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RESOURCES SUPPORT SERVICES AGREEMENT BETWEEN AGENCY FOR INTERNATIONAL DEVELOPMENT

AND U.S. DEPARTMENT OF AGRICULTURE

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BELTSVILLE *RHIZOBIUM* STUDY AND COLLECTION CENTER

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I. OVERALL OBJECTIVES

1. Collection, characterization, documentation and preservation of *Rhizobium*.

In CY 1981 the following new germplasm was added to the collection:

4 isolates from *Leucaena leucocephala* nodules collected in Tanzania;

18 isolates of *R. japonicum* from nodules of *G. max* and *G. soja*, collected in China by American Soybean Association tour.

20 strains of *R. japonicum* from nodules of *G. soja* collected in China, T.S.Hu.

28 strains of *R. japonicum* from nodules of *G. max* collected in China, T.S. Hu.

Among the *R. japonicum* isolates from the Fehr-Hinson collection (1979), T.S. Hu's collection and one isolate from Tanner's collection (provided by J. Burton)were found several fast-growing, acid producing cultures. These have all come from mainland China in the past 3 years from different collection trips. In collaboration with B. Ben Bohlool (Univ. of Hawaii) these unique and previously undescribed cultures were characterized in pure culture and symbiotic tests. The pure culture tests indicate these fast-growing isolates are very similar to other fast-growing *Rhizobium* (e.g. *R. leguminosarum*, *R. trifolii* and *R. meliloti*)and quite distinct from other *R. japonicum*. These isolates produce nodules on siratro, wild soybean (*G. soja*) and soybean, and do not nodulate hosts of other cross inoculation groups. However, while they are effective at fixation of N_2 with *G. soja* and *G. max* cv Peking, they are ineffective with the 10 commercial cultivars of soybean tested so far. These new cultures should prove very valuable in studies on genetics of *R. japonicum* and for studies of host determined factors of N_2 fixation. Further tests of their serological affinity with slow-growing *R. japonicum* and effectiveness within the 'cowpea cross inoculation group' will be conducted.

We examined several strains of R. japonicum for their effectiveness and relative efficiencies (net H_2 evolution) on hosts of the cowpea cross inoculation group. Some of the strains which are only moderately effective soybean were found to be very effective with siratro and cowpea. Also, we found evidence for host influence on the expression of hydrogenase activity in comparisons of strains USDA 61 and 74 on soybean and cowpea. The strain USDA 33 which is ineffective on soybean was very effective on siratro, while the effective soybean strains USDA 110, 122 and 136 were ineffective with siratro. Further, USDA 94 was found to be effective with Alysicarpus vaginalis, Crotalaria sericea, Glycine wightii, Macroptilium lathyroides, Strophostyles helvola, Vigna mungo, Vigna acontifolia, and Macrotyloma uniflorum. In comparison, USDA 110 was ineffective with all these hosts. This suggests that some of the less effective R. japonicum are better symbionts with the cowpea group, and were merely assigned to the soybean group due to their isolation from nodules of soybean. These results indicate that it is necessary to recognize possible competition between strains of R. japonicum and cowpea miscellaneous rhizobia for nodule sites on the same host in soil where both are present.

Symbiotic evaluation was completed on all accessioned R. meliloti in the collection, about half of the R. trifolii isolates and many of the miscellany rhizobia for cowpea, mungbean, peanut and lima bean.

Experiments were conducted on our freeze-drying system to evaluate the procedure. We are using an Edwards 'self sealing' manifold which accomodates 300 flat bottomed serum vials in place of the more typical manifold which accomodates 48 ampolues. All vials were found to maintain their vacuum after the process, and approximately 10^6 cells survived in each vial. Their symbiotic capacity appeared unaltered. We are going to proceed in converting the collection held on agar slants to parallel collections of freeze-dried vials and broth in glycerin (held at -80° C) both for culture distribution (vials) and storeage of stock cultures. We consulted the Bacteriology Department of the American Type Culture Collection on these matters and received excellent advice. Further, we intend to cooperate with ATCC on a project to determine any damage to DNA (chromosomal and plasmid) in rhizobia damaged by these processes, as very little is known about this except in E. coli and yeast, where damage has been found in some cultures. This is a fairly important question, as most major Rhizobium collections depend upon freeze-drying for permanent storeage of stock cultues in a genetically stable condition.

2. Distribution of cultures of Rhizobium for research and inoculum production in DCs and LDCs.

In CY 1981 the Collection serviced 117 requests for 764 cultures:

R. japonicum	- 72 requests,	367 cultures	(48%)
R. species	- 47 requests,	247 cultures	(32%)
R. meliloti	- 9 requests,	27 cultures	(3½%)
R. trifolii	- 10 requests,	34 cultures	(4%)
R. leguminosarum	- 13 requests,	33 cultures	(4%)
R. phaseoli	- 16 requests,	51 cultures	(7%)
Azsopirillum	- 2 requests,	3 cultures	(1%)
Azotobacter	- 1 request,	1 cultres	(½%)

The Collection serviced requests from 25 countries other than the U.S., 46 requests for 299 cultures. These countries were Zimbabwe, Nigeria, Yugoslavia, India, Spain, Vietnam, Ireland,Malaysia, England, Italy, Canada, Brazil, Mexico, Colombia, South Africa, Senegal, Egypt, Poland, Argentina, Turkey, W. Germany, Austria, Australia and New Zealand. Thirteen of the requests were from private companies (inoculant producers and biotechnology firms), the remainder from federal, state, local or University research stations.

Below is a summary of the requests serviced by the Collection over the past 6 years, with the exception of 1979 (we can't find the damn file):

Year	Requests	Cultures
1976	31	193
1977	61	320
1978	47	227
1979	-	-
1980	57	508
1981	117	764

The increase in the number of cultres requested in the most recent years undoubtedly reflects increased research on Rhizobium as well as distribution of the USDA Collection Catalogue.

3. Serve as a depository for microbial germplasm of useful nitrogen fixing organisms.

No new germplasm was deposited in CY 1981 for safekeeping.

4. Train researchers and technicians in techniques and subject matter for applied research and technology of Rhizobium biology, and consult with and provide technical assistance to those who work with rhizobia in LDCs.

In CY 1981 training was given at the Collection Center to 3 visiting scientists:

a. Seydou Sanogho from Mali, received instruction in serological techniques, effectiveness evaluation, strain competition and field investigation. Dr. Sanogho has some previous training in rhizobiology from IITA, Nigeria, and developed 2 research projects in addition to regular activities: a field investigation of competition between fast and slow-growing R. japonicum for nodule occupancy with G. max cvs Peking and Lee, and cultural and symbiotic characterization of new isolates from wild soybean (G. soja). Dr. Sanogho's tenure will finish in CY 1982, and he plans to return to West Africa.

b. Frank Shao from Tanzania, whose program is described in the CY 1980 Annual Progress Report, finished his tenure and returned to his research assignment there, and we plan to continue cooperation in exchange of material and information, and hopefully a cooperative research project.

c. T.S. Hu, from Beijing, China, completed his tenure in CY 1981, and returned to China via Ben Bohlool's lab and the NifTAL project.

In September, 1981, Dr. Keyser was invited by J. Gritzner of the National Academy of Sciences' Board on Science and Technology for International Development to be a panel member in an assessment of the establishment of a Microbiological Resource Center for West Africa to be housed in Senegal. In Dakar, Senegal, the panel (Gritzner, Keyser, and E. DaSilva from UNESCO) met with Senegalese officials and researchers, and with the AID personnel in Dakar concerning the MIRCEN. It was concluded that the most immediate prospects for success of a MIRCEN there would be emphasis on Rhizobium and mycorrhizae inoculation. UNESCO is in the process of contacting agencies to provide seed money for the establishment of the MIRCEN. The Senegalese appear enthsiastic to house the MIRCEN in their country, and can provide the necessary physical facilities. The main benefits of such a MIRCEN to West African agriculture would be in better peanut production, a source of inoculant for seedling legume trees used throughout the area, and a center for training in applied agricultural microbiology.

II. RESULTS OF RHIZOBIUM FIELD TRIALS

1. Rhizobium-lima bean trial conducted at Soil Conservation Service Farm, USDA Beltsville, Maryland, 1981. Seed applied peat inoculant on Fordhook 242.

Rhizobium strain	Yield - Pounds of shelled bean / acre*
USDA 3259	5183 a
TAL 22	4735 ab
USDA 3316	4582 ab
USDA 3260	4404 ab
USDA 3314	4120 ab
USDA 3313	3998 ab
USDA 3257	3887 ab
Uninoculated	3880 ab
USDA 3258	3848 ab
USDA 3261	3788 ab
USDA 3256	3722 ab
USDA 3315	3592 b

* Yields flanked by the same letter are not significant at the 5% level of probability according to Duncan's new multiple range test.

This field was just cleared and prepared the previous year, and the relatively fertile condition (such as mineralization of organic matter) may have contributed to the nonsignificant response for all but one strain above the least effective strain, and none were different from the uninoculated control. Also, some of the variability in reps within treatments were due to deer grazing, which ruined a soybean trial in this same field.

2. Soybean - Rhizobium field trials conducted at Maryland Tobacco Research Station Upper Marlboro, Maryland 1981. Seed applied peat inoculant.

ESSEX		KENT	
Strain	Yield (bu/acre)*	Strain	Yield (bu/acre)*
Y1a	43.4 a	Hu 113-2	38.0 a
USDA 143	43.4 a	USDA 143	37.9 ab
WB 69	43.4 a	USDA 6	37.7 abc
USDA 6	43.2 a	PRC 143	37.3 a-d
CB 1809	42.9 a	USDA 122	37.3 a-d
J507	42.9 a	USDA 142	36.8 a-d
PRC 127	42.8 a	PRC 127	36.7 a-d
USDA 122	42.1 ab	Y1a	36.6 a-d
315	42.1 ab	PRC 160	36.5 a-d
WB 19	42.1 ab	USDA 110m	36.4 a-d
USDA 138	41.8 ab	J507	36.3 a-d
PRC 3	41.8 ab	WB 19	36.2 a-d
S1	41.7 ab	315	36.1 a-d
USDA 110	41.6 ab	Hu 005	36.0 a-d
THA 1	41.6 ab	PRC 3	35.9 a-d
R54a	41.5 ab	Y1	35.3 a-d
Y1	41.4 ab	USDA 110	35.1 a-d
USDA 110m	41.3 ab	CB 1809	35.1 a-d
587	41.1 ab	WB 69	35.1 a-d
Y2	41.0 ab	PRC 43	34.8 a-d
29W	40.7 ab	Y2	34.3 a-d
Hu B15	40.6 ab	USDA 138	43.1 a-d
PRC 160	40.6 ab	R54a	34.1 a-d
PRC 74	40.5 ab	Hu B15	34.0 a-d
Hu 113-2	40.2 ab	PRC 74	33.8 a-d
PRC 43	40.2 ab	29W	33.7 a-d
PRC 143	40.0 ab	S1	33.4 a-d
Hu 005	39.8 ab	THA 1	32.3 bcd
USDA 142	39.3 ab	Uninoculated	32.2 cd
Uninoculated	37.7 b	587	32.0 d

* Yields flanked by the same letter are not significantly different at the 5% level of probability according to Duncan's new multiple range test.

Marlboro 1981 field trials, continued.

MILES		WILLIAMS	
Strain	Yield (bu/acre)*	Strain	Yield (bu/acre)*
315	41.2 a	USDA 6	41.3 a
USDA 142	40.7 a	315	40.6 ab
WB 69	40.3 a	USDA 143	40.2 abc
PRC 127	40.3 a	29W	40.2 abc
Y1a	40.2 ab	Hu B15	40.1 abc
USDA 110m	40.0 ab	PRC 143	39.7 a-d
USDA 110	39.3 ab	WB 69	39.7 a-d
THA 1	38.9 ab	PRC 160	39.6 a-d
USDA 143	38.7 ab	CB 1809	39.3 a-d
USDA 122	38.7 ab	USDA 142	39.1 a-e
Hu 005	38.6 ab	USDA 122	38.9 a-e
USDA 6	38.6 ab	R54a	38.7 a-e
Hu 113-2	38.5 ab	Y1a	38.7 a-e
PRC 143	38.3 ab	PRC 127	38.3 a-e
PRC 43	38.3 ab	WB 19	38.3 a-e
R54a	38.2 ab	USDA 110m	38.1 a-e
CB 1809	38.1 ab	587	38.0 a-e
PRC 74	38.0 ab	PRC 3	38.0 a-e
Hu B15	38.0 ab	USDA 138	37.7 a-e
PRC 3	37.7 ab	PRC 43	37.6 a-e
Y1	37.6 ab	J507	37.5 a-e
USDA 138	37.5 ab	Y1	37.4 a-e
WB 19	37.4 ab	PRC 74	37.4 a-e
PRC 160	37.3 ab	Y2	37.4 a-e
J507	37.3 ab	USDA 110	36.3 b-e
S1	36.9 ab	Hu 113-2	36.6 b-e
Uninoculated	36.7 ab	S1	36.3 b-e
587	36.3 ab	Hu 005	36.1 cde
Y2	36.2 ab	Uninoculated	35.4 de
29W	34.6 b	THA 1	35.1 e

* Yields falnked by the same letter are not significantly different at the 5% level of probability according to Duncan's new multiple range test.