PN-ABB-178

MEMORANDUM

Date 24 May 88

TO: PPC/CDIE/DI, Tina Wilson/Acquisitions - 209 SA-18

FROM: S&T/AGR/RNR, Lloyd R. Frederick

SUBJECT: Reprints of Articles with Partial Support of A.I.D. Contracts/Grants

Attached is one copy of the following article with the supporting contract/grant number:

1987 Annual Report for
World Rhizobium Study and Collection Center

Contract/~~Grant~~ RSSA Number: BST-4109-R-AG-3164

Project: 936-4109

Attachments: a/s

cc: T.Gill

Lloyd Fredenck

WORLD *RHIZOBIUM* STUDY AND COLLECTION CENTER

1987 Report

An A.I.D.-U.S.D.A. interagency project (RSSA BST-4109-R-AG-3164) associated with the Beltsville *Rhizobium* Culture Collection, Nitrogen Fixation and Soybean Genetics Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland

Harold H. Keyser, Deane F. Weber and Richard F. Griffin

ABSTRACT

Since 1912 the Agricultural Research Service has maintained a collection of the important agricultural bacterium, Rhizobium. These bacteria provide nitrogen to legumes and are an essential component of the global nitrogen pool. The collection has grown to become one of the world's major germplasm resources for Rhizobium and Bradyrhizobium.

The purpose of the collection is to provide a safe and perpetual depository and collection center for Rhizobium and Bradyrhizobium cultures and make them available to users worldwide. The emphasis of the collection is on agricultural legumes, but bacterial germplasm is also available that has been isolated from wild legumes. Currently, we have 2,659 cultures isolated from 325 species of 85 genera of the Leguminosae. We distribute between 600-800 cultures per year, about half of which are sent to foreign countries. A comprehensive, second edition catalog describing all strains is available. The collection is a source of strains for much of the research on nitrogen fixation in the U.S. and abroad.

In 1976, the U.S. Agency for International Development (A.I.D.) contracted with the U.S.D.A. to establish the "World Rhizobium Study and Collection Center" to complement the activities of the Beltsville Rhizobium Culture Collection. The establishment of this project with A.I.D. extends the activities of the collection to research scientists in developing countries. This cooperation provides opportunities for training and research collaboration at Beltsville, and assistance in projects related to the legume-Rhizobium symbiosis.

INTRODUCTION

Bacteria of the genera Rhizobium and Bradyrhizobium are able to convert atmospheric dinitrogen (N_2) into a biologically useful molecule (NH_4) when they are in symbiotic association with plants of the family Leguminosae. This process of symbiotic nitrogen fixation occurs within legume root nodules, and provides the host with its nitrogen requirement for growth. Symbiotic nitrogen fixation is an important component of the global nitrogen cycle, and is widely used in agriculture through inoculation of legume seed at planting. Inoculation attempts to provide legume crops with specific and highly effective strains of the bacteria.

Microbiologists with the USDA began collecting and evaluating strains of Rhizobium in 1912. The ensuing collection of rhizobia was passed on from one microbiologist to another through the years. Early isolations were made primarily from pasture and green-manure legumes. With the growing importance of soybeans in the 1950's the emphasis of the collection in turn shifted to the rhizobia for soybean, Bradyrhizobium japonicum. The Collection provided strains and information to both researchers and inoculant manufacturers, thereby assisting in the establishment of the soybean industry in the U.S. Today, the Beltsville Rhizobium Culture Collection is recognized worldwide as having the best collection of well characterized B. japonicum.

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In 1981 the Beltsville Rhizobium Culture Collection was designated a Rhizobium MIRCEN. MIRCEN is an acronym for Microbiological Resources Centers, a network of centers established by UNESCO/UNEP to preserve microbial gene pools, promote use of microbial biotechnology in developing countries, foster training of personnel, and promote the exchange of germplasm.

GERMPLASM HOLDINGS

Table 1 shows the contents of the collection by bacterial species. The 2,659 cultures were isolated from 325 species of 85 genera in the Leguminosae.

Although the collection has historically emphasized the bacteria for temperate climate legumes important to American agriculture, we are now adding germplasm to make the collection more comprehensive. The vast majority of the 20,000 species representing 650 genera in the family Leguminosae are tropical, and only about 15 % of these species have been studied with regard to their bacterial symbionts.

We recently acquired the research collection of the late Dr. Joe Burton. This collection of rhizobia isolated from agricultural and wild legumes from all over the world was accumulated while he was with the Nitragin Company, and represents a valuable germplasm pool which would be extremely difficult to replace. Many of the habitats in the regions of diversity for legumes and their rhizobia in Africa and South America are rapidly disappearing, so additions to the germplasm banks held in public collections, such as that of Dr. Burton's, are quite valuable.

The germplasm in the collection is permanently preserved in two forms, lyophilized and frozen in glycerin. For both, the mother cultures are kept at -80°C. Strains sent upon request are provided as lyophilized cultures.

In March, 1987 we published the second edition of the Beltsville Rhizobium Culture Collection Catalog, and copies are available upon request.

In addition, we are now completing the conversion of all strain information, such as origin or efficiency, to a Dbase III system. This file will be made available to other institutions on request.

GERMPLASM DISTRIBUTION

Table 2 shows the requests filled by the collection staff during the past 18 years. Over the last 15 years approximately half (53 %) of the cultures requested have been B. japonicum (rhizobia for soybean). Since the inception (1976) of the A.I.D. project, 7,088 cultures have been provided to 80 different countries. While the largest user group is researchers at universities and research stations, requests from the private sector (including rhizobial inoculant manufacturers) have averaged about 14 % annually.

AFFILIATED SERVICES

Through the support of the A.I.D.-U.S.D.A. project, training and opportunities for collaborative research are offered in the Nitrogen Fixation and Soybean Genetics Laboratory to scientists from developing countries. Aspects of training are tailored to the needs of the individual, with emphasis on the area of work the visitor will be engaged in upon return to their home country. Those visitors with sufficient scientific background can participate in collaborative research with the staff scientists with opportunities in several areas of nitrogen fixation research available.

Since the interagency project began, eleven visiting scientists have come to Beltsville for the in-depth training and research opportunities. Most of these scientists are still directly involved in nitrogen fixation research or technology in their home country.

The two staff scientists on this project (Drs. Weber and Keyser) have also assisted nitrogen fixation projects in developing countries in several other ways. They assisted in the development of West African MIRCEN in Dakar, Senegal, and participated in training workshops given by the Board on Science and Technology for International Development. Drs. Weber and Keyser have made numerous on-site visits for review and consultation and have provided information on the applied technology of inoculant preparation and use.

Special services of the collection include:

- Lyophilization of cultures for scientists in developing countries that do not have access to freeze-drying equipment.
- Serological identification of certain species of Rhizobium and Bradyrhizobium.
- Deposition of valuable collections of rhizobia if they are in danger of being lost.
- Duplicate storage of rhizobial cultures from other collections.

AFFILIATED RESEARCH

Since the collection began it has also served as an exploitable base for research on the legume-*Rhizobium* symbiosis by ARS scientists. Research from Beltsville scientists associated with the collection has demonstrated genes in soybeans that control specificity of nodulation (1, 2 and 3), the existence of a plant-damaging toxin produced by certain strains of *B. japonicum* (4), the significance of interstrain competition for nodulation of soybeans (5 and 6), host-strain specificity for expression of hydrogenase (7), and the discovery of a new species of fast-growing soybean rhizobia, *Rhizobium fredii* (8). Additionally, the collection provides many of the strains used in research published by workers at universities and other institutions. The accumulated research on strains in the collection adds to its inherent value, and demonstrates the importance of a public collection available to all interested persons throughout the world.

Table 1. Current holdings of the Beltsville *Rhizobium* Culture Collection.

Genus and species [1]	Host legume	Cultures
Bradyrhizobium japonicum	soybean	446
Bradyrhizobium sp.	many tropical & temperate legumes	1,198
Rhizobium meliloti	alfalfa	109
Rhizobium leguminosarum		
biovar trifolii	clovers	382
biovar viceae	pea	121
biovar phaseoli	common bean	67
Rhizobium fredii	Asian soybean	11
Total [2]		2,345

[1] The genus *Rhizobium* was recently split into two genera, *Rhizobium* for the fast-growing bacteria, and *Bradyrhizobium* for the slow-growing bacteria.

[2] We also have 314 unaccessioned cultures, bringing the grand total to 2,659.

Table 2. Culture requests over the past 18 years from the Beltsville *Rhizobium* Culture Collection.

Year	Number of cultures sent upon request	Number of countries receiving cultures
1970	170	16
1971	164	10
1972	177	8
1973	113	8
1974	258	21
1975	361	16
1976	389	17
1977	332	17
1978	319	16
1979	515	16
1980	508	16
1981	764	25
1982	842	26
1983	786	23
1984	614	41
1985	852	22
1986	666	22
1987	601	24

REFERENCES

1. Caldwell, B.E. 1966. Inheritance of a strain-specific ineffective nodulation in soybeans. Crop Sci. 6:427-428.

2. Vest, G. 1970. Rj3 - A gene conditioning ineffective nodulation in soybean. Crop Sci. 10:34-35.

3. Vest, G., and B.E. Caldwell. 1972. Rj4 - A gene conditioning ineffective nodulation in soybean. Crop Sci. 12:692-693.

4. Erdman, L.W., H.W. Johnson, and F. Clark. 1956. A bacterial induced chlorosis in the Lee soybean. Plant Dis. Rep. 40:646.

5. Johnson, H.W., and U.M. Means. 1964. Selection of competitive strains of soybean nodulating bacteria. Agronomy Journal 56:60-62.

6. Boonkerd, N., D.F. Weber, and D.F. Bezdicek. 1978. Influence of Rhizobium japonicum strains and inoculation methods on soybean grown in rhizobia-populated soil. Agronomy Journal 70:547-549.

7. Keyser, H.H., P. van Berkum, and D.F. Weber. 1982. A comparative study of the physiology of symbioses formed by Rhizobium japonicum with Glycine max, Vigna unguiculata, and Macroptilium atropurpurem. Plant Physiol. 70:1626-1630.

8. Keyser, H.H., B.B. Bohlool, T.S. Hu, and D.F. Weber. 1982. Fast-growing rhizobia isolated from root nodules of soybean. Science (Washington, D.C.) 215:1631-1632.