is complex to assess the needs of, or potential benefits from, applying AMiGRs in agriculture because responses are often species and environment specific. This is the greatest challenge in embracing AMiGRs. AMiGR usage in developing countries is often limited by lack of manufacturing capacity and quality control. This needs to be addressed. Developing countries often subsidize imported N[itrogen] fertilizer to make this affordable for their farmers. An alternative is to develop RNB. A global benefit of this is that legume N fixation does not contribute to greenhouse gas emissions, whereas the manufacture of 1 tonne of urea burns 1 tonne of fossil fuels.

[ES.10] AMiGR adoption in developing countries would benefit from the availability of a 'core set' of AMiGRs with which to experiment. This would remove the initial time-consuming need to authenticate cultures and to establish their phenotype. A core set of AMiGRs could readily be developed by scientists who have collaborated in microbial germplasm exchange and evaluation programmes with the CGIAR centres. Countries that hold AMiGRs, for example in *in situ* repositories, may not yet be exploiting them, and thus the cost of conserving germplasm is borne by them for no immediate reward. Developed countries are prominent users of AMiGRs, mainly RNB, but they distribute a narrow range of organisms over vast acreages, and this has implications for loss of microbial biodiversity in these regions.

[ES.11] It is difficult to foresee where (geographically) the next range of exploitable microbes may arise. For example, Australia's microbes may become globally useful in bioremediation, and hence her current role as a net user of agricultural genetic resources (without contributing to the cost of their preservation) might well be reversed. Although the usage of AMiGRs in some agricultural systems might be routine (e.g. RNB), and the benefit of this application may be high (as estimated by the monetary cost of replacing N fixed by RNB with fertilizer N), the wholesale value of manufactured microbes is much lower, and thus any royalties levied on production are not likely to be of high value. The major obstacles to uptake of AMiGRs in developing countries are discovering, preserving and cataloguing the available AMiGR biodiversity, accurately ascertaining the beneficial properties of any AMiGR, and then manufacturing, distributing and utilizing high quality inoculants.

[ES.12] The USDA has moved to centralization of curatorial responsibility for MGRs in the USA. There is evidence that South American countries are utilizing this centralized facility. It could be possible to assign curatorial responsibility for one mainstream group of AMiGRs to each continent.

[ES.13] There has emerged an 'official' approach to acquisition and exchange of AMiGRs over the last decade, with Material Transfer Agreements (MTAs) and Memoranda of Understanding (MOUs) covering acquisition and exchange as well as future control of any commercial outcomes.

[ES.14] The taxonomy of microbes, particularly bacteria, in the 21st century, is unsettled. It is difficult to develop standards for the identification for many microbes, except for type strains whose genome is fully sequenced. AMiGRs may be separated from MGRs on the basis of functionality, but there is overlap, and for some microbes this distinction may have to be at the species level. AMiGRs are delivered live, whilst MGRs generally transform a process and are then eliminated—a major difference between AMiGRs and MGRs that can influence the possibility of obtaining and enforcing patents for MGRs used in food, medicine and industry. The situation for AMiGRs is clouded by their rapid rate of reproduction, and potential change during culture.

[ES.15] Quarantine and biosecurity concerns are reducing the extent of germplasm exchange, more so than issues of 'ownership'. Commercial entities in the 21st century are patenting AMiGR manufacturing and delivery technologies, rather than the microbes themselves. This reflects that a major challenge in utilization of AMiGRs is the development of appropriate manufacturing and delivery technologies.

PART 1

I. INTRODUCTION: HISTORICAL PERSPECTIVE

[1] Although the Romans wrote of the beneficial effects of cultivating nitrogen-fixing legumes such as lupins and pulses in rotation with cereals, the deliberate utilization of microbes in agriculture awaited the advances in manufacturing technologies that were developed towards the end of the 19th century. The invention of fine instrumentation for observing microorganisms, the subsequent development of specific growth media and then microbe purification enabled the microscopic world to be studied in detail. The root nodule bacteria (RNB) for legumes were almost certainly the first group of agricultural microbes to be studied at the microscopic level, and this was in the same decade that proof emerged (in 1883) that microbes such as *Vibrio* spp. were the causative agents of serious human and animal illnesses, such as cholera. RNB were, in fact, manufactured as agricultural amendments within a few years of Beyerinck isolating and growing the bacteria, and Wilfarth and Hellriegel identifying their role in legume nodulation and nitrogen fixation in 1887. This was only 6 years after Koch first cultured bacteria on gelatin. The early adoption of RNB inoculants was achieved by transferring soil from field to field, or soil to seed before planting, but this was quickly replaced by the supply to farmers of pure cultures on agar slants, then as broths. The first inoculant industries for RNB developed in the 1920s, with peat carriers available from the 1950s (Deaker et al. 2004). Global inoculation of legumes with RNB is valued at in excess of US$ 10 billion annually (calculated on the basis of the cost of replacing RNB-fixed N with manufactured N; Herridge 2005). This equation does not include the additional benefit that legume N fixation is a net user of greenhouse gases, whereas the manufacture of fertilizer N is energy demanding, and thus a net producer of greenhouse gases.

[2] Concomitant with the isolation of RNB from nodules, the understanding of the diversity of microbes interacting in symbioses with plants was expanded with the discovery of the relationship between certain fungal hyphae and plant nutrient acquisition. Frank described the fungus-root interaction with mycorrhizae in 1885, and it is now realized that about 95 percent of all vascular plants are involved in symbiotic associations with fungi. The most notable of these roles is with vesicular arbuscular mycorrhizae (VAM) and ectomycorrhizae in the acquisition of phosphate. It was not long into the 20th century before the role of the soil microflora in the development of plant disease and also in nutrient cycling in the soil ecosystem could be quantified. The concept of the ‘rhizosphere’ and its role in plant growth was described in the 1950s, and the capacity for rhizosphere organisms to affect plant growth by hormone production, diazotrophy (non-symbiotic N fixation) or nutrient acquisition reported soon after. Rumen microbiology had become a discrete science by the 1970s, and the molecular communication between microbes and plant roots (or animal cells), leading to regulation of gene cascades, was revealed in the 1980s. The latest phase in the discovery of microbes as plant symbionts is in their role as intercellular endophytes. Within (or between) plant cells, secondary metabolites from endophytic microbes elicit plant responses. The best described of these associations is with *Aceotobacter* sp. in Brazilian sugar-cane systems, which has the capacity to provide N in excess of 30 kg/ha. Unfortunately, difficulties with culturing the endophytic VAMs (for phosphate acquisition) has restricted their widespread adoption.

[3] It is now accepted that without these multiple aspects of microbial activity in the soil and rhizosphere, as well as in plant and animal tissues or cells, healthy plant and animal growth would not be possible. However, another facet of AMiGRs which is in a phase of development is in the use of microbes as indirect agents of plant growth (i.e. restricting a competitor or predator, rather than as plant symbionts or initiators per se). Thus, we now see a range of AMiGRs being considered as biocontrol agents for crop insects (e.g. *Metarhizium* spp.; nuclear polyhedrosis viruses) and fungal plant pathogens (e.g. *Bacillus subtilis*) to protect crop plants from disease. This field is termed ‘entomopathogenicity’ and there are several registered products currently on the market. One of these, *Metarhizium anisopliae,* has been used successfully to avert grasshopper plagues developing in outback Australia, prior to them moving towards farmers’ crops. This sort of application of AMiGRs, together with RNB, entomopathogens and VAM, has great potential in developing countries. It is

worth noting here the historical widespread use of two classes of AMiGRs in China, India and the former USSR: organisms that stimulate root growth through hormone production or through diazotrophic N production, commonly termed 'yield increasing bacteria' (YIB), have gained substantial acceptance in the rural communities of these nations.

[4] Thus, AMiGRs have been used, in one way or another, since antiquity, with the science of their interactions with plants, insects and animals only elucidated in the last 125 years. There is still much to learn about the microbes that enhance and protect animal growth in both natural and agricultural settings.

II. SCOPE OF THE REVIEW

[5] The scope of the review was defined by the Terms of Reference (see Appendix 4).

III. MICROBIAL GENETIC RESOURCES FOR FOOD AND AGRICULTURE AS A DISTINCT SUBSET OF MICROBIAL GENETIC RESOURCES

[6] Following the development of RNB as inoculants in the late 19th century, other microbes are now applied in agriculture, in a relatively wide variety of roles. These disparate roles can be summarized from a functional perspective, and then compared with microbes used in food, industrial processes and in medicine production. Differences between the two groups of microbes are considered, and there is a discussion of how effectively we have captured these roles to enhance agricultural production.

[7] The main functional roles of microbes in agriculture are considered to be as:

- plant microsymbionts;
- associative organisms (i.e. eliciting or enhancing a positive reaction or effect when in intimate proximity to a plant or animal);
- rumen organisms;
- biocontrol agents (pathogens of weeds, fungi, insects or nematodes);
- pathogens of plants or animals;
- agents for nutrient solubilization, bioremediation or biodegradation;
- agents for production of biofuels; or
- agents facilitating DNA or gene transfer.

Examples of some AMiGR within these functional roles are given below, with more details to be found in Appendix 1.

Plant microsymbionts (specifically RNB) — overwhelmingly the most successful AMiGR in agriculture

[8] RNB, like legumes, are found on all continents. The RNB nodulate the Leguminosae, which is one of the largest families of flowering plants, with more than 18 000 species classified into 650 genera (Sprent, 2001), just under one-twelfth of all known flowering plants. RNB tend to colonize the soils in association with their host legumes, although there is speculation (and indeed evidence) that some species of RNB 'invade' soils well in advance of their host. Not all the legumes fix atmospheric N, however, and amongst the subfamilies of the Leguminosae, the species within the Fabaceae are recognized as those of greatest agricultural importance. Some of our most valuable food crops, such as pea (*Pisum* spp.), beans (*Phaseolu*s spp.), ground-nut (*Arachis* spp.) and soybean (*Glycine* spp.) are Fabaceae, producing high-protein grains for human consumption. Of all the plants that man uses for

food, perhaps only the grasses (Graminiae) are more important than the legumes (Graham and Vance, 2003).

[9] The symbiotic association between RNB and legumes plays a significant role in world agricultural productivity by annually converting approximately 100 million tonnes of atmospheric nitrogen into ammonia (Herridge and Rose, 2000), and saving $US 10 billion in fertilizer N. This is a critical issue, as many countries (both developing and advanced) have not fully embraced biological nitrogen fixation and are substantially reliant upon fertilizer nitrogen. This lack of adoption of RNB is attributed to many factors: from a lack of knowledge and expertise in growing and inoculating legumes with rhizobia (Giller, 2001), to government subsidies in both developing and advanced economies that militate against the use of biological nitrogen fixation. Sadly, with the price of fossil fuels inevitably increasing, small economies will be faced with either food shortages or an inflated bill for fertilizer N. Many developing countries rely upon buying urea for rice production (Thein and Hein, 1997). Their declining purchasing power in real terms will be deleterious for food production; this must be addressed, as current reviews forecast that food production will need to double by 2020 to feed our expanding population (Byerlee and White, 2000).

Vesicular arbuscular mycorrhizae (VAM) and ectomycorrhizae

[10] Approximately 90 percent of all flowering plant species belong to families that form mycorrhizal associations. Mycorrhizae can be either endophytic (exist within cells) or grow between cells (ectophytic) of plant roots. Both patterns of development can be viewed as providing an extension of the plant root systems for the purpose of exploring a greater soil volume for nutrient uptake. Mycorrhizae and their interactions profoundly affect forest site productivity through capture and uptake of nutrients, protection against pathogens, maintenance of soil structure and buffering against moisture stress. The nutrients that are most often limiting plant growth are fixed nitrogen (N) and phosphorus (P), and it is for alleviating deficiencies of the latter that mycorrhizae have proven efficacious. Where soil P levels fall to 1 or 2 ppm, plant growth is usually constrained. Unfortunately, many heavily leached tropical soils are at or below this level and it is in these environments, as well as in severely eroded regions, that applications of mycorrhizae can be effective. Although the VAM are difficult to culture, they are the preferred type of inoculant, so we see cottage industries in tropical and subtropical countries where soils containing VAM are used to inoculate trees in nursery situations. When planted out into degraded lands, the VAM-inoculated seedling trees have a distinct advantage over uninoculated trees. VAM utilization has not spread to broad-acre crops for two main reasons. Firstly, it is difficult to inoculate crops with soil containing VAM over wide acreages, and, secondly, P fertilizers can effectively replace VAM. Despite this, VAM is a *bona fide* AMiGR in horticulture and forestry applications, and in rehabilitation exercises.

Microalgae, including Cyanobacteria

[11] Cyanobacteria (formerly termed blue-green algae) are photosynthetic prokaryotes, usually unicellular, some of which have the capacity to fix atmospheric nitrogen. The capacity of Cyanobacteria to fix N has long been utilized in paddy rice fields to provide additional N to the rice-growing system, reducing the need to supply all the crop N requirements from combined fertilizer. The Cyanobacteria utilize the water and phosphorus applied to the rice crop, and sunlight as an energy source. The species of Cyanobacteria most commonly utilized in paddy fields is the filamentous algae *Nostoc* spp., which forms a symbiotic association with the water fern Azolla in paddy fields. *Nostoc* spp. may also associate with *Gunnera* spp. and the terrestrial Cycads. *Nostoc* spp. has been transformed by the addition of *Bacillus thuringiensis* (*Bt*) genes to investigate the potential of this alga to control insects in rice production.

Associative organisms: Plant Growth Promoting Rhizosphere (PGPR) organisms or Yield Increasing Bacteria (YIB)

[12] China, India and the former Soviet Union have a long history of experimenting with, reporting and even manufacturing microbes that can be classified as PGPR or YIB. These microbes fit within functional group 2 (see Figure 1). The microbes are generally bacteria that form close associations with plant root systems, but may also be actinomycetes, fungi or endophytes. As a result of a plentiful supply of nutrients exuded from the roots in the rhizosphere, the PGPR have the capacity to grow and produce of enzymes such as ACC deaminase, whose action reduces the production of ethylene under stress conditions. Hormones, such as indole acetic acid (IAA), which affect root growth, branching and hair formation, are also commonly produced by PGPR, together with some N fixation (albeit in small amounts). There are many more mechanisms in which PGPR may benefit their hosts, from disease protection, nutrient solubilization to controlled exchange of mutually desirable proteins. With the cloning era, it has become possible to investigate more elaborately the relationship between PGPR and the host plant, and it is becoming obvious that many of the relationships are established by a complex pathway of low molecular weight (LMW) biochemical signals that control gene expression.

[13] Many of the commonly reported PGPR microorganisms are ubiquitous and it is possible to isolate them from garden, farm and forest soils. Because of the ease of isolation of the common PGPR, there is little exchange of this sort of germplasm per se. For those more difficult to culture, such as the actinomycetes and endophytes, there is substantial laboratory-to-laboratory exchange. Appendix 1 contains descriptions of some of the microbes commonly referred to as PGPR. The Pseudomonads have been used extensively in broad-acre agriculture for many years, but there is very little hard and convincing data that proves yield enhancement from their application. Similarly, *Penicillium* spp. have been developed as agents for solubilization of soil-bound phosphate, although modern studies have questioned this role and attributed their efficacy to direct impacts on plant growth.

Rumen organisms

[14] Some animals have a second stomach called the rumen, in which a suite of microbes assist in the breakdown of otherwise indigestible forages. The best researched rumen microbes are those that enable the digestion of forage containing high tannin levels, but other rumen microbes enhance fibre and cellulose digestion, and mitigate anti-nutritional factors. In cellulose degradation, a complex suite of microbial-mediated actions is initiated by anaerobic prokaryotes and protozoans, which liberate carbohydrates from cellulose. The carbohydrates are then fermented to gaseous end products. There is continuing research to select rumen microbes that minimize the release of methane (a greenhouse gas) to the atmosphere. The rumen microbes eventually overflow into adjacent stomach compartments, where their degradation by stomach acids yields amino acids and sugars that provide animal nutrition. Apart from minimizing methane production, other research interests include modifying the rumen microflora to metabolize toxic compounds found in some forages, such as the fluoroacetate found in many legumes. There is evidence that the rumen microflora can naturally evolve in response to the nutritional environment of their host, and that this response can be transferred from animal to animal.

Biocontrol agents, such as Metarhizium anisopliae (an insecticide), Bacillus subtilis (a fungicide) and B. thuringiensis (an insecticide)

[15] There are approximately 15 different biopesticides in current commerce, with *Bacillus thuringiensis* (*Bt*) accounting for approximately 45 percent of the market. A related species, *B. subtilis,* has been developed as a root-active fungicide, for protecting horticultural plants from pathogens. *B. subtilis* is sold as a fungicide for application to flower and ornamental seeds, and to agricultural seeds including cotton, vegetables, ground-nut and soybean. The bacterium colonizes the developing root system of the plant and competes with fungal disease organisms. The fungal genus *Metarhizium* is another AMiGR that has long shown promise as an insecticide. The successful mass culture of *M. anisopliae* and development of methods of mass-producing infective spores has led to the commercial uptake of this fungus as a microbial ‘insecticide’. *M. anisopliae* is grown on a large scale

in semi-solid fermentation and the spores are then formulated as a dust suspended in oil. This may be aerially applied to insect plagues. In Australian trials, application of *M. anisopliae* from aircraft in remote Queensland controlled a developing locust plague by killing 90 percent of the insects.

Pathogens of plants or animals

[16] Plant and animal pathogens need to be considered as AMiGRs because they are held in germplasm collections to facilitate breeding or selection programmes to find resistance to them. For plants, the pathogens are dominantly fungi, bacteria and viruses, most of which are ubiquitous at the genus level, but many of which have distinctive 'landraces' that are geographically separated. The transport of agricultural plants and animals to new geographical locations is now strictly regulated to control transfer of such pathogens, yet it appears the transfer of pathogenic microbes eventually follows the movement of their hosts. For example, the development of *Cicer arietinum* (chickpea) as an industry in Australia flourished in the early 1990s, but has since been seriously constrained by the development of Ascochyta blight disease, which was previously unrecorded in that country. There has been substantial success in managing the unwanted transfer of animal pathogens. For example, the Foot-and-Mouth virus, in the genus *Aphthovirus,* has been effectively excluded from many major meat producing regions by restrictive quarantine efforts.

AMiGRs as agents for nutrient solubilization, bioremediation or biodegradation

[17] This group of AMiGRs can be considered as separate from the associative organisms in Functional group 2 (Figure 1) principally because they interact with inanimate and inorganic targets (in contrast to the plants or animals that host the associative microbes). Targets for this group of AMiGRs include the (substantial) pool of inorganic phosphate held in the soil, toxic chemicals inadvertently accumulated or deposited in the soil, such as DDT, heavy metals and fossil fuels.

AMiGR for production of biofuels

[18] Biofuels, such as ethanol, have been considered an expedient alternative to fossil fuels since the petroleum fuel crisis of the 1970s. Essentially, carbohydrates derived from sugar-rich plants such as cassava, sugar beet or sugar-cane are fermented to ethanol by yeast in anaerobic respiration, but also occasionally by some bacteria. These microbes might be considered as AMiGRs because of their strong linkages to broad-acre agricultural enterprises.

AMiGRs facilitating DNA or gene transfer

[19] Although bacteria have been exchanging DNA since life formed on the planet, the cloning era began in earnest post-1985 with the deliberate laboratory transfer of whole genes, or parts of genes, between bacteria. Such transfer is now routine in many laboratories, between almost all higher lifeforms. There are universal vehicles for facilitating the transfer of DNA. The most common vectors in agricultural research are *Agrobacterium* spp. for plant-to-plant transfer and *Escherichia coli* for inter-bacterial transfer. Thus, these microbe vectors should be considered as AMiGRs because of their direct relevance to agricultural research.

[20] Fuller descriptions of some of the microbes that fill these functional roles can be found in Appendix 1. It is noteworthy that by far the most successful AMiGRs in broad-acre agriculture appear to be those that are endophytic, i.e. they invade the tissues of their host, for all or part of their life cycle, rather than residing on the surface of the target plant or animal. On the surface they may become exposed to competition from resident organisms that are, perhaps, better adapted to that particular environment.

Grouping the AMiGR into functional roles

[21] AMiGR may be broadly grouped as in Figure 1. Functional roles 6 and 7 in Figure 1 group together microbes that interact with nutrients, biomass or pollutants for bioremediation or fuel production. The beneficial symbiotic organisms (1, 3) can be grouped with those that also increase growth of plants or animals as associative microbes (2). The pathogens (4, 5) can be grouped, whether they are directly beneficial or not, because their modes of action are similar (i.e. they decrease growth of the target organism). These last-named two functional groupings (highlighted) contain those microbes that have seen major exploitation in agriculture.

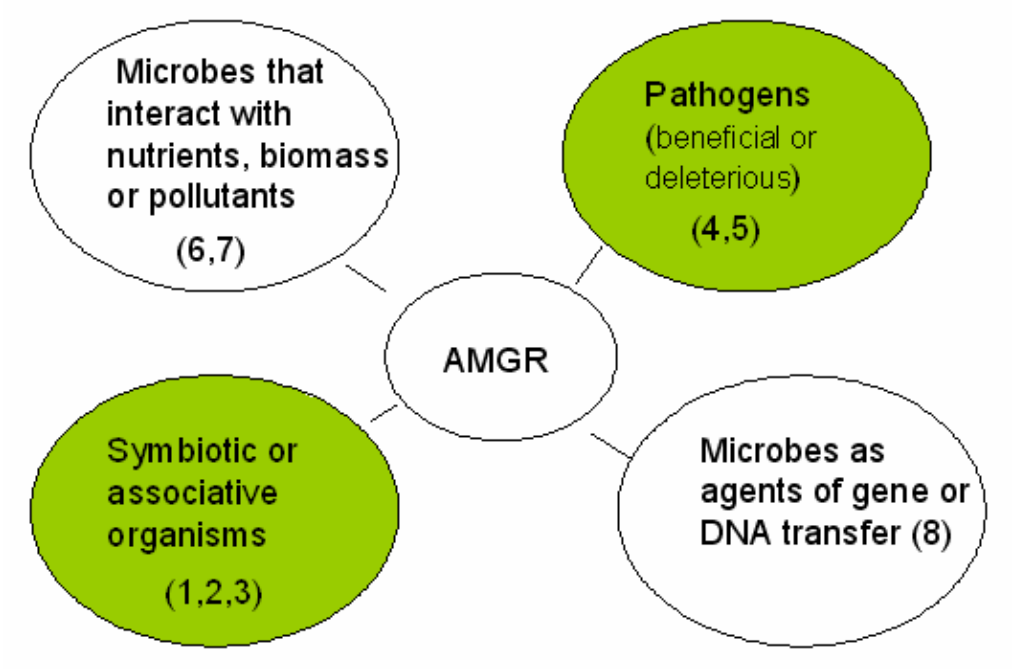

Figure 1. AMiGRs assigned to functional groups, with the highlighted groups being those most exploited in agriculture.

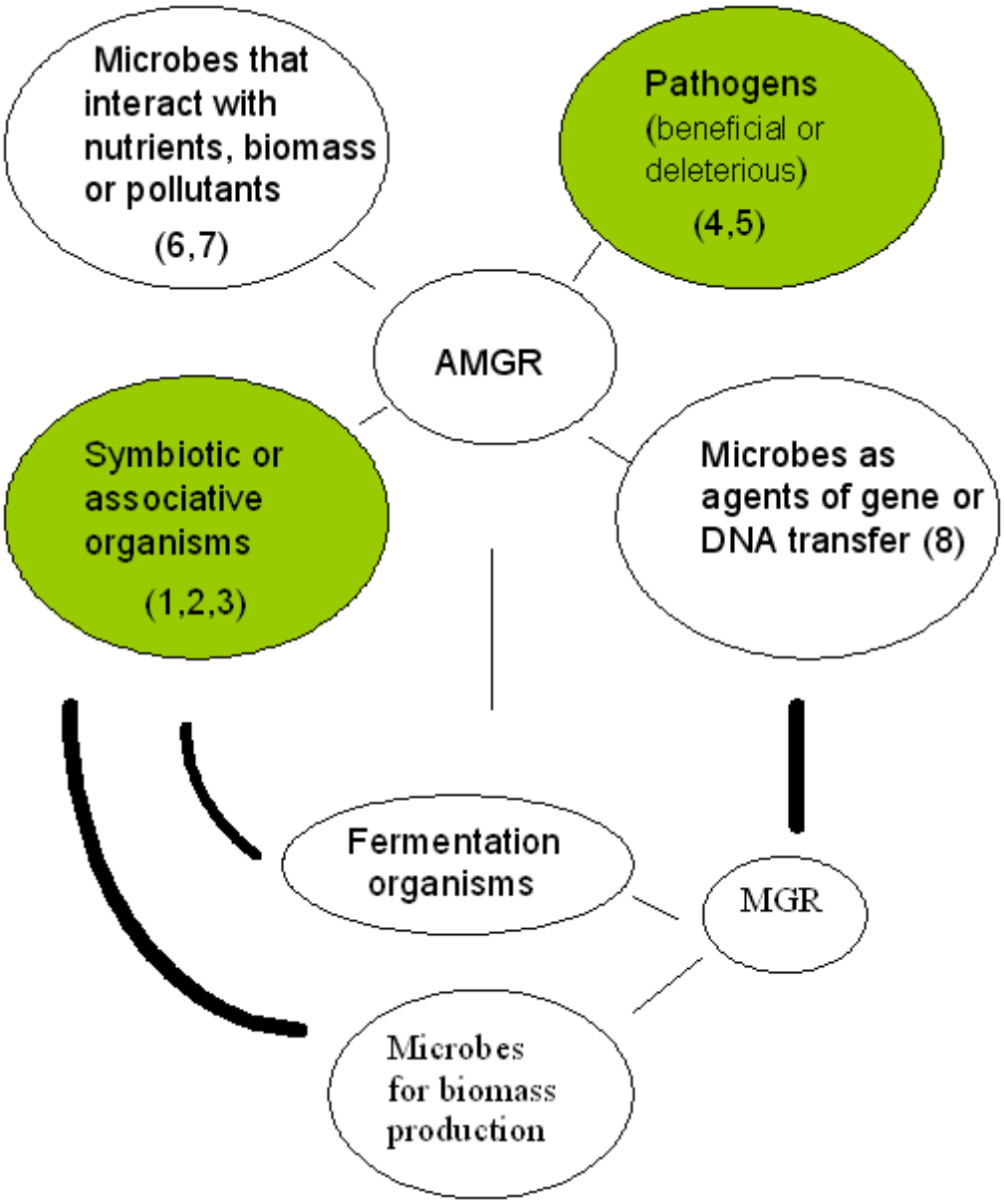

Figure 2. Functional groups of MGR added to those of AMiGR, and with an indication of areas of overlap with those used in Agriculture (thick bars).

Microbes in food, medicine or industry

[22] The main functional roles for microbes in food, medical science and industry (i.e. MGRs) are considered to be in:

- fermentation of foods and beverages;
- manufacture of medicines and pharmaceuticals;
- gene or DNA transfer; or
- mass culture as a source of pigments or antioxidants, or as a feed base for higher organisms.

[23] Fuller descriptions of these functional roles can be found in Appendix 1, but for comparison they are placed alongside those used in agriculture in Figure 2.

Areas of overlap. How may the agriculturally relevant groups be best separated from those utilized in food or medicine?

[24] There appear to be at least three functional roles that directly overlap between agriculture and either food, medicine or industry. The first is the use of microbes as agents for transfer of genes or DNA. Examples are *E. coli* and *Agrobacterium* spp., both of which are used extensively as carriers of plasmids holding DNA. *Agrobacterium* spp. is well described as a parasite of agricultural relevance. The second overlapping role is in microbes (usually microalgae) used in mass culture, that might also be associated with plants in agricultural settings. Specifically, microalgae can be utilized to produce pigments or antioxidants (e.g. beta-carotene, astaxanthin), fine chemicals (e.g. phycocyanin from *Spirulina* spp.) or to produce bulk feed in aquaculture industries (e.g. *Chlorella* spp.). However, the Cyanobacterium genus *Nostoc* is widely utilized to fix atmospheric nitrogen in association with rice production. Thirdly, there is significant overlap in the functions of fermentation for foods and associative organisms for plants. Microbe genera that overlap in these two groups include *Penicillium* spp. and *Acetobacter* spp., which are used in the production of fermented dairy products, as well as being important rhizosphere or endophytic microbes for plants. Yeasts, of course, are essential in fermentation of food and beverages, but are also a key microbe in the production of ethanol as a biofuel.

[25] However, it might be pragmatic to delineate AMiGRs from MGRs on the basis of their role in primary production. Thus AMiGRs might be considered (*vide* Figure 1) as:

> "microbes that are utilized, directly or indirectly, to assist the production of plants or animals in agricultural settings"

Adherence to this definition would separate those MGRs utilized for biomass production in aquaculture (e.g. microalgae) or for food fermentation from those microbes utilized *in situ* in agricultural settings. Microbes routinely used for gene or DNA transfer (such as *E. coli* and *Agrobacterium* spp.) and for fermentation would then overlap both sectors, as shown in Figure 2.However, can the groups realistically be separated in this way, or are the overlaps just too numerous? If we look further there are other areas of overlap in industry, where *E. coli* and *Clostridium* spp. are used in ethanol or butane production (which is a fermentation process), *Penicillium* spp., yeasts and *E. coli* are exploited in production of antibiotics, alkaloids, steroids, insulin and growth hormones (outside of agricultural settings), and *Aspergillus* spp. and *Bacillus* spp. produce enzymes utilized in food or health processes. All of these genera are, or may be, utilized as AMiGRs. The challenge in defining a distinct set of AMiGRs then becomes one of separating the functional groups at the species rather than the genus level. It is an outstanding question as to whether this enterprise is warranted.

IV. THE PHYSICAL NATURE OF COLLECTIONS AND HOW THEY DIFFER

[26] Microbe 'collections' can be considered as either *in situ* or *ex situ. In situ* collections may be of two types:

- the remaining undisturbed areas of the globe where microbes evolved and remain to this day relatively undisturbed as a component of the natural biodiversity; or
- in disturbed sites where, because of the general resilience of microbes, the perturbation to the environment has not eliminated them.

There is some evidence that in perturbed sites, such as long-term polluted sites, the microbe populations have been enriched in those organisms capable of remediating the pollutants.

[27] In both forms of *in situ* repository, the microbes are probably dependent upon some form of host interaction for their survival and multiplication, whether with plants, animals, insects or other microbes. Few microbes are competent saprophytes in isolation.

[28] *Ex situ* collections are of three major forms and the major difference from *in situ* repositories is that in these collections the microbe is usually cultured in pure form, in the absence of any host. The full metabolic requirements of the microbe must be met from artificial sources. *Ex situ* collections may be:

- collections amalgamated and fostered in the care of an individual;
- collections associated with institutions, and, more correctly, departments of institutions, which accept curatorial responsibility for them; or
- in association with commercial entities that exploit the microbes.

V. THE HISTORY AND ACTUAL GLOBAL PATTERNS OF DISTRIBUTION OF THESE ORGANISMS

General considerations concerning AMiGR distribution and exchange patterns

[29] Many AMiGRs are microscopic bacteria, or form spores, and it is difficult to contain such microbes geographically. As for pathogens, AMiGR will cross borders in aerosol form, in dirt or as unintentional contaminants. This re-distribution of microbes has been concomitant with exploration of the globe by man. The implications of this are that the geographical origin of many microbes is difficult to ascertain, and, further, that widespread application of an AMiGR will eventually lead to the widespread availability of that AMiGR. This is compounded by the fact that AMiGRs, unlike most microbes used in food reactions, are delivered to their target in a live state. Without a comprehensive and expensive border quarantine effort, it is unlikely that any unwanted re-distribution of an AMiGR could be prevented. For example, DNA is currently exchanged between laboratories through postal services by simply applying a small quantity of DNA to a sheet of paper and circling that spot on the letter. The recipient simply elutes the DNA from the paper and then amplifies it for use via the polymerase chain reaction (PCR) process.

[30] CGIAR, university and institutional scientists have historically and routinely exchanged AMiGRs or components of them (plasmids, DNA) for centuries. Culture repositories around the globe now hold thousands of cultures that have been accumulated in this fashion. By doing so, the science and exploitation of AMiGRs has rapidly advanced. The implications of this are that whilst there has been valuable preservation of genetic material *ex situ,* control of AMiGR at the species level has become clouded. Further, the value of any particular AMiGR is attached overwhelmingly to its manufactured form rather than to its germplasm form. As an example of this, culture collections sometimes contain over 1000 representatives (strains) of an organism. Individuals of this collection only become valuable after special attributes of them are identified, and the strain subsequently commercialized.

[31] In recognition of the above points, a policy of facilated exchange of AMiGR with a harmonized form of multilateral benefit sharing seems most practicable. Proof of geographical origin and of strain identity will, in many cases, be impossible to provide and thecosts of enforcing a rigid constrainment policy will also far exceed the value of the AMiGR, and will reduce the global exploitation of beneficial microbes. There may be some resistance to this approach by countries who perceive themselves as the countries of origin of AMiGRs. To counter this, the development of a core set of authenticated AMiGRs for facilitated distribution, with benefits flowing to developing countries in general, but not to particular suppliers, is suggested.

[32] A policy of facilitated exchange of AMiGR must be seen as separate to individual country policies on microbial biosecurity, as those policies might logically be applicable to any manufactured product, or to importation and distribution of pathogenic microbes.

GLOBAL CHANGES

Root Nodule Bacteria

[33] Without doubt, the greatest global changes in RNB distribution have come about with man's exploration of the world in the 18th century and then with their use as inoculants for legumes, particularly in the 20th century. Massive changes have occurred in the tropics, subtropics and warm temperate zones of Africa, Asia and America, where *Glycine max* (soybean) inoculated with *Bradyrhizobium japonicum* now dominates grain legume production. There is nearly 70 million tonne of inoculated soybean produced annually in the USA, in addition to 34 and 53 million tonne in Argentina and Brazil, respectively (USDA, 2005). This compares with the global trade in cool season grains of approximately 60 million tonne (Kelley et al., 2000), which suggests that soybean is probably the single largest traded legume commodity in the world. The RNB inoculants for this crop, which probably evolved in China, have thus been distributed over more than 150 million hectares of the Americas in the last 30 years.

[34] Similarly to soybean in the Americas, large tracts of land have been cleared of their native vegetation in central Asia, temperate America and southern Australia and planted to cool season forage legumes from two main genera, *Trifolium* (clovers) and *Medicago* (medics). Again, the majority of these legumes have been inoculated at some stage in their production with (the AMiGR) RNB. The perennial forage *M. sativa* (alfalfa; lucerne) has wide adaptation to soil and climate and because of this has spread from its centre of origin (believed to be in the temperate zones of Persia) to become the dominant forage on all continents in the last three millennia, carrying its RNB with it. No perennial form of a *Trifolium* species has achieved such prominence. Annual clovers and medics were established across 25 million hectares of arable land throughout southern Australia in the 19th and 20th centuries, with RNB inoculants available since 1896. As for the tropics, this represents a massive global change in distribution of RNB.

[35] At the same time, despite these examples of success in legume breeding and adoption, it is of concern that there are perhaps only 50 species of forage legumes and less than 15 species of grain legumes in wide global commercial trade (Kelley et al., 2000). Is it prudent, from a gene conservation perspective, to cover the globe so completely with only 65 of a potential 18 000 species of legume inoculated with only relatively few strains of RNB? We have evidence that these inoculants displace the original RNB. What is this doing to the *in situ* conservation of AMiGR biodiversity?

[36] The Australian usage of RNB AMiGR provides a good example for analysis of some of the issues relevant to this review. The value of RNB to Australian agriculture is estimated at AUD$ 3 billion annually, in terms of N fixed estimated by the replacement cost of N as urea fertilizer. All of this N fixation is by strains that were originally exotic to Australia, originating from the Mediterranean basin and western Asia for the temperate strains, and a range of tropical origins, including Africa and South America, for the tropical inoculants. Further, almost all of the strains that are commercially manufactured in Australia and that have been developed over the last 40 years have come from germplasm collected either *in situ* in focused collection missions, or *ex situ* from genebanks. This suggests a commercial exploitation of AMiGR by Australian agriculture from resources held by developing countries. However, the manufacturing industry that produces these inoculants has a wholesale value of less than AUD$6 million. Thus, the $3 billion benefit accrues from a $6 million industry, and it is the latter from which returns could be made to the country of origin of these inoculants. However, there is one pertinent example in this scenario that cannot be ignored. The lupin inoculant accounts for over 55 percent of RNB sales in Australia. The strain utilized, WU425, was originally isolated from naturalized serradella nodules found in Western Australia. It is believed that both the serradella and the rhizobial strain arrived by accident on Australian shores in the 19th century transport of animal fodder. This illustrates the difficulty of attempting to manage AMiGR movements around the globe, because microbes have moved accidentally with the development of global shipping. To reinforce this, recent genetic analysis of 50 lupin nodule isolates from Western

Australian fields examined by Thomas Strepkowski in Poland revealed that all of the isolates were from Europe, and that none had been deliberately introduced to Australia.

[37] So managing AMiGR exploitation by developed countries in such a way that the country(ies) of origin of the microorganism (often developing countries) may benefit faces dual difficulties, namely that:

- the value of AMiGR manufacture may be several orders of magnitude less than the value of their impact; and
- AMiGR (and pathogens) demonstrably transit country borders unaided

[38] This example of lupins in Australia is paralleled by that of soybean in the USA and South America, i.e. the current commercial inoculant strains evolved outside the geographical boundaries of these countries (actually in China), and were unintentionally transmitted to the New World, originally as contaminants on seed or in trash. All three soybean inoculants in America came from isolates made from naturalized soil populations. A similar scenario exists with alfalfa (lucerne). The movement of plant pathogens such as rusts (*Puccinia* spp.*)* and blights (e.g. *Phytopthera* spp.) from continent to continent is strong evidence for microbial transfer in aerosol form via the stratosphere.

[39] The scenario with newly developed legumes and their inoculants differs from the examples given above. In Australia there has arisen a 'second generation' of pasture legume species in the last decade (Howieson et al., 2000). Several species that form this second generation are new to agriculture and hence their inoculants have not always accidentally been carried around the world. For these legumes, the inoculants arose following targeted acquisition activities and their pedigree can be clearly traced. It is likely that some of these new species will ultimately be sown across tens of millions of hectares. However, the wholesale value of their inoculant manufacture will be measured in the tens of thousands of dollars per annum, and thus royalties from these, were they to be imposed, would be almost insignificant. Royalties are not currently paid on commercially manufactured rhizobial inoculants in Australia and the strains are distributed to manufacturers on the basis of a non-exclusive licence.

VAMs and ectomycorrhizae

[40]Uptake of VAMs and ectomycorrhizae has been significant, particularly in subtropical and tropical agriculture in Asia, where aid programmes have demonstrated the benefits of inoculation in the nursery phase. As with RNB, there is not always a response to inoculation with mycorrhizae, because many soils already contain naturally effective strains. The challenge in utilizing VAMs more widely is to develop regional knowledge of where positive responses are likely to occur, and to develop strains of VAM that are adapted to both the soils and crops of interest. This has happened, for example, in rattan plantations in southern China, where selection of locally effective VAM strains has resulted in increased production of rattan. There appears to be a gradual increase in VAM application around the globe and this may spread to developed countries as P fertilizers become more expensive.

Biocontrol agents, such as Metarhizium spp., B. subtilis and B. thuringiensis

[41] *Bacillus subtilis* is naturally widespread globally, and was actually one of the first bacteria to have its genome fully sequenced. The uptake of this AMiGR has been predominantly in horticulture or intensive agriculture in developed countries. Of greater impact has been the related species *B. thuringiensis,* used as an insecticide in many countries since the 1950s. *B. thuringiensis* produces a range of crystal proteins with varying degrees of toxicity to coleopteran and lepidopteran insects. Genes isolated from *B. thuringiensis* have been incorporated into commercial plant genomes for protection against insect pests, the most notable of which is the cotton boll weevil. The Pasteur Institute has a broad collection of both genes and strains of *B. thuringiensis* available for research purposes. Several genes have been patented since 1980. Although target organisms evolve resistance

to *Bt* toxins, the combined application of chemicals and biopesticides such as *B. thuringiensis* is a seen as a desirable development in integrated pest management.

[42] *Metarhizium* fungal spores can be produced in large-scale fermentors, but they can also be grown on sterilized rice in plastic bags for small-scale production. One limitation to widespread *Metarhizium* development is its sensitivity to temperature extremes; spore viability decreases as storage temperatures increase and virulence decreases at low temperatures. However, the broader application of *Metarhizium* to control cockroaches and white ants may increase its uptake. As for mycorrhizae, there has been a slow but steady uptake of AMiGRs as biopesticides since the 1950s, when the environmental implications of widespread chemical pesticides were first understood and publicized.

China and India, and the use of PGPRs or YIBs

[43] There has been historical acceptance of PGPRs in China, India and the former Soviet Union agriculture, with a research effort dating back some 50 years. The majority of these applications are of the diazotrophic microbes, in search of N accretion. It appears the use of PGPRs is static in these countries, neither declining nor becoming a mainstream activity. This influence is now spreading to South-East Asia, where co-inoculation of rice paddy fields with PGPR microbes (again predominantly diazotrophs) is gaining acceptance. There is certainly substantial research activity exploring the role of PGPR in rice growing in this region. Analysis of the published data on PGPR globally suggests that in more than 30 percent of reported applications of PGPR (generally associative N fixing *Azotobacter, Azospirillum* or *Clostridium*), a yield increase of 5 to 10 percent has been statistically demonstrated. It is difficult to gauge how much unreported experimentation with PGPRs is undertaken, and the range of the results of this work.

[44] In developing countries, the focus of PGPR application is on phosphate solubilization, stimulation of root length and early root growth, disease suppression, and nodulation enhancement. There is little doubt that inoculation of agricultural plants with PGPR can elicit a measurable response in the plant for all these factors. It is more problematic to transfer this plant response into an actual increase in grain yield.

VI. SURVEY TO ASSESS THE PHYSICAL NATURE OF CGIAR CENTRES HOLDINGS OF AMIGRS

Current status

[45] The Street (2000) review of AMiGR holdings in CGIAR Centres reported the breakdown of the microbial resources held at that time. A comparison is provided with the current situation in Table 1.

Table 1. Microbial resources held in CGIAR Centres in 2000 compared to 2005.

Microbe or functional group	Number in 2000†	Number in 2005
RNB	7780	6816
Animal pathogens	1326	na
Aquatic free-living N fixers	740	na
Plant pathogens	Undocumented	>1000
Entomopathogens	Undocumented	125
Mycorrhizae	Undocumented	>100
Rumen microorganisms	Undocumented	na
Non symbiotic beneficial microbes	Na	>600
Total (documented)	9846	8641

NOTES: † Data from 2000 derives from the review by Street (2000).na = data not available.

[46] From information received for the CGIAR survey (December 2005), the situation has altered somewhat since the Street (2000) review. ICRISAT, for example, in addition to 715 RNB, now holds significant numbers of plant pathogenic fungi (>1000), as well as a range of PGPR microbes (306). Interestingly, ICRISAT has also accumulated a number of entomopathogens (120) in the last few years. This evolution reflects the changing global patterns of AMiGR research quite well (although global usage of AMiGR is still dominated by RNB). The number of PGPR microbes held by ICRISAT is also consistent with the historical acceptance of these forms of AMiGR in Indian agriculture. During the mid-1980s and until 1995, ICARDA had as many as five scientists working with AMiGRs, predominantly with RNB. There are now no scientists active in this area at ICARDA, and no projects are being serviced from the collection. However, the RNB germplasm has been lyophilized and an electronic database is kept updated. There is, however, activity in integrated pest management using biopesticides, so this represents a further indication of trends in AMiGR usage in the CGIAR system. The downturn in active research with RNB at ICARDA has coincided with an increase in the usage of RNB in west Asia and North Africa, where farmers are inoculating pulses with cultures of rhizobium strains selected and manufactured locally.

[47] To provide a contrast to the response of the CGIAR Future Harvest Centres vis-à-vis other organizations, the questionnaire was also circulated within Australia. In Australia, holdings of RNB numbered approximately 7000, whilst there were collectively approximately 2500 plant pathogenic fungi, bacteria and viruses. The major institutions in Australia (e.g. CSIRO, State Departments of Agriculture, large universities) held collections of PGPR microbes and plant pathogens. The Grains Research and Development Corporation (GRDC) had implemented an AUD$ 10 million programme on Soil Biology (2003–2008), a large proportion of which is allocated to studying microbe-plant interactions.

[48] It seems incongruous that many projects (seven at the time of the 2005 survey) were built around microbial germplasm repositories that were uniformly poorly resourced. In Australia, a current research emphasis on the development of novel perennial legumes would be severely constrained without immediate access to RNB germplasm**.**

PART 2

VII. BASIC NEEDS AND CHALLENGES IN USING THESE AMIGRS IN THE GENERAL CONTEXT OF AGRICULTURAL DEVELOPMENT FOR THE COMING YEARS

[49] The primary needs and challenges can be distilled down to four:

- Discovering, preserving and cataloguing the available AMiGR biodiversity.
- Accurately ascertaining the beneficial properties of any AMiGR.
- Manufacturing, distributing and utilizing high quality AMiGR inoculants.
- Ensuring equitable access to AMiGR and sharing benefits associated with their use .

These challenges are discussed below. However, a substantial aid to the adoption of AMiGRs by developing countries would be the availability of a core set of AMiGRs (perhaps with representatives from each of the functional groups) that could satisfy the first two of the four requirements.

Preserving biodiversity

[50] CBD sets out principles of conservation and accesss and benefit sharing concerninggenetic resources. The application of the access and benefit sharing principles of the CBD is challenging in relation to AMiGR because microbes can easily transcend borders, as described earlier. CBD also suggests scientific experiments should be undertaken within the country of origin of the genetic resources, where possible. This is likely to be a difficult or impossible undertaking with AMiGRs because response to inoculation is likely to be species and environment specific.

[51] *In situ* repositories are, of course, relatively inexpensive to maintain, but there are substantial sociological, legislative and community consultation procedures to work through to ensure they succeed. With continued development of arable land, are we certain that maximum genetic diversity can be protected in these repositories? *Ex situ* collections are the converse: with relatively low diversity and expensive to maintain. The very positive outcomes of the current ICARDA project in biodiversity conservation with *in situ* repositories should provide a framework for further development of such collections. AMiGRs for plants are inevitably preserved wherever *in situ* repositories are proclaimed, but they must be large to preserve microbes associated with animals. There is some debate as to how many *in situ* repositories are required to capture a wide sample of AMiGRs. While many of the AMiGRs are ubiquitous at the genus level, stress-tolerant strains or species of AMiGR usually evolve in the presence of that stress, and these situations may be local.

[52] It is also pertinent here to discuss the loss of hosts for AMiGRs as an issue relative to loss of physical habitat of the AMiGRs. We can sometimes fall into the error of considering the AMiGRs in their habitat, but in isolation from their hosts. In reality, the loss of the host is more a threat to conservation of AMiGRs than the loss of diverse habitats, and this is more likely with animals than with plants. It is realistic to assume that whenever higher forms of life become extinct on this planet, then there is the strong likelihood that specific microbes associated with these lifeforms are also lost.

[53] The biosecurity aspects of exchange of AMiGRs cannot be ignored. The key issues here are, from the recipients' viewpoint, the potential loss of microbial biodiversity *in situ* following the application of an AMiGR to a new environment (i.e. competition for survival of microbes within that environment), the introduction of unwanted microbes, and the introduction of known pathogens. These are clearly matters of concern for sovereign governments, but are subjects of internal policy that should not be confused with the global exchange of AMiGRs.

Differentiating strains of AMiGRs

[54] While species of AMiGR may be nearly ubiquitous, strains vary considerably. For example, strains of RNB that belong to a single species and that nodulate a single species of legume can differ greatly in their N fixation and ecological properties. Molecular techniques, usually based upon some form of PCR (such as PCR-RFLP [polymerase chain reaction - restriction fragment length polymorphism]) can reliably differentiate microbial species at the strain level, yet not all microbes are amenable to PCR. Techniques for reliably differentiating strains within the broad suite of AMiGRs (fungi, bacteria, archae, viruses, algae, etc.) would need to be developed. These techniques are almost certainly likely to be based upon molecular methods.

Classifying microbes

[55] As with the discussion on differentiating microbes, despite the wealth of molecular tools available, microbial taxonomy is in a state of rapid change as we learn more about lateral transfer of genes on mobile genetic elements. There is little consensus amongst microbiologists on how to reliably classify many microbes below the genus level, particularly the bacteria. Nomenclatural changes have the potential to unwittingly confuse the origins of some AMiGRs in collections.

Handling AMiGRs

[56]Microbes replicate very quickly and the conditions under which they are cultured can lead to genetic change (drift), mainly through loss of plasmids or DNA units bearing non-essential genes. Bacteria and fungi can be readily freeze dried or lyophilized in glass ampoules, and this should be the preferred mode of preservation. If ampoules are kept below 15°C, the microbes commonly have a life span of over 50 years. However, not all microbes can be lyophilized or stored at -80°C. The microalgae are one such class of AMiGR that must be routinely subcultured, which is expensive and unreliable. So the optimal methods for handling some types of microbes for long-term storage needs further research.

Code of conduct

[57] With MOSAICS [Microorganisms Sustainable use and Access management Integrated Conveyance System – an EU initiative], a voluntary and guiding code of conduct already exists to assist suppliers and receivers of materials ensure that they are in compliance with the basic tenets of the CBD, namely that materials are accessed subject to prior informed consent (PIC) and on mutually agreed terms. This covers access to and circulation of MGRs, a pathway that tracks utilization and potential commercial benefits arising from exploitation of MGRs. This could be adopted for AMiGRs. MOSAICS is premised on the notion that suppliers and access seekers will negotiate new terms and conditions for each case. One possibly very useful value-added approach would be to develop a harmonized, pre-agreed set of terms and conditions that could be used for exchanges between a wide range of parties for specified purposes, such as research, conservation, etc. Such a harmonized approach would usefully complement the development of an internationally publicly available core set of AMiGRs, as discussed elsewhere.

Institutional continuity

[58] The world AMiGR collections appear to be associated with individuals rather than institutions, and thus when the individual relinquishes their position, the germplasm collection suffers. This seems to be the case for most CGIAR collections, which are ‘working collections’ rather than genebanks per se. The contrast here might be made with herbaria, seed banks or some microbial collections, such as those at USDA-ARS and Ghent (Laboratorium voor Microbiologie, Universiteit Ghent) where there is substantial funding for long-term curatorial purposes. Of significance is that a well-maintained culture collection is the product of many work-hours of collection, propagation, preservation, experimentation, authentication and documentation. It represents intellectual property that should not

be summarily dispensed with, and successional planning through the appointment of a curatorial position is the best way to achieve security.

Trends in amalgamating AMiGR collections

[59] In both the USA and Australia, the trend over the last two decades has been towards amalgamation of collections, particularly for RNB. With the withdrawal of CSIRO from rhizobiology, the Australian CSIRO collections (prefix CB (Brisbane), and CC (Canberra)) have been amalgamated into the WSM (curator Howieson, Perth), SARDI (curator Ballard, Adelaide) and US (curator Kennedy, Sydney) genebanks. However, only the lyophilized cultures were transferred (some 1000 cultures), with those held on agar slopes being destroyed. The Sydney US genebank is considered to be vulnerable, with the imminent retirement of Professor Ivan Kennedy. This situation reflects the generally poor long-term planning in relation to germplasm of AMiGR at the global level, even where the value of these microbes is acknowledged.

Recognizing and attaching value to AMiGRs

[60] In many traditional disciplines of biology, the value or role of microbes is not (transparently) recognized. For example, in the International Union of Forest Research Organizations (IUFRO) there is the IURFO Root Physiology and Symbiosis Unit. This unit has no public policy on the preservation of forest microbial genetic resources. It seems that most collections of forest microbes are privately owned and held in universities.

[61] However, in the USA, the USDA ARS has assumed responsibility for the majority of RNB collections held on the North American continent (curator Peter van Berkum). This raises the possibility of a model for AMiGR collections, with one repository per continent being nominated as the key core collection.

[62] A more detailed look at the USDA Agricultural Research Service (ARS) system in relation to AMiGR is provided below.

USDA ARS NATIONAL MICROBIAL GERMPLASM PROGRAM

[63] The goal of this programme is to ensure that the genetic diversity of agriculturally important microorganisms is maintained to enhance and increase agricultural efficiency and profitability. The programme will collect, authenticate and characterize potentially useful microbial germplasm; preserve microbial genetic diversity; and facilitate distribution and utilization of microbial germplasm for research and industry [Author's note: this is in the context of benefit to the USA as presented in the US Farm Bill outlined in Appendix 3].

[64] ARS in fact maintains several microbial germplasm collections, including:

- ARS Culture collection
- ARS Collection of Entomopathogenic Fungal Cultures (ARSEF)
- ARS National Rhizobium Genetic Resource Center
- ARS National Fungus Collections

The ARS National Rhizobium Genetic Resource Center has allocated funding of US$ 140 000 per annum in addition to the salary of its curator.

[65] Some aspects of the management and policies of these collections are relevant to this review:

➢ Identifying and acknowledging ARSEF strains in publications

> 'We ask that all publications using or referring to strains obtained from ARSEF acknowledge the ARSEF culture collection and state the ARSEF accession numbers of these strains. We would greatly appreciate receiving reprints of all past, current, and future publications involving ARSEF strains.'

Accession numbers of strains from commercial culture collections, such as the American Type Culture Collection (ATCC), Centralbureau voor Schimmelcultures (CBS), CAB International Mycological Institute (IMI), and the University of Alberta Microfungus Collection (UAMH), are listed in this catalogue only for the sake of providing complete information. Cultures received from ARSEF should be referred to by their ARSEF numbers only. Citation of cultures obtained from ARSEF by any corresponding ATCC, CBS, IMI or UAMH accession numbers they may also have is a violation of trademark laws; persons doing so are subject to prosecution.

➢ Updated, special, and electronic catalogues

Periodic updates of the general and special ARSEF catalogues and the update to the printed 1992 catalogue will be mounted on the Web page. Printed copies of the 1992 catalogue of ARSEF isolates (covering isolates up through 3736) are available without cost upon request to the curator. Complimentary copies of the ARSEF database and the customized application used to manage it can be obtained upon consultation with the curator of the ARSEF collection. It was anticipated that a fully interactive, searchable version of ARSEF culture accession data would be made available on the Web site in 2004.

➢ Depositing and exchanging cultures

The ARSEF culture collection encourages deposition of entomopathogenic fungal cultures—particularly strains used in published studies—as well as of voucher and reference specimens to its herbarium. Depositors may reserve the right to limit redistribution of any culture deposited with ARSEF for specified periods upon consultation with the curator. Depositors can receive subcultures of their own depositions at any time; these cultures do not affect any allowances for free cultures. Exchanges of cultures between ARSEF and other research or general collections of fungal cultures are encouraged and are not subject to numerical limits.

[66] Prior to shipping cultures from countries outside the United States, contact the Curator to obtain the appropriate needed importation permit from the U.S. Department of Agriculture, Animal and Plant Health Inspection Services, Plant Protection and Quarantine. When sending cultures and/or specimens to ARSEF, it is very important to include as much of the following information as possible:

- Scientific name (and taxonomic authority) of the fungus.
- Common and scientific name (with taxonomic authority) of the host.
- Order and family of the host. [This is essential information!]
- Date and site of collection.
- Name of collector.
- Date and name of isolator.
- Any collection, accession, or other identifier number(s) applied by the collector or sender.
- Medium on which a culture is sent.
- Any special requirements or conditions for growth (such as medium, temperature, pH).

➢ Diagnostic Services for Cultures and Specimens

Specimens and cultures of unidentified fungi from invertebrates can be submitted to ARSEF for diagnosis. This service is an important function of the ARS Collections of Entomopathogenic Fungi and is provided without charge. Identifications and information about the disposition of specimens will be mailed to the sender.

➢ Release of ARSEF Cultures from Containment or Quarantine

Neither the curator nor any employee of ARSEF or of the Plant Protection Research Unit is entitled to authorize the release of any culture it provides from laboratory containment or quarantine in the United States or elsewhere. Recipients of ARSEF cultures are responsible for obtaining all appropriate and necessary permissions from or for providing official notifications to State and Federal regulatory agencies.

The pragmatic value of a core set of authenticated AMiGRs

[67] This document hypothesises that a core set of 'authenticated' AMiGR might be developed by scientists and institutions who have historically collaborated in exchange of microbial germplasm. A core set would be different from a type set: the latter providing a taxonomic basis, the former providing a proven phenotype. The benefits of a core set would be two-fold. Firstly, it has been identified that the development of AMiGRs is hindered by the need for researchers to devote substantial time to procurement of microbes, followed by purification (if the organism is not from a reliable source), identification, laboratory or glasshouse evaluation, and finally *in situ* experimentation. The steps between procurement and *in situ* experimentation are considered as authentication. The second advantage is that developing countries (from which many AMiGR have been sourced) would perhaps be great beneficiaries of such a scheme, as the authentication steps can be difficult. As an example, if a research group were interested in developing a plant growth promoting organism based on the enzyme ACC deaminase, there may be several work-years required for isolation, purification, development of the bioassay for production of the enzyme, then selection of isolates for evaluation *in situ.* This same process has been undertaken in many laboratories over the last 20 years, and by now there should be available a set of strains, probably representing many species, that are well characterized for this enzyme. Selections from among these would represent a core set of ACC deaminase strains from which new projects might be developed. They could be thought of as 'control' strains for comparison with new isolates, or possible strains for commercial development in their own right. The concept of a core set parallels the 'type' strains available for serious diseases, or cancerous cell lines, which are widely distributed in medical research laboratories. It differs from current taxonomic 'Type Strains' in culture collections in the sense that the phenotypes of the core set of microbes would be substantially well researched. For example, the taxonomic Type Strain for *Sinorhizobium meliloti* is Sm1021. Although much is known about Sm1021 genetically, it is poor at nitrogen fixation when in association with many species of its host genus *Medicago.* Sm1021 would thus not be very useful as a core strain for evaluation in agricultural settings.

[68] So, how might this core set concept work in practice? The concept might initially be floated at the major international microbiology conferences. If there was general enthusiasm for the concept at the individual level, which was then supported at the institutional level, there would follow development of a working party to assess which AMiGR groups might be suitable for inclusion in the core set. Obvious candidates are the RNBs, PGPRs, pathogens, pathogen suppressors and probably others from the major functional groups 1–8 in Figure 1. An ensuing Web-based activitymight then be suitable for the process of deciding which AMiGR groups, and then which individual species and strains, might be accepted as the core set for each group. The strains finally accepted into the core group would be based upon agreed standard levels of authentication and, importantly, *in situ* performance from a number of valid tests.

[69] The major costs in developing and then servicing a core set of AMiGR are difficult to predict. The development phase might be potentiated by direct donations of strains from individuals or institutions. The costs of servicing the core set would be determined to a substantial extent by the demand. An estimate of the cost might be gained from enquiry through the USDA in relation to their RNB, or the Pasteur Institute for their *Bacillus thuringiensis* collection.

[70] Suitable partners in developing a core set of AMiGRs in the initial phase would be the CGIAR genebanks and public institutions such as the USDA, which have demonstrated a willingness to hold publicly available materials and supply them internationally. If the concept were favourably received (and there was appropriate recognition for acceptance of an organism into a core set, such as journal publication), it is possible that donations to the core set might rapidly gather momentum.

[71] A further consideration concerns how much of the usefulresources currently held by organizations could actually be globally, publicly distributed. This is a question that would require extensive review of each accession's legal status with reference international laws, national laws, intellectual property ownership, and the conditions under which those materials were supplied (and by whom) to the organizations concerned.

VIII. OBSTACLES FOUND IN USING AMIGRS, WITH EMPHASIS ON DEVELOPING COUNTRIES

Accurately ascertaining the beneficial properties of any AMiGR and demonstrating bona fide responses to inoculation of AMiGR

[72] The data for AMiGR response, apart from RNB and mycorrhiza, is seldom convincing. For RNB, the USDA NifTAL programme, and its follow up, the Worldwide Rhizobial Ecology Network, noted that where rhizobial populations of compatible strains were less than 10 per gram of soil, 93 percent of experiments produced yield increases in excess of 140 percent. However, where soil numbers were higher, 10 to 100 per gram of soil, the response dropped dramatically, to 68 percent frequency and 8 percent magnitude (Herridge, 2005). Determining the need to inoculate and then the response for other AMiGRs represents a substantial barrier to their scientific credibility and their adoption. As noted previously, for associative diazotrophes the frequency of response to inoculation drops to around 30 percent of published reports, but the magnitude to an alarmingly low 10 to 30 percent. These responses are difficult to accurately measure. In China, India and the former USSR, the relatively widespread use of AMiGRs seems to be more a cultural phenomenon than scientifically based. Perhaps there is merit in accepting that responses to AMiGRs will rarely, if ever, be comparable with those from RNB.

Decision-making in relation to the opportunities or benefits arising from application of AMiGRs

[73] The information explosion has delivered a multitude of reports relating to successes or failures with AMiGRs. Access to these reports is becoming more efficient, with on-line journals, although the information transfer to developing countries is certainly slower than for developed countries. Notwithstanding this, most reviewers acknowledge that responses to inoculation with AMiGRs are site and species specific. Thus, a major obstacle in developing countries to uptake of AMiGRs is in assessing whether there is likely to be local benefit from them. Although decision-making of this kind is not simple, Figures 3 to 5 illustrate a model developed for legumes and inoculation with RNB that could be adapted by regional scientists for application to a broader range of AMiGRs.

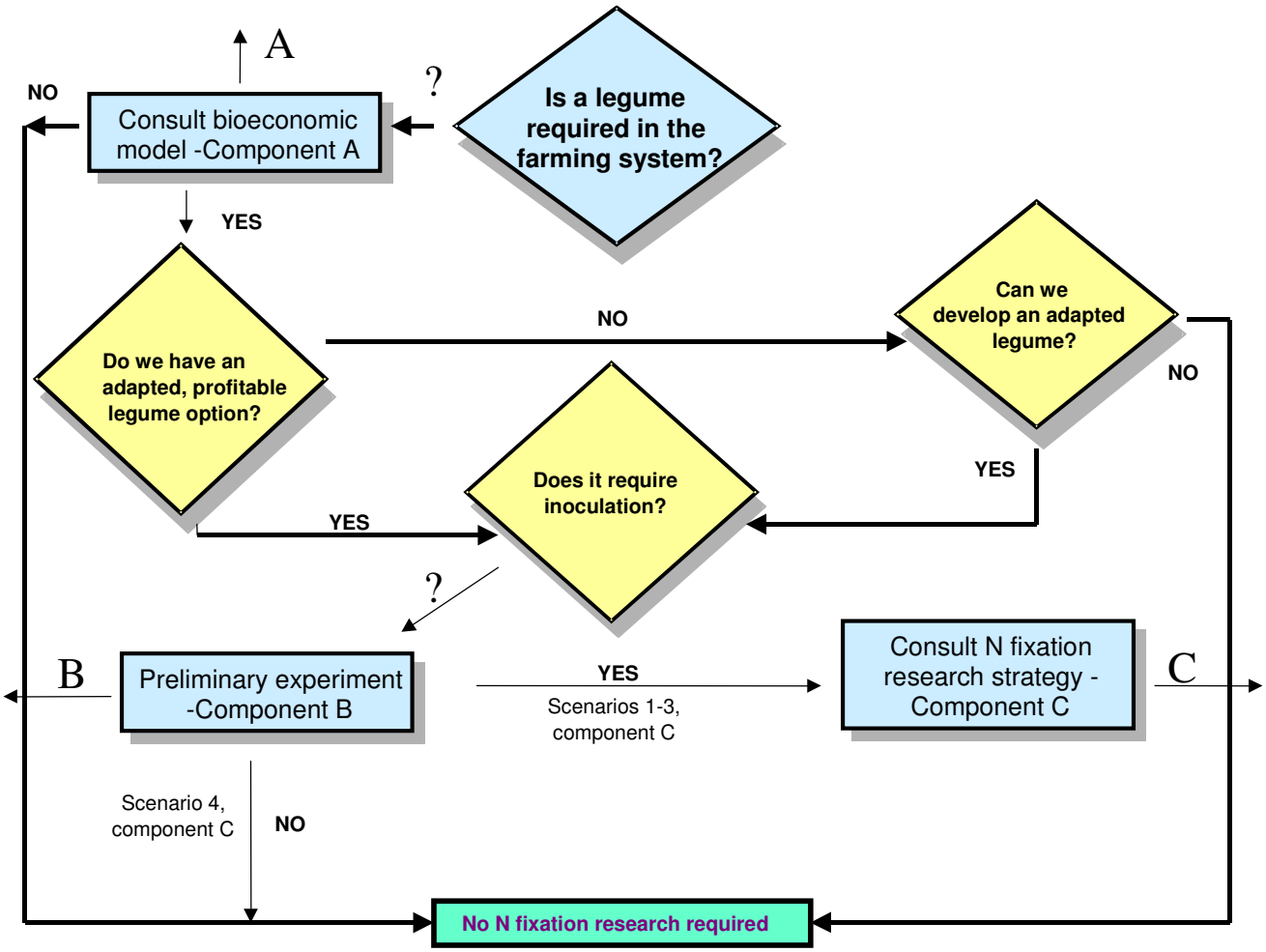

Figure 3. A flow chart illustrating the range of decisions required prior to initiating a legume or rhizobial selection programme.

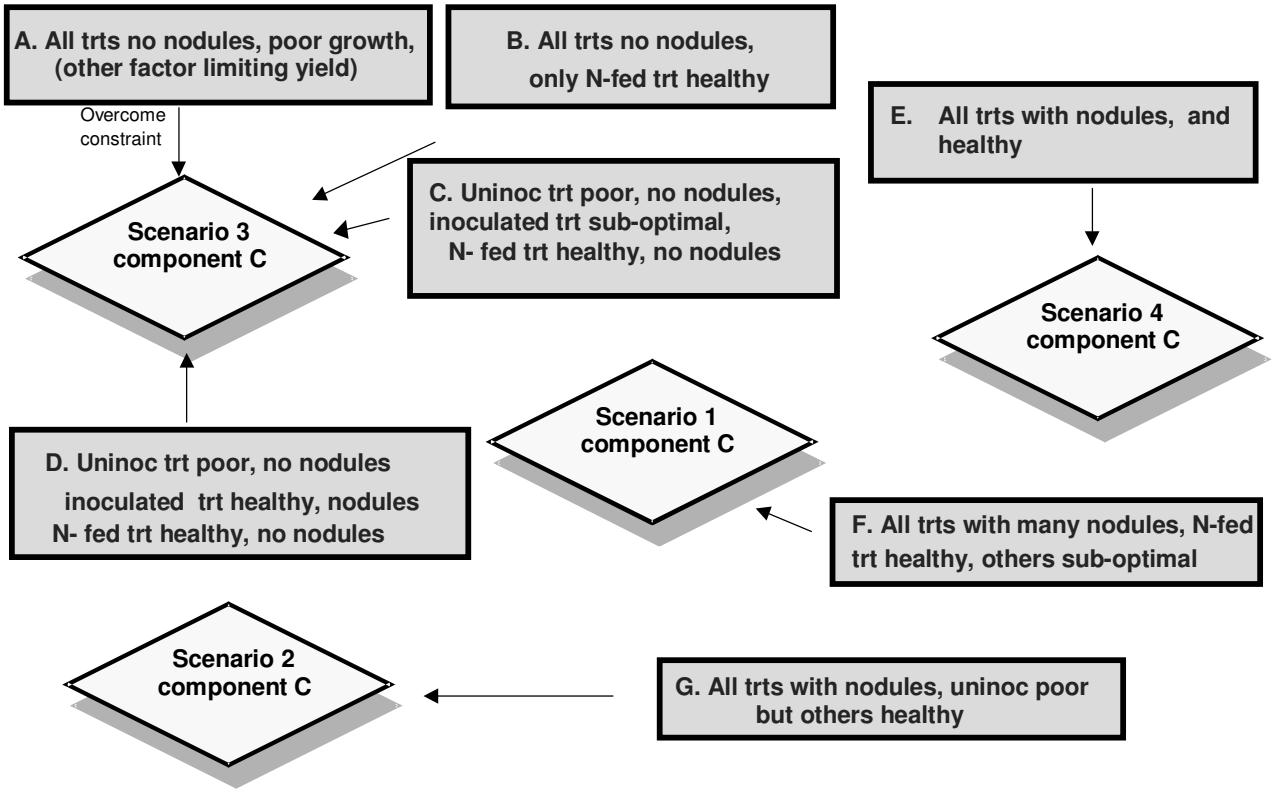

Figure 4. Component B. The possible outcomes of a preliminary inoculation experiment to determine if a legume requires inoculation in a particular soil. The experiment has three legume treatments- uninoculated, inoculated with a "best bet" strain and N-fertilized.The ensuing research requirements are represented in Component C (see Figure 5).

Notes: trt(s) = treatment(s). uninoc = uninoculated.

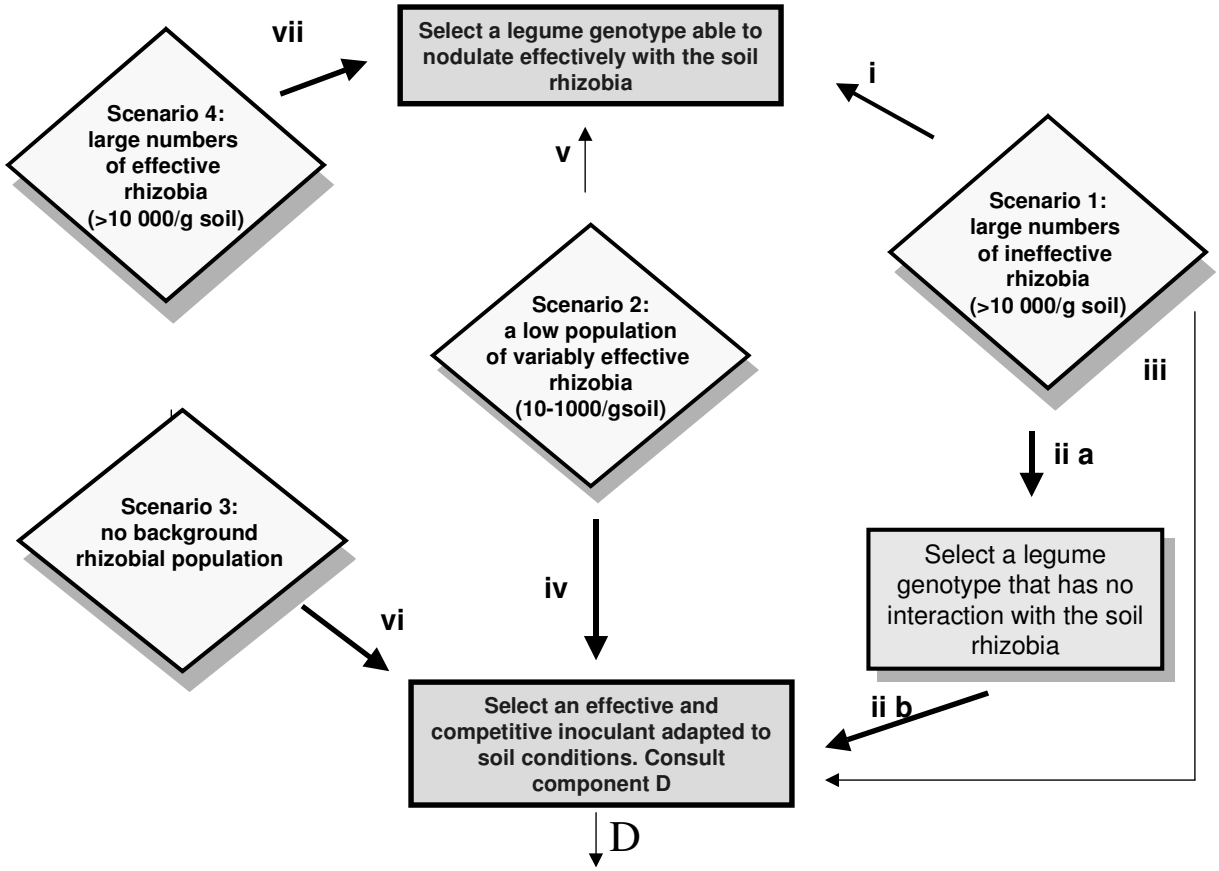

Figure 5. Component C. Research strategies for increasing N2 fixation (after Sessitsch et al., 2002).

[74] As previously mentioned, the availability of a core set of AMiGR with which to experiment within the boundaries of this decision-making model would perhaps greatly benefit the uptake of AMiGRs in developing countries. A core set of AMiGRs could readily be developed by scientists who have collaborated in microbial germplasm exchange and evaluation programmes with the CGIAR centres.

MANUFACTURING, DISTRIBUTING AND UTILIZING MICROBES

[75] After recognizing the value of AMiGRs and demonstrating responses to inoculation with them, the next step in utilizing AMiGRs is to manufacture them in sufficient quantity and with sufficient quality to ensure their adoption. In developed countries, the factors militating against adoption include the ease of applying alternatives to AMiGRs (chemicals, fertilizers). In developing countries, the problems are more microbiological.

Problems with manufacturing technologies in developing countries

[76] If the decision is made to manufacture AMiGR, then bacterial or fungal inoculants need to be produced in a fermentation process, usually under conditions of controlled sterility. The key challenges in manufacture of AMiGRs include:

- ensuring that the right organism is cultured during the fermentation step, i.e. that the inoculant is the desired organism and the growth phase is uncontaminated; and
- ensuring the fermentation is carried to completion (i.e. to achieve high numbers) and harvested without injury to the microbe.

[77] After fermentation, the microbes must be stored in a 'carrier' material until applied to seed, plants or soil. Carriers include:

- soils, such as ground peat, coal or lignite;
- plant material, such as charcoal, composted straw, bagasse, rice husks or coir dust;
- inert materials, such as vermiculite, perlite, bentonite, clay, phosphate rock, talc or alginate; or
- combinations of the above, such as a mixture of soil, clay and compost.

[78] The type of carrier developed usually depends on the availability of materials in reasonable proximity to the fermentation facilities. The key challenges in carrier selection are:

- ensuring the carrier protects the organism for a period sufficient to utilize the inoculant; and
- ensuring the carrier maintains high numbers of the inoculant capable of engaging with the target plant, insect or animal.

[79] Low rates of usage of AMiGR in many countries may reflect problems of supply or regional access to AMiGR, rather than reflect the actual intent of the farmer, who might be amenable to the purchase of a bona fide inoculant. Thus, lack of a reliable infrastructure for AMiGR production may restrict adoption, even if the organisms have proven efficacy.

Documentation and databases to aid transfer and to track acquisition and usage

[80] As with any scientific pursuit, it is essential that records be kept of experimental outcomes. However, because the nature of AMiGR research is long term, it is even more essential that good databases are developed to record the information generated with any series of experiments with AMiGR. This is essential where AMiGR repositories mature to become associated with institutions rather than individuals. In the case of the CGIAR system, the use of electronic databases to record and track the use of AMiGR acquisition and outcomes of experiments with them is strongly recommended.

IX. INFORMAL (NON-LEGALIZED) CUSTOMS DEVELOPED FOR THE ACQUISITION, DISTRIBUTION OR EXCHANGES OF AMIGRS

Record-keeping

[81] Many curators historically recorded the distribution of their cultures, more as a thorough record-keeping exercise than as a legal requirement. There was generally an understanding between scientists that the culture would be referred to with its initial accession number in any publication in an international forum and this provided some tracing of cultures. This has changed somewhat over the last 15 years. There currently exists relatively substantial record-keeping relating to acquisition and exchange of at least some AMiGRs. In the case of RNB, acquisition activities by institutions are now only undertaken with the full knowledge and cooperation of the country of origin. Material from collecting missions is then usually shared between collaborators at the point of collection, or after isolation and preservation has been achieved. The acquisition activity is usually preceded by written declarations of intent that describe the intended scope of the activities. This scenario differs significantly from that which existed pre-1994, where acquisition activities were frequently undertaken without the written consent of legally constituted authorities in the country of origin and following established access and benefit sharing laws, since relevant international standards and national laws generally did not exist.

X. TOWARDS CODIFICATION OF ACTIVITIES: DIRECTIONS AND ORGANIZATION TYPES GENERALLY INVOLVED

MTAs and MOUs

[82] There is now a general requirement for the preparation and signing of Memoranda that deal exclusively with the acquisition, exchange, research and future commercialization of any AMiGR. These documents are usually inter-institutional, rather than inter-governmental. In the case of the distribution of cultures from germplasm resource centres, requests for cultures may now often be met with Material Transfer Agreements (MTAs) that specify, amongst other things, that negotiation is required with the 'owners' of the material before commercial activities are to be undertaken. Usually, AMiGRs cannot be forwarded to a third party. An example of a current MTA is appended (Appendix 3). The exchange of AMiGRs has thus moved substantially towards an official activity, with record keeping and commitments by both parties.

Re-selection

[83] The issue of re-selection is significant. Microbes may divide and double their number within 30 minutes, and the offspring may be slightly different to the parents, depending upon how they are cultured. For example, at a mutation rate of 1 in 100 million, which is not high, any plate of bacteria is likely to have up to ten colonies that differ from the parents. It is, for example, a very basic step in microbiology to select for natural antibiotic resistance mutants. This rapid rate of change clearly has the potential to make claims for intellectual property ownership a significant challenge.

XI. POSSIBLE DIFFERENCES AMONG CODIFICATIONS APPLICABLE TO AMIGRS AND TO MGRS

AMiGRs differ from MGRs

[84] AMiGRs are generally delivered live to their target (soil, plant, insect, animal) whilst MGRs transform a process and then are eliminated. This is a major difference between AMiGRs and MGRs that affects their codification. For almost all AMiGRs, the organism itself is manufactured then utilized in a live state. Thus, to elicit the required response the microbe is distributed by inoculation of or placed in the vicinity of the target organism, using live cells, or fruiting bodies that should develop into live cultures. This contrasts with the utilization of MGRs, the vast majority of which act as microbial catalysts in a production sequence where the end product contains no live cells of the microbial agent. For example, yeasts ferment grape juice into wine or champagne and then die, with no live cells usually present in the final product. Similarly, whilst *Agrobacterium* might produce transformed cells, the bacteria itself is ultimately removed from the target organelle (although there are exceptions, such as the lactobacillus used in yoghurt manufacture). Because MGRs are generally utilized within a contained process, there has followed the "ownership and protection" of MGRs. Breweries have their favourite yeasts, which they closely guard, and laboratories store their unique transformation vehicles. The same protection is not available to AMiGRs, because once they are released into the environment, it is generally a simple matter to recover the organism.

XII. IMPACTS OF NATIONAL QUARANTINE LAWS

Labelling

[85] The primary requirements for import and export of agricultural microbes relate to labelling, in particular in relation to any potential hazardous substance. These must be disclosed and penalties for not doing so may be applied to both the exporting and importing agent. For animal pathogens, in particular those that are the subject of global quarantine efforts, access to microbial germplasm remains as strictly controlled as that of animal shipment. Aside from this, there is very little

monitoring of the exchange of agricultural microbes in most countries, and the unintentional trade of microbes across the globe continues to increase in association with shipments of grain, animals and fodder. However, those countries with strict quarantine laws are becoming increasingly stringent about the importation of microbes. Thus AQIS, the Australian Quarantine Inspection Service, prohibits the import of AMiGRs without special permits, and this is having a substantial impact upon the development of AMiGRs in that country.

XIII. TRENDS IN PATENTING OF UNMODIFIED AND MODIFIED MICROBIALS

AMiGRs and intellectual property

[86] In any handful of soil, from most places on the planet, there is likely to be in excess of 100 populations of different microbes, some of which will exceed 1 billion individuals in that handful. From this handful of soil there is the potential to develop one or more AMiGR inoculants. The widespread availability or natural distribution of microbes has several implications. The first is that it is almost impossible to demonstrate the origins of an AMiGR unless that AMiGR is highly specific. An example of a highly specific AMiGR might be the bacteria from which DNA polymerase for many PCR reactions originates, i.e. from thermal pools, which are a relatively restricted environment. Other than these rare examples, AMiGRs from common environments are ubiquitous. The intellectual property in their development, therefore, is associated with proving theirutility/industrial application rather than discovering the organism per se. This in turn means that it might be difficult to protect the intellectual property associated with many AMiGRs. For example, it is public knowledge that RNB fix nitrogen, RNB are ubiquitous, and therefore it is a relatively simple matter to isolate RNB from legumes to develop inoculants that simply can not be protected by intellectual property (IP) rights legislation. Whilst procaryotic AMiGRs can be patented, this is not a common practice.

[87] As noted earlier, microbes routinely double their number within 30 minutes when grown under favourable conditions. This provides the opportunity to generate inoculants within days, and the implications of this are that a competent manufacturer may develop a commercial-quality inoculant from a starter culture within a very short time-frame. This makes AMiGRs uniquely attractive as a small business opportunity in developing countries where local fermentation expertise is available. This is why many aid programmes, such as USAid (through NifTal), have focused upon AMiGRs. There is, however, a down side to rapid reproduction. The first consideration is that with the rapid rate of reproduction comes a potential for rapid mutation, or change. If the altered genotype is favoured in the production environment, the new genotype will soon dominate the population (this could be considered evolution). This, in turn, has implications: firstly, if the change is not beneficial then the inoculant may not be efficacious (and hence AMiGR production requires stringent quality control), and, secondly, if the original AMiGR was protected by patent, it is unlikely that the patent would apply to the evolved genotype. A similar scenario can occur for microbes delivered to soil. There is substantial acceptance and donation of DNA between even distantly related organisms, which leads to relatively rapid evolution or change. A major implication of rapid reproduction, then, is that it brings with it difficulties in intellectual property protection associated with the difficulties in proving identity.

Trends in patenting

[88] It seems commercial manufacture of AMiGRs is accompanied by patent applications, more so than through the activities of the genebank curators themselves. Executives of Becker Underwood in Australia were contacted on 10 August 2005. Becker Underwood are a major global manufacturer of AMiGRs. They had patented the use of microorganisms as biocontrol agents (although not RNB) in the USA and in Australia. These patented AMiGRs are not genetically modified and are occur naturally in the environment. The AMiGRs under patent have been selected in research programmes for specific purposes (e.g. *Metarizium* as an inseticide). This suggests that some patent laws now recognize and offer protection forinvestment in scientific researchof microbes that have been isolated from the environment and used in specific ways.

[89] A second manufacturer of AMiGRs in Australia, ALOSCA Pty Ltd were contacted on 14 August 2005. At that time, they had patented their delivery technology rather than specific microbes.

[90] New strains developed through scientific research and then made available for commerce are provided to Australian manufacturers free of charge under the conditions of a non-exclusive licensing arrangement, but only to those manufacturers who are participants in the Australian quality control programme (ALIRU).

Procaryotes protectable as intellectual property

[91] Although this document presents some pragmatic challenges associated with patenting of AMiGRs, the case of *Diamond v Chakrabarty* in the US Supreme Court has shown prokaryotes may be patent protectable under law in the USA, a decision that remains unchallenged today. The most current issue of Bergey's Manual (2005) has a paper by R.D. Meredith that examined the 1998 position on protecting IP in prokaryotes. In summary, at that time, prokaryotes were protectable if they were considered new inventions, of practical value and not simple variants of an entity already anticipated in the public domain. It was noted by Meredith that the law is evolving. The case was decided in favour of the applicant (5-4), which, in the opinion of the author, is indicative of a challengeable position. In the sense of AMiGR, it would be difficult in many cases to establish that a similar entity was not anticipated in the public domain. Procaryotes that are deliberately genetically altered to deliver a unique product (e.g. insulin) would be an obvious exception.

XIV. CONCLUSIONS

[92] Drawing on the preceding sections of this paper by Dr Howieson, the Genetic Resources Policy Committee of the CGIAR seeks to highlight a number of potentially important issues:

1. It is possible to develop a working definition of agricultural microbial genetic resources (AMiGR) on the basis of the function for which those resources are used, i.e. the fact that that they assist in the production of plants or animals, either directly or indirectly, in agricultural settings.
2. Because of a combination of factors concerning microbes used in agriculture—for example, their deployment in open environments; their extremely fast rates of reproduction and variation; their small size and portability; and historical patterns of use and distribution—it is difficult, and often impossible, to subject them to legal forms of control or appropriation. A large number of patents, however, have been granted in some countries over microbes as well as genes and proteins derived therefrom.
3. AMiGR are potentially extremely important for the sustainable improvement of productivity in developing countries, subject to biosafety considerations. However, they are as yet not widely exploited in a systematic manner in developing countries.
4. One possible way to increase the availability to, and use of AMiGRs by, developing countries would be to develop a 'virtual' core collection of screened materials currently held by public organizations around the world that wanted to participate. A critical aspect of this enterprise would be to agree upon harmonized terms and conditions for the distribution of those materials, in conformity with international law. The process for considering the establishment of such a base collection and the terms and conditions for its use would need to be highly participatory, with costs, legal status, partners, administrative responsibilities and other issues identified and exhaustively considered.

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APPENDIX 1

A BRIEF DESCRIPTION OF SOME COMMON AMIGRS WITHIN THEIR ASSIGNED FUNCTIONAL GROUPS

PLANT SYMBIONTS

[A1.1] Plant symbionts are microbes whose actions directly improve plant growth, usually by supply of otherwise limiting nutrients such as nitrogen or phosphorus. Root nodulating bacteria (RNB) are the best-researched example of microsymbionts for plant growth, reducing inert di-nitrogen gas in the atmosphere to a form that legumes can metabolize, usually amino acids. There are six main genera of RNB, including the phyllosphere microorganism *Azorhizobium* that forms stem nodules on Sesbania. The stem nodules and their microbial occupants may also be photosynthetic. Actinorhizae are fungi that form Frankia-type nodules on non-legumes, within which N fixation also takes place. The microbe genus *Frankia* can now be cultured on complex media and hence *Frankia* spp. are suitable as AMiGRs and can be applied to at least seven families of non-leguminous plants, the most utilized plants being in the genera *Casuarina* and *Alnus*.

[A1.2] The Cyanobacteria may also be listed under this heading, as they have the capacity to form symbiotic associations with eukaryotes and to fix atmospheric N. *Nostoc* is the most exploited genus of this group.

[A1.3] Mycorrhizae are root-fungus associations that effectively extend the rooting-zone of plants. There are six major types of mycorrhizae. The endomycorrhizae are of particular interest, although they can not be grown without the plant and therefore remain difficult in an AMiGR context.

RUMEN ORGANISMS

[A1.4] The rumen of methane-producing animals such as sheep and cattle contains a large and diverse microbial community of anaerobic fungi, such as *Neocallimastix,* prokaryotes, ciliates and protozoans. There may be as many as 1×10^{12} organisms per millilitre of rumen fluid. These microbes act together to break down the cellulosic plant components, mainly through the action of anaerobic prokaryotes and protozoans. Other bacteria then ferment carbohydrates to volatile fatty acids, carbon dioxide and methane, which the Archaea produce from acetate, carbon dioxide and hydrogen gas. Having performed their tasks, the rumen microorganisms are digested in the adjacent stomachs to yield amino acids and sugars for ruminant metabolism.

ASSOCIATIVE ORGANISMS

[A1.5] Associative organisms are organisms that elicit or potentiate a positive reaction or effect when in intimate proximity with a plant or animal. The best known are Plant Growth Promoting Rhizosphere (PGPR) organisms and Yield Increasing Bacteria (YIB)

[A1.6] The most common of these are the diazotrophs, including *Azotobacter, Azospirillum, Acetobacter, Azoarcus, Clostridium*, Enterobacteriaceae and *Herbaspirillum*, as well as the facultative nodule bacteria *Burkholderia, Rhizobium* and *Azorhizobium,* which have been shown to have additional associative effects in cereals. Most of these associative organisms may supply small amounts of N to crop plants, which may be useful in N-deficient systems, and this can be measured using the %ndfa natural abundance technique. *Azospirillum* has been shown to increase yield by 5 to 30 percent in about 70 percent of reported trials. However, they may also have a range of other functions related to hormone, siderophore or chelate production, or nutrient solubilization. Another class of microbes that is becoming well-researched in contemporary laboratories is the ACC group. This group deaminates 1-amino cyclopropane -1-carboxylate, which is a precursor to ethylene. Ethylene may be injurious to plants grown under stress. Avoidance of exposure to ethylene can

increase plant growth. The most studied ACC organism is *Pseudomonas putida*. There are accepted methodologies to assay for these functions.

BIO-CONTROL AGENTS (PATHOGENS OF WEEDS, FUNGI, INSECTS OR NEMATODES)

[A1.7] The use of microbes to control pests through parasitism, pathogenicity or competition is considered an environmentally sound use of AMiGRs, with significant potential in agriculture. A well documented bioinsecticide is *Bacillus thuringiensis,* which produces toxin crystals effective in controlling coleopteran and lepidopteran insects. *Bt* has been utilized for over 20 years in cotton crops to control the Boll Weevil, and *Bt* toxin genes have been transferred into both plants and bacteria for similar purposes. A large collection of *Bt* toxin genes are maintained by the Pasteur Institute in Paris. Other examples of the current application of AMiGRs as biocontrol agents include *Bacillus subtilis* as a pathogen of fungi; *Agrobacterium* cured of the *Ti* plasmid as a competitor against Crown gall-inducing *Agrobacterium*; *Pseudomonads* as weed control agents; *Metarhizium* as a control agent for locusts and grasshoppers; and the twist fungus as an inhibitor of nematode and *Corynebacterium* induced toxicity of annual ryegrass. The nuclear polyhedrosis viruses (NPVs) have proven effective against lucerne and celery loopers, and could be employed in genetic modification studies to control insect pests of agricultural plants.

FERMENTATION OF FOODS AND BEVERAGES

[A1.8] Yeasts are used in bread, beer and wine manufacture; *Streptococcus* and *Lactococcus* in dairy products such as cheese and yoghurt, as well as in nisin production, which may be used as an anti-spoilage treatment; *Penicillium camamberti* is used in the later stages of camembert production; *Acetobacter* in wine-vinegar production; *Lactobacillus* in production of fermented meats; *Aspergillus* and *Rhizopus* in soy fermentation. Many of these genera have a role elsewhere in Agriculture.

MASS CULTURE OF MICROALGAE AS A SOURCE OF PIGMENTS OR ANTIOXIDANTS, OR AS A FEED BASE FOR HIGHER ORGANISMS

[A1.9] Mass culture of microalgae is routinely undertaken in aquaculture facilities for production of feed-base to provide bulk for fish, cattle, pig or poultry feed. Examples include *Chlorella* spp., *Isochrysis* spp. and *Pavlova* spp. Microalgae may also be grown in high volume culture for fine chemical production, such as phycocyanin from the Cyanobacterium *Spirulina*; beta-carotene from *Dunaliella salina*; and astaxanthan from *Haematococcus pluvialis.* These are global aquacultural industrial processes. Cyanobacteria have a key role in rice production.

APPENDIX 2

AN EXTRACT FROM THE CURRENT US FARMBILL

US Farmbill

Appendix I
104 STAT.3744
Public Law 101-624-Nov. 28, 1990
Title XVI

Subtitle C--National Genetic Resources Program

7 USC 5841.

SEC. 1632. Establishment, Purpose, and Functions of the National Genetic Resources Program

(a) IN GENERAL.--The Secretary of Agriculture shall provide for a National Genetic Resources Program.

(b) PURPOSE.--The program is established for the purpose of maintaining and enhancing a program providing for the collection, preservation, and dissemination of genetic material of importance to American food and agriculture production.

(c) ADMINISTRATION.--The program shall be administered by the Secretary through the Agricultural Research Service.

(d) FUNCTIONS.--The Secretary, acting through the program, shall--

(1) provide for the collection, classification, preservation, and dissemination of genetic material of importance to the food and agriculture sectors of the United States;

(2) conduct research on the genetic materials collected and on methods for storage and preservation of those materials;

(3) coordinate the activities of the program with similar activities occurring domestically;

(4) unless otherwise prohibited by law, have the right to make available upon request, without charge and without regard to the country from which such request originates, the genetic material which the program assembles;

(5) expand the types of genetic resources included in the program to develop a comprehensive genetic resources program which includes plants (including silvicultural species), animal, aquatic, insect, microbiological, and other types of genetic resources of importance to food and agriculture, as resources permit; and

(6) engage in such other activities as the Secretary determines appropriate and as the resources of the program permit.

APPENDIX 3

AN EXAMPLE OF AN MATERIAL TRANSFER AGREEMENT (MTA) THAT RELATES TO MICROBES

The CHIEF EXECUTIVE OFFICER OF THE INSTITUTION, a body corporate under the *xxxxx Act 1988* (COUNTRY) having its offices at xxxxxxxxxxxxx and the Recipient requires the following Details set out in Schedules 1, 2 and 3 to be provided to allow for the exchange of Genetic Material (hereinafter called 'Material') under the Terms and Conditions of this Agreement.

	Item	SCHEDULE 1: Details		
MATERIAL	1	Description of Material to be transferred (If further details are attached please tick the box below and complete Schedule 2) □ Further Details Provided in Schedule 2		Common Name: Species: Identifying Codes: Other attributes:
	2	Quantity and form of Material		Quantity:
	3	Nominated Delivery Date		Date:
AGREEMENT DETAIL	4	Recipient's Details	Organization: Delivery Address: Contact Name	
	5	Purposes for which the Recipient can use the Material (Please place an "X" in only one of the boxes on right, and complete Schedule 3 if Purpose 5 is chosen)	Purpose 1 ☐ Purpose 2 ☐ Purpose 3 ☐ Purpose 4 ☐ Purpose 5 ☐	As parental material for crossing with genetic material only As reselection material only As testing and evaluation material only As genetic manipulation material only For any Purpose above where special conditions apply, a combination of Purposes listed above apply, or where Material is to be used for a purpose not covered above
	6	Commencement Date:		7 Expiry Date:
	8	INSTITUTE Authorised Signatory	Name: Position: Telephone: Email:	
	9	Recipient's Authorised Signatory	Name: Position: Telephone: Email:	

EXECUTION CLAUSE	By countersigning below, both parties agree to the Terms and Conditions of this Agreement and have provided the Details as required in Schedules 1, 2, and 3	
	Dated this day of 20__	Dated this day of 20__
	Signed for and on behalf of INSTITUTE ……..…….. Authorised Signatory (signature) …… Witness (signature) …… Witness Name and Title …… Manager Business Development (counter-signature)	Signed for and on behalf of the Recipient ……..…… Recipient Authorised Signatory (signature) …….... Recipient Witness (signature) …….... Recipient Witness Name and Title …….. Recipient Authorised counter-signatory, if applicable (signature)
Office use only GMTA ID……		GMTA prepared by:……

SCHEDULE 2: Further information describing the Material to be supplied

SCHEDULE 3: Further information describing the purposes for which the Material may be used and subsequent obligations of both parties
1. Purpose(s) for which the Material may be used:
2. Special conditions relating to the use of the Material:

SIGNING	**By countersigning below, both INSTITUTE and the Recipient's Authorised Signatory have checked the information set out in Schedule 2 and 3 and any associated attachments and agree that all information is true and correct and in accordance with the wishes of their organization.**	
	Dated this day of 20__	**Dated this day of 20__**
	..... INSTITUTE **Authorised Signatory (Signature)**	..... Recipient **Authorised Signatory (Signature)**

TERMS and CONDITIONS

All Item numbers referred to in the Terms and Conditions refer to Items within Schedule 1, 2 or 3 unless otherwise stated.

By providing the Details and countersigning Schedule 1 and if applicable, providing further information and countersigning Schedule 2 and 3, both INSTITUTE and the Recipient agree to the following:

1. GENERAL OBLIGATIONS

1.1) The Recipient acknowledges it accepts the Material at its own risk and that INSTITUTE is supplying the Material without any expressed or implied warranty as to the utility of the Material for the Purpose

1.2) INSTITUTE hereby grants the Recipient (Item 4 of Schedule 1) the right to use the Material (Item 1 of Schedule 1 or Schedule 2, as applicable) solely for the purposes defined in Item 5 of Schedule 1 or Item 1 of Schedule 3, as applicable.

1.3) The quantity and form of Material (Item 2 of Schedule 1) shall be delivered at INSTITUTE expense to the Delivery Address of the Recipient (Item 4 of Schedule 1) by the Delivery Date (Item 3 of Schedule 1) or as soon as practicable thereafter.

1.4) The Recipient shall take all necessary precautions to ensure the security of the Material, including but not restricted to adequate confidential identification as mutually agreed. The Recipient must detail such security measures in reports as required in Clause 1.8.

1.5) If the Recipient ceases to have a use for the Material, or if this Agreement expires or is terminated, or if INSTITUTE so requests, all Material shall be destroyed or returned to INSTITUTE (at INSTITUTE's election) and evidence to INSTITUTE's satisfaction of such destruction shall be immediately forwarded to INSTITUTE.

1.6) INSTITUTE shall have access to the Material and the relevant trialling, testing, modification or experimenting sites and all associated results, information and data at any point in the duration of the Agreement, subject to reasonable notification being given by INSTITUTE to the Recipient.

1.7) The transfer of any other material from the Recipient to INSTITUTE including, where applicable, crossbred breeding lines whose parent is the Material supplied by INSTITUTE will occur on the basis of like terms and conditions to those set out in this Agreement.

1.8) The Recipient shall deliver to INSTITUTE an identical copy of all summary reports produced by the Recipient on the performance and security of the Material at least every twelve (12) months following the Commencement Date, for the duration of the agreement.

2. DURATION OF THE AGREEMENT

2.1) This agreement shall commence on the Commencement Date (Item 6 of Schedule 1) and expire on the Expiry Date (Item 7 of Schedule 1). The Recipient shall complete all obligations under this Agreement by the Expiry Date (Item 7 of Schedule 1).

2.2) CONFIDENTIALITY

2.3) For the duration of the Agreement and for a period of three (3) years thereafter INSTITUTE and the Recipient shall keep confidential all information in relation to the supplied Material and all subsequent testing, modifications or experiments in relation to the Material. Either party may reveal information within the confidentiality period upon written approval from the other party.

2.4) Nothing in this Agreement prevents or inhibits INSTITUTE from providing information to the Minister of the Crown in right of the COUNTRY having responsibility for the INSTITUTE. Further, nothing in this Agreement prevents or inhibits that Minister of the Crown from providing to the Parliament of COUNTRY information concerning any conduct or operation of INSTITUTE in such a manner and to such an extent as the Minister thinks reasonable and appropriate.

3. OWNERSHIP OF MATERIAL AND INTELLECTUAL PROPERTY

4.1) Notwithstanding the Recipient's right to use the Material to the purposes defined in Item 5 and Item 1 of Schedule 3 as applicable, and unless Clauses 5.2 and/or 5.5 of this Agreement apply, the Recipient acknowledges and agrees that the Material and all associated industrial and intellectual property rights are owned in perpetuity by

INSTITUTE and the Material cannot be transferred by the Recipient to any third party, under any circumstances.

4. SPECIFIC OBLIGATIONS

4.2) The Recipient may only use the Material for the purposes set out in Item 5 of Schedule 1 or Item 1 of Schedule 3 as applicable, and accordingly agrees to the following specific obligations:

4.3) **As parental material**: If the Material is used in its supplied form as parental material for crossing with other genetic material being either breeding lines or commercial plant varieties (Purpose 1 of Item 5 of Schedule 1) the Recipient does not need any further approval for such activity. Provided that any new material is not considered to be essentially derived within the meaning of the Plant Breeder's Rights Act 1994 (Cth), any new material that results from such crossing with other genetic material will be solely owned by the Recipient provided that the Recipient agrees that progeny derived from material received by INSTITUTE from the Recipient shall be owned solely INSTITUTE. INSTITUTE's role in the parentage of the new material should be acknowledged in any subsequent trialling, modifying, Plant Breeders Rights registration or commercialization process.

4.4) **For reselection**: If the Material is used for reselection purposes (Purpose 2 of Item 5 of Schedule 1) any new material that results from such reselection will be solely owned by INSTITUTE. Such new material cannot be modified, improved, experimented on, or commercialised without both parties entering into a meaningful agreement allowing such activity.

4.5) **For testing and evaluation**: If the Material is used for testing and evaluation purposes (Purpose 3 of Item 5 of Schedule 1) the Recipient does not need any further approval for such activity. Any new material that results from such testing and evaluation shall be solely owned by INSTITUTE. Such new material cannot be modified, improved, experimented on, or commercialised without both parties entering into a meaningful agreement allowing such activity.

4.6) **For genetic manipulation**: If the Material is used in its current form as genetic manipulation material for the insertion of extraneous deoxyribonucleic acid or 'DNA' (Purpose 4 of Item 5 of Schedule 1) the Recipient does not need any further approval for such activity. Any new material that results from such genetic manipulation and DNA insertion, will be jointly owned by INSTITUTE and the Recipient. Such new material cannot be modified, improved, experimented on, or commercialised without both parties entering into a written agreement allowing such activity

4.7) **For some other purpose or combination of purposes**: If the Material is used for any other single purpose or combined purposes (Purpose 5 of Item 5 of Schedule 1) as specified in Schedule 3 the Recipient shall meet all of the obligations set out in Schedule 3.

APPENDIX 4

THE TERMS OF REFERENCE DEFINING THE SCOPE OF THE REVIEW

Terms of Reference for a Consultant to conduct study on technical issues related to developing harmonized management policies, guidelines and practices concerning acquisition, use and distribution of Agricultural Microbial Genetic Resources (AMiGR)

The consultant will:

1. Participate in a research initiation brain-storming session, either in person or over the phone, with members of the Genetic Resources Policy Committee (GRPC), Inter-Center Working Group – Genetic Resources (ICWG-GR), FAO, MOSAICC and other interested parties concerning the research activities he or she will undertake;

2. Address the question whether there is a distinct subset of microbial genetic resources that can be called microbial genetic resources for food and agriculture, or agricultural microbial genetic resource (AMiGR). In this context, the consultant will, among other things, consider data concerning the physical nature of AMiGR and their broad categories and uses, the history and actual patterns of the distribution of AMiGR around the globe, and other possible factors that may distinguish AMiGR from other forms of MGRs, for example, those that are used for pharmaceutical or industrial purposes. It is understood that it is probably not possible to exhaustively define the outer limits of this sub-class of resources, given the highly dynamic nature of their various sources, uses and distribution. Of course, it also the case that some MGRs are used for both agricultural purposes and other purposes. However, it is desirable to at least establish a 'moving' definition. We may also consider the use of the MGR as a discriminating factor (as for PGRFA).

3. Conduct a survey of management policies, guidelines and practices of organizations concerning the acquisition, use, collection and distribution of AMiGR materials. In this context, the consultant will focus on:

- Future Harvest Centres holding AMiGRs. Regarding the Future Harvest Centres, the consultant will use the System-wide Genetic Resources Program (SGRP) report entitled "A Discussion Paper on the Status of Microbial Genetic Resources Held by the CGIAR Centres," by Dr Kenneth Street as a starting point. The consultant will also develop a questionnaire and circulate it through the SGRP of the CGIAR to obtain new data and critical reflections from AMiGR managers within the Future Harvest Centres; and
- other organizations studying, holding or using similar AMiGRs, including the World Federation of Culture Collections, ICIPE, the United States' Department of Agriculture, the Belgian Coordinated Collections of Micro-organisms (which coordinated the development of the Micro-Organisms Sustainable use and Access regulation International Code of Conduct (MOSAICC)), Biological Resource Centres as developed by the OECD, organizations that have developed codes of responsible behaviour for forestry plantation management concerning uses of MGRs, the International Union of Forest Research Organization (IUFRO), including working group 2.01.13 "Root physiology and symbiosis", and so on. The consultant will identify a range of other similar AMiGR collection-holders and/or users whose management policies, guidelines or practices could usefully be reviewed in the context of this study.

4. Address the following questions:

- What are the basic needs and challenges in using these AMiGR in the general context of agricultural development for the next years?
- What obstacles are countries having in using these AMiGR, (with a special emphasis on developing countries)?

- Are there informal (non-legalized) customs developed for the acquisition, distribution, and or exchanges of these AMiGR? Are there movements towards codification of those activities? In what direction are they moving and what kinds of organizations are generally involved in developing those codes?
- Are the informal customs and movements towards codification with respect to these AMiGR different than those resources being used, e.g., biological control, bio fertilization, food industry, etc? If so, why?
- Is there evidence that national access laws are having an impact on the transfer, use of, and research concerning, these resources?
- What are the trends in patenting of unmodified and modified microbials?