PN-AAX-469

ANNUAL PROGRESS REPORT

1983

RESOURCES SUPPORT SERVICES AGREEMENT
BETWEEN
U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT
AND
U.S. DEPARTMENT OF AGRICULTURE,
AGRICULTURAL RESEARCH SERVICE

RSSA BST - 4109-R-AG - 3164

'World Rhizobium Study and Collection Center'
Nitrogen Fixation and Soybean Genetics Laboratory
Beltsville Agricultural Research Center
Beltsville, Maryland

Annual Progress Report - CY 1983
RSSA BST - 4109-R-AG-3164

Current Staff

Deane F. Weber, Microbiologist
Harold H. Keyser, Microbiologist
Richard F. Griffin, Biological Laboratory Technician
Harris Prager, Biological Aid (half time)

I. OVERALL OBJECTIVES

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

In 1983 emphasis was placed on the permanent preservation of the assessioned collection. Survival studies from *R. japonicum* either lyophilized or frozen in glycerin (10%) in 1982 showed excellent survival and very minimal contamination when stored at -60°C. Therefore, we proceeded to preserve every strain in both the freeze dried and frozen in glycerin state in quadruplicate. This will be the permanent mother stock collection. Also, each culture will be lyophilized and stored at -20°C for servicing requests. Users of the collection have indicated to us that the lyophilized cultures are convenient and satisfactory for their purposes. We use flat bottomed ampules with a split ring stopper with a 3 level manifold Edwards Modulyo. The unit accomodates up to 300 ampules per run.

An initial trial was conducted on the rhizobial requirements of 15 tree legumes. A mixture of 7 broad spectrum strains (USDA76, 94, 3404, 3427, 32H1, CB756, 176A22) was used to inoculate seedlings in vermiculite. After 8 weeks roots were examined for nodulation and top growth was compared to treatments receiving combined N. The results are given below:

Legume	Top growth	Nodulation
Acacia albida	I = N	Normal
A. aneura	I<< N	Very sparse
A. nilotica var. adansonii	I < N	Normal
A. nilotica var. tomentosa	I < N	None
A. raddiana	I = N	Sparse
A. senegal	I = N	Normal
Leucaena leucocephala K8	I > N	Normal

L. leucocephala K500	I > N	Normal
Parkinsonia aculeata	I = N	None
Prosopis africana	I = N	None
P. alba 0166	I = N	Normal
P. alba 0388	I < N	Normal
P. glandulosa	I = N	Normal
P. juliflora	I > N	Normal
P. velutina	I = N	Normal

While most of the trees were well nodulated, specificity for infectiveness and effectiveness was apparent. (Parkinsonia sp. have been reported to be non-nodulating). To follow-up this research we will obtain seed and rhizobial germplasm in order to carry out a methodical study on the specificity and cross-effectiveness groupings among important tree legume species.

2. Distribution of cultures of Rhizobium for research and inoculum production in DC's and LDC's.

In CY 1983 the collection distributed 786 cultures of Rhizobium for 127 requests, serving 23 coutries. A summary of the 1983 requests follows:

R. japonicum	442 cultures	56%
R. sp.	149 cultures	19%
R. phaseoli	54 cultures	7%
R. leguminosarum	43 cultures	5%
R. trifolii	43 cultures	5%
R. lupini	36 cultures	4%
R. meliloti	19 cultures	2%

Below is a summary of Rhizobium requests serviced by the collection over the last 8 years:

Year	Requests	Cultures
1976	31	193
1977	61	320
1978	47	227
1979	69	515
1980	57	508
1981	117	765
1982	124	842
1983	127	786

Serve as a depository for microbial germplasm.

No germplasm was deposited by outside sources in CY 1983.

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 LDC's.

In CY 1983 Dr. Mohammed El-Sayed Hassan from Giza, Egypt, received training at Beltsville from March 15 to June 24. A summary of his activities is attached in a separate training report.

Dr. Lucien Bordeleau was hired as a consultant to meet with MIRCEN and other Senegalese scientists and administrators regarding the support and development of the West African MIRCEN. Dr. Bordeleau's expertise is valuable and necessary to the success of the MIRCEN. His report is attached.

II. A summary of field research conducted in CY 1983.

SOYBEAN - R. JAPONICUM YIELD TRIALS AT UPPER MARLBORO, MARYLAND
AVERAGE YIELD (BU/A) OF CV. KENT AND ESSEX

	1 YR AVE. 1983	2 YR AVE. 1982-83		3 YR AVE. 1981-81-83		4 YR AVE. 1980-81-81-83	
CB1809	40.0	CB1809	37.7	CB1809	38.8	CB1809	36.3
PRC127	38.4	PRC127	37.6	PRC127	38.6	PRC127	35.9
WB69	36.1	113-2	36.1	USDA122	37.5	WB69	35.1
USDA122	35.9	USDA122	36.1	USDA143	37.0	113-2	34.6
PRC143	35.5	J507	35.4	J507	37.0	PRC160	34.2
113-2	35.0	PRC160	34.8	WB69	36.9	USDA6	34.1
USDA110	34.5	USDA143	34.7	113-2	36.9	USDA110	34.0
USDA138	34.4	USDA110	34.6	PRC160	36.6	WB19	33.7
USDA143	34.3	WB69	34.6	R54a	36.4	PRC143	33.6
100 lbs. N	33.7	-	-	-	-	-	-
J507	33.7	R542	34.5	29W	36.3	PRC3	33.5
29W	33.0	29W	34.3	PRC143	36.2	Y18	32.8
PRC3	33.0	PRC143	34.1	USDA110	36.1	-	-
USDA6	32.8	USDA138	33.5	USDA6	35.9	-	-
PRC160	32.7	USDA142	33.3	USDA138	35.6	-	-
USDA142	32.6	587	33.3	USDA142	35.5	-	-
WB19	31.5	PRC3	33.1	PRC3	35.4	-	-
R542	31.5	WB19	32.8	587	35.4	-	-
587	31.0	USDA6	32.7	Y1a	35.2	-	-
Y1a	31.0	Y1a	32.3	WB19	35.1	-	-
USDA24	28.8	-	-	-	-	-	-
Uninoc.	25.3	Uninoc.	29.5	Uninoc	30.1	Uninoc.	29.4
USDA31	24.0	USADA31	26.3	-	-	-	-
Non-nod	15.1	Non-nod	26.2	-	-	-	-

The following summarizes the activities of M. E. Hassan while he was a visiting scientist at USDA-ARS Beltsville in cooperative studies with Drs. Harold H. Keyser and L. David Kuykendall.

I. Selection of antibiotic resistant strains of fast-growing soybean rhizobia

The objective of this study was to select genetically marked strains of fast-growing soybean rhizobia so that soil ecology studies could be undertaken in non-sterile soil, using a direct recovery technique.

Sequential selection for resistant mutants was performed with naladixic acid and spectinomycin. Three of the fast growers (USDA 191, 192, and 205) yielded doubly-marked isolates. These isolates were then compared with their parent strains for any significant change in growth rate, as well as with a slow-growing strain (1-110) and its triple-resistant mutant (110-ARS).

Rhizobium strain	Specific growth rate	Generation time (hr)
191	0.206	3.36
191 ns^5	0.281	2.46
192	0.303	2.28
192 ns^4	0.244	2.84
205	0.306	2.26
205 ns^5	0.178	3.70
1-110	0.128	6.43
110-ARS	0.119	5.83

The growth rate test was carried out in glycerol-yeast-salts liquid medium. As can be seen from the above table, there were no major differences in growth rates between the antibiotic resistant isolates and their parent strain. Therefore, these isolates can be used in soil for studies of growth rates in rhizospheres in comparison with slow growers.

Further, testing of the marked isolates with the fungal inhibitor 'pamaricin' indicated that the fast-growers were slow to develop on solid medium at 100 μg/ml, indicating further selection is necessary.

Preliminary studies in non-sterile soil with 110-ARS indicate this isolate could be successfully recovered and identified on the selection medium at levels of approximately 10^3 cells per gram of soil.

II. Characterization of Rhizobium japonicum isolates from China

Native isolates of R. japonicum from China were evaluated for symbiotic effectiveness on soybean cv. Kent. Thirty-four isolates were inoculated in sterile Leonard jars in triplicate, plants grown for 28 days in the greenhouse, and evaluated for plant dry weight accumulation as compared to uninoculated controls and an effective reference strain (USDA122).

The data of dry weight of soybean shoots clearly showed that the tested strains markedly differed among them in their efficiency to accumulate more plant dry matter. In this respect the strains can be classified into three groups. The first group includes four strains as well as the uninoculated treatment. In this group the dry weight of the plants didn't exceed 2.90 g/4 plants. This represented about 71% of the dry weight of the control strain (USDA122). The second group contains the majority of tested strains. In this group the dry weight of the plants ranged between 3.08 to 3.87 g/4 plants. The fertilized treatment was included in this group and gave a dry weight of 3.08 g/4 plants. It was noticed that any of the strains of this group gave higher dry weight matter as compared to the fertilized treatment. The third group includes the most eleven efficient strains as compared to the other two groups as they accumulated more dry matter ranging from 4.09 up to 4.43 g/4 plants.

Four of the Chinese isolates produced foilar symptoms of Rhizobium induced chlorosis, a specific reaction due to the amount of photo-toxin produced in nodules by certain strains and serogroups of R. japonicum.

Also, seven of the isolates were examined for hydrogenase activity from freshly crushed nodules. Only one isolate (HT1) is classed as Hup^+. This isolate gave very low levels of H_2 evolution compared with the other isolates, and it oxidized some of the supplied H_2 as well.

An in vitro assay for Hup^+ determined chemolithotropic growth using methylene blue indicated that all 90 of the Chinese isolates were Hup^- in comparison to the reference Hup^+ strains USDA122 and 136.

In somatic agglutmation tests against 13 antisera of R. japonicum, 4 of 13 isolates reacted. Two reacted with serogroup 6, one with serogroup 123, and one with the double group 76-123. The lack of reaction of other isolates indicates the possibility of new somatic serogroups present in these Chinese rhizobia.

Report on the second mission of Dr Lucien M. Bordeleau on the establishment of a Microbial Resource Center (MIRCEN) in Senegal for the West African Region

August 23 - September 2, 1983

Purpose of the mission

To meet with Senegalese authorities and with the Director of the Senegalese Microbiological Resource Center (MIRCEN) regarding implementation of the MIRCEN training program, the possible relocation of the MIRCEN to a more convenient place near Dakar, and the MIRCEN's research program development with other MIRCEN's and with the National Academy of Sciences, Washington, D.C.

On my arrival, I have meet with Moctar Touré, DRAAI, Republic of Senegal, at Dakar, to discuss of the implementation of the MIRCEN after signature of the contract with UNESCO. Few papers are still missing with UNESCO (Activities Program & Budget), otherwise everything is in order and funds from Paris/UNESCO Office should be sent soon. We discussed also the location of the MIRCEN de l'A.O. at Bambey and difficulties of having a functional laboratory at CNRA/Bambey. These aspects will be discussed with ISRA's authorities (Institut Sénégalais de la Recherche Agronomique), namely Mamadou Sonko and M. NDOYE. I was urged by Moctar Touré to look at the preparation of two research projects within the MIRCEN program, one dealing with Voandzeia subterranea and addressed to National Research Council, Washington; and the second dealing with demonstration projects on Biological Nitrogen Fixation for plant proteine production at the farmer scale and financed by PNUE, FAO and AGFUND. Most of my mission was at CNRA/Bambey where the MIRCEN Coordinator, Mamadou GUEYE, is located.

The Voandzeia subterranea project was finalized and I sent a copy to the attention of Dr Michael P. Guene, Washington, for immediate consideration by the Committee on Research Grants. The funding of this project is essential to actively initiate the MIRCEN program of West Africa. The PNUE/FAO/AGFUND project was looked after for its final preparation and it should be submitted soon. Again, this project should provide opportunities to establish the logistic for inoculant manufacturation in West Africa, and should provide the support to organize training courses within the MIRCEN's activities. I should have follow up on the development of these projects.

The functioning of the West Africa MIRCEN at CNRA/Bambey is presently difficult. The responsible, Dr Mamadou GUEYE, must travel to ORSTOM/Dakar every week to look after the culture collection and to do the necessary lab manipulation. Quite often, he must assume his own expenses for travelling and housing because of lack of funds to assure him a per diem. After discussion with the Scientific Director of ISRA, Mamadou Sonko, and the Director of "Production Végétale", Mbaye NDOYE, I was told that the Rhizo/lab will be renovated soon and will be functional. The fact that the Soya Program will move to Casamance will liberate some lab space. I was also told that they are improving the electrical system to avoid power failures. Therefore, in a few months, the MIRCEN should have a functional lab at Bambey. As it is right now, microbiological laboratory work is very difficult at Bambey; Ibrahima NDOYE is trying to isolate Rhizobium strains that were used in the soyabean field trial. The room is equipped with a laminar flow cabinet, but there is no ventilation or exhaust for

evacuation of the toxic gases that accumulate on the use of gas burners. After 5-10 minutes work, temperature reaches over 40°C and the air is filled with carbon dioxide, this constitutes a health hazard and must be corrected by installing an air conditioning unit in the room. It is imperative that the microbiological laboratory be remodeled and properly equipped. Otherwise, the MIRCEN program will be seriously compromised. ISRA has two possibilities: (1) to fix the lab at CNRA/Bambey or (2) to finance the functioning of the CDH at Cambéréne and relocate the MIRCEN in these new facilities. Still, the training program cannot be given at either place, and ISRA should negotiate with ENSUT (Dakar University) to assure a close collaboration for the training part of MIRCEN's activities. ENSUT is well equipped for training courses in microbiology, and they offered there collaboration on the MIRCEN's program.

I visited field plots of peanuts and beans at CNRA/Bambey to evaluate the biological nitrogen fixation. Since specific Rhizobium inoculant is not available, these crops are not inoculated and their nodulation depends on the natural population. Plants suffered from lack of water and nodules were scarce on the root system. BNF could certainly be improved.

I was asked to review the manuscript prepared by Dr Mamadou Gueye for his civil service examination, and I prepared an appreciation report to the attention of ISRA's authorities. Although the lack of logistics to establish the West Africa MIRCEN at Bambey, the responsible could start a culture collection by taking advantage of ORSTOM's laboratories at Dakar, quite often travelling at his own expenses from Bambey. From the report, I concluded that Dr Mamadou Gueye is scientifically and intellectually prepared to belong to the community of research professionals.

I prepared a tentative schedule for my future missions, assuming that NAS will pursue this consultancy program for the West Africa MIRCEN. I also assume that this MIRCEN will have a functional laboratory in a few months.

1 - From February 1st to 21st 1984: Follow up on NAS and AGFUND research projects - revision of culture collection - meeting with West African Microbiologists to regionalize the MIRCEN - preparation of the 1st conference of African Association for Biological Nitrogen Fixation - training on manufacturation and quality control of legume inoculant - planning field plots for inoculant trials

2 - September 1984: Site visit of field plots - sampling and strain identification training - follow up on projects - preparation strains catalogue - report of MIRCEN's activities to UNESCO

3 - February 1985: Training course and lecture on BNF for West Africa MIRCEN - follow up on projects - manufacturation and quality control of legume inoculant - meeting with West Afica microbiologists involved with the MIRCEN - site visit of a few laboratories of participating West African Countries.

Lucien M. Bordeleau, Ph.D.
Microbiologist
NAS Consultant for W.A. MIRCEN

September 6, 1983

PROGRAMME MIRCEN

1er JUIN 1983 AU 30 AVRIL 1984

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JUIN 1983 :

- Rapport de la réunion sur la microbiologie
- Etab lissement de la première liste de la collection de culture de Rhizobium et de Mycorhizes.
- Préparation de la production d'un inoculum contrôlé pour Vigna unguiculata CV 58-185 (souche de Rhizobium ORS 407 ; support de tourbe).
- Mise en place d'une expérience sur la cinétique de l'absorption du phosphore par Vigna unguiculata CV 58-185cultivé sur sol stérile inoculé par Rhizobium ORS 407 et Glomus mosseae.
- Discussion sur l'octroi de parcelles d'essais au champ à Nioro (Sénégal) pour les essais de 1984.
- Préparation d'une lettre et d'une fiche technique du MIRCEN en vue de sa régionalisation.
- Finalisation du programme Voandzeia subterranea.

JUILLET 1983 :

- Suivi de l'expérience mise en place en juin 1983.
- Préparation et suivi des parcelles d'essai à Nioro : précédents culturaux.
- Production d'un inoculum contrôlé pour Vigna unguiculata CV. 58-185
- Visite d'une pépinière d'agrumes à Mboro (Sénégal) : contact avec Mademba SY pour l'acquisition de la maîtrise technique de multiplication de la souche de champignon mycorhizien Glomus mosseae.
- Préparation du rapport de titularisation
- Proposition du programme Voandzeia subteranea à l'ISRA et à la NAS.

AOUT 1983 :

- Visite probable de L. BORDELEAU, consultant UNESCO (21 au 29 Août)
- Visite parcelles d'essai à Nioro
- Soutenance rapport de titularisation (entre le 25 et le 29 Août).

SEPTEMBRE 1983 :

- Fin précédents culturaux sur les parcelles d'essai à Nioro
- Collecte échantillons de sols et nodules
- Préparation des cours pour le stage du Water Resources Department (WRD) à Banjul (GAMBIA)
- Préparation de la réunion de l'"African Association for Biological Nitrogen Fixation" : Contact Dr S.O KEYA MIRCEN de Nairobi.

OCTOBRE 1983 :

- Préparation du bulletin "Revue du MIRCEN de l'A.O" (1982), 1.
- Isolement des souches de Rhizobium à partir des échantillons de sols et nodules collectés en semptembre.
- Participation au stage du WRD-Banjul (24 Oct. - 12 Novembre 1983)

NOVEMBRE 1983 :

- Test et fichage des souches de Rhizobiumisolées en Octobre
- Préparation du bulletin "Revue du MIRCEN de l'A.O" (1982) 1.

DECEMBRE 1983 :

- Publication et distribution de "Revue du MIRCEN de l'A.O" (1982), 1
- Préparation de la première réunion pour la régionalisation du MIRCEN.
- Préparation des contacts avec l'Animation Rurale, Département Nioro. (transfert de techniques d'inoculation)

JANVIER 1984 :

- Régionalisation des activités du MIRCEN : première réunion
- Préparation de la liste de la collection de cultures
- Début probable du programme Voandzeia subteranea.

FEVRIER 1984 :

- Publication de la liste des souches
- Préparation du rapport d'activités

MARS 1984 :

- Proposition activités MIRCEN pour 1984 et 1985.

AVRIL 1984 :

- Rapport d'activités MIRCEN pour le 1er contrat UNESCO.

BUDGET MIRCEN - 1983 - 1984

DESIGNATION	en dollars US
Etablissement et entretien de la collection de culture de Rhizobium du MIRCEN de l'Afrique de l'Ouest au laboratoire de microbiologie ORSTOM-DAKAR	
- boîtes de petri jetables	360
- produits chimiques	900
- verrerie	500
- indemnités de déplacement	1 000
2 - Octroi de parcelles d'essai à Nioro pour 1984	
- précédents culturaux (1983)	600
3 - Participation au stage du Water Resources Department à Banjul (GAMBIA)	
- frais de mission (chercheur + chauffeur)	250
4 - Collecte d'échantillons de sols et de nodules (10 jours)	
- frais de mission (chercheur + chauffeur)	200
5 - Matériel et produits de laboratoire	
- Lyophilisation des cultures de Rhizobium	
. cannes de verres (10 kg)	90
. chalumeau à 3 feux	300
- gaz	500
- coton	60
- sacs en plastique (en rouleau)	50
- fer à souder	40
6 - Matériel et Fourniture de bureau	
- papèterie	550
- climatiseur	750
7 - Publication de la fiche technique du MIRCEN	1 500
8 - Carburant	1 500

9 - Affranchissement		50
10 - Rapport d'activité		800
	TOTAL	10 000
Revue du MIRCEN *		1 200

* : La publication de la "Revue du MIRCEN" est financée par l'UNESCO sur un budjet indépendant (voir DRAFT PROJECT DOCUMENT FOR THE ESTABLISHMENT OF A MICROBIOLOGICAL RESOURCES CENTER (MIRCEN) IN SENEGAL FOR THE WEST AFRICAN REGION. AUGUST 4, 1982).

RÉUNION DE SYNTHÈSE SUR L'ÉTABLISSEMENT DU MIRCEN DE L'AFRIQUE DE L'OUEST AU SÉNÉGAL, LE 7 JUIN 1983

Tenue à la Direction Générale de l'ISRA à Dakar sous la présidence de Mamadou SONKO.

Sont présents:

Dr Lucien M. Bordeleau, consultant UNESCO/NAS pour le MIRCEN
Mamadou ND'IAYE, SR/Rhizo, ISRA, CNRA - Bambey
Francis GANRY, SR/Sol N, CNRA - Bambey
P. Léopold SARR, SR/Sol N, CNRA - Bambey
Jacques LARCHER, Programme soja, CNRA - Bambey
Jos. WEY, SR/Rhizo, CNRA - Bambey
Ibrahima N'DOYE, MRST/DRAAI
Pierre VIAUD, ENSUT-DAKAR
Mamadou GUEYE, ISRA-MIRCEN, CNRA - Bambey
Mbaye NDOYE, D/PV, ISRA - Bambey
Abdou Aziz MBAYE, CDH, ISRA - Camberene

La réunion débute à 16 heures et L. Bordeleau propose les points suivants pour discussion, secondé par SONKO:

1 - Faire le point de Mission du Consultant, voir rapport L.B.
2 - Prérequis établis par le MIRCEN
3 - Site le plus favorable pour le MIRCEN
4 - Encadrement scientifique du responsable du MIRCEN
5 - Projets pendants de la NAS, PNUE
6 - Financement complémentaire de l'ISRA
7 - Coordination et collaboration pour les travaux du MIRCEN
8 - Programme de Microbiologie à l'ENSUT
9 - Potentiel des Microbiologistes pour le MIRCEN

Les discussions font ressortir le bien fondé du MIRCEN à Bambey, ainsi que les inconvénients majeurs en ce qui regarde la non-fiabilité du pouvoir électrique, le bris des équipements existants, le manque d'espace pour les cours de formation, le manque d'encadrement scientifique et le manque d'équipements auxiliaires.

Il ressort nettement que Bambey ne convient pas pour le MIRCEN, en fonction des prérequis établis. Le consultant devra faire des recommandations éventuelles à l'ISRA, une fois que le contrat avec l'UNESCO sera paraphé.

Apparemment, l'ISRA pourra assurer le financement complémentaire, directement ou indirectement par des projets financés de l'extérieur. Dans l'immédiat, le MIRCEN a besoin d'une automobile, d'une dactylographe et service de secrétariat et d'une ligne téléphonique.

Différentes opinions sont émises sur la relocalisation du MIRCEN. Il est bien entendu que le MIRCEN doit rester au sein du Ministère de la Recherche scientifique et technique et que l'ISRA en sera l'organisme hôte, ceci pour permettre l'accès aux projets financés par l'étranger et pour assurer la régionalisation des activités en

Afrique de l'Ouest. L'INDR de Thiès ne conviendrait pas car il est sous la tutelle de l'Enseignement Supérieur, les coûts de fonctionnement seraient trop élevés à cause des déplacements et des communications, il n'y a pas de système d'information et il faudra créer un programme de microbiologie. L'ENCR de Bambey manque de moyens financiers pour assurer la marche des installations et aucun équipement valable ne s'y trouve, tout au plus on y trouverait des locaux pour abriter le MIRCEN. L'ENSUT de l'Université de Dakar offre ce qu'il y a de mieux et complet pour toutes les activités du MIRCEN; déjà on y monte un programme de microbiologie des sols et de biotechnologie; il s'y trouve toutes les salles de travaux pratiques bien équipées, un système complet de graphistes et de montage pour publication de documents, un très bon système de préparation documentaire pour la télévision. De plus, l'ENSUT est à réaménager des salles spécifiquement pour la recherche en microbiologie. Une rencontre complémentaire avec le directeur de l'ENSUT, Prof. S. SECK, et le Prof. P. VIAUD a permis de constater l'intérêt de l'ENSUT pour recevoir le MIRCEN. Il existe déjà une étroite collaboration entre l'ORSTOM et l'ENSUT.

Il est de mon opinion que l'ENSUT serait l'endroit idéal pour localiser le MIRCEN de l'A.O. au Sénégal. Tout l'équipement et les requis pour la conservation des souches microbiennes, pour la formation des chercheurs et l'entraînement, la diffusion d'informations, la production d'inoculants. De plus, l'encadrement scientifique du MIRCEN serait assuré. Compte tenu que le MIRCEN doit rester au sein du Ministère de la Recherche scientifique et technique, la localisation du MIRCEN à l'ENSUT demanderait la détachement du responsable du MIRCEN de l'ISRA et une entente avec l'ENSUT. Ce serait la meilleure solution à envisager et que je préconise en premier lieu.

Une deuxième solution est la localisation du MIRCEN au Centre de Développement Horticole (CDH) de l'ISRA à Cambérène. Physiquement, ce centre offre presque tout l'équipement majeur pour la microbiologie, à l'exception du petit équipement de laboratoire. Ce CDH est neuf et spacieux et offre certainement un grand potentiel pour le développemenmt du MIRCEN. Cependant, certains éléments sont défavorables au CDH pour abriter le MIRCEN, entre autres: le volet formation et entraînement de personnel n'est pas possible avec l'aménagement actuel, il n'y a pas de logements à proximité, pas de restauration et pas de transport en commun. Ce dernier élément est particulièrement un problème, étant donné que le responsable du MIRCEN aura à se déplacer pour aller vers l'ORSTOM et l'ENSUT pour l'encadrement scientifique. Cependant, une entente pourrait être prise avec l'ENSUT pour assurer le volet formation des activités du MIRCEN et pour aider dans la préparation de matériel de diffusion technique et de vulgarisation. C'est une deuxième solution à envisager.

Dans un cas (MIRCEN à l'ENSUT) comme dans l'autre (MIRCEN au CDH avec collaboration de l'ENSUT), il va de soit que les volets forestier au CNRF et agricole à Bambey doivent être assurés par une collaboration étroite des institutions. L'avantage de la collaboration de l'ENSUT réside dans le fait que cette institution développe un programme en microbiologie et en biotechnologie, notamment sur le bio-gaz et les fermentations, ce qui encadre très bien le MIRCEN dans ses activités futures. Rappelons qu'une collaboration très étroite existe entre l'ORSTOM et l'ENSUT, et que le MIRSEN renforcera la collaboration entre l'ENSUT et l'ISRA et ce pour le bénéfice des deux parties et du peuple sénégalais, puisque la théorie et la pratique scientifiques se marieront bien.

Le professeur P. VIAUD de l'ENSUT a fait état du programme de formation en Microbiologie (Protection des Végétaux et Génie Sanitaire), et de l'accord de coopération avec l'ORSTOM, de même que l'installation d'une unité microbiologique à l'ENSUT. Le sérieux de cette opération mérite une attention particulière en ce qui regarde la localisation définitive du MIRCEN et la collaboration quasi essentielle de l'ENSUT.

Il a été discuté brièvement des deux propositions de recherche pour soumettre à la National Academy of Sciences (Washington), l'une en foresterie, l'autre en agriculture. Ces deux projets sont en phase finale de préparation et seront soumis bientôt pour approbation ministérielle avant leur envoi à Washington. Le projet de la PNUE sera préparé en différents volets, un volet sera préparé par le CNRA de Bambey avec la collaboration de Ibrahima N'DOYE (MRST/DRAAI).

Une liste partielle de microbiologistes sénégalais formés ou en formation a pu être constituée. Cette liste permettra de maintenir des contacts plus étroits en vue du développement de la microbiologie au Sénégal et en Afrique de l'Ouest. La liste est attachée au présent rapport.

Préparé par

Dr Lucien M. Bordeleau
Consultant UNESCO/NAS

8 juin 1983

MICROBIOLOGISTE SÉNÉGALAIS

Mamadou GUEYE	Dr 3e cycle	ISRA, responsable du MIRCEN, Bambey
Mamadou ND'IAYE	DEA	ISRA, SR/Rhizo, CNRA, Bambey
Ibrahima N'DOYE	DEA	MRST/DRAAI, Dakar
Abdrelaye DIACK	Ing. Agronome, avec Stage de Rhizobiologie à Gembloux	Chômeur
Emanuel TINE	Dr 3e cycle	ENSUT, Dakar, pédologie, micro.
Alioune DIAGNE	Dr 3e cycle	SODEP, agropédologie
Ousmane DIAGNE	DEA	ORSTOM, Bel Air, Dakar
Bassirou SOUGOUFARA	DEA (en préparation)	ORSTOM, Bel Air, Eau et Forest.
Pape Leopold SARR	Dr 3e cycle	ISRA, Bambey, bio-gaz
Henry DIARRA	Agronome (Azolla-Sesbania)	ADRAO, Richard Toll.

APPRECIATION DU RAPPORT DE STAGE DE TITULARISATION DE MAMADOU GUEYE ISRA/CNRA-BAMBEY

Après lecture d'une copie manuscrite qui m'a été remise, je crois que ce rapport illustre bien le travail du candidat, au cours des mois d'essais, dans ses responsabilités envers le MIRCEN au sein de l'ISRA. Compte tenue des difficultés à mettre sur pied une nouvelle structure qu'est le MIRCEN de l'Afrique de l'Ouest, le candidat a bien oeuvré entre le CNRA de Bambey et le laboratoire de l'ORSTOM à Dakar. Il s'est bien acquitté de sa première tâche qui était de mettre en plan une collecte de cultures microbiennes, et d'un système de fiches techniques de la collection.

Le fond de chacun des trois sujets présentés dans ce rapport me semble logique. Il m'est difficile d'apprécier à sa juste valeur ce rapport de stage, car je n'ai pas en main une copie finale. Mais à la lecture du texte présenté, j'en conclus que le candidat démontre une maturité intellectuelle suffisante et une honnêteté scientifique digne d'un professionnel de la recherche en microbiologie ; il se gagne ainsi le respect du milieu scientifique/

Bambey, le 30 Août 1983

Dr Lucien M. BORDELEAU
Microbiologiste

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Rhizobium japonicum Serogroup and Hydrogenase Phenotype Distribution in 12 States

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A survey was conducted in 1980 on 972 isolates of *Rhizobium japonicum* obtained from 65 soybean field locations in 12 states. Isolates were examined for the hydrogenase (Hup) phenotype and somatic serogroup identity. Only 20% of the isolates were Hup$^+$, with a majority of Hup$^-$ isolates occurring in 10 of the 12 states. The most predominant serogroup was 31 (21.5%), followed by 123 (13.6%). Although most serogroups contained a majority of Hup$^-$ isolates, marked differences occurred. None of the isolates in serogroup 135 were Hup$^+$, but 93% of the isolates in serogroup 122 were Hup$^+$. The serogroups with relatively high frequencies of Hup$^+$ isolates (122 and 110) constitute only a small part (<5% each) of the *R. japonicum* field population in the 12 states.

ne presence of an active hydrogen uptake (Hup) system 'hizobium japonicum may be a desirable characteristic in energy-efficient, nitrogen-fixing symbiosis with soy- ıs (*Glycine max* L. Merr.) (1, 16, 19, 25). In 1979, a large ey of *R. japonicum* isolates from 28 states which make he major U.S. soybean production area showed that a ority (>75%) were lacking the Hup system (18).

1980, a second survey was conducted in those 12 states h in the 1979 survey had the highest percentage of Hup tes. The purpose of the second survey was to confirm low percentage of Hup$^+$ isolates in those states, to logically identify the isolates, and to determine any ciation between the Hup phenotype and serogroups of *ıponicum*. The Hup phenotype distribution in the 1979 1980 surveys was reported elsewhere (22); this paper presents the results of the 1980 survey regarding serogroup distribution and association between serogroups and the hydrogenase phenotype.

MATERIALS AND METHODS

Nodule collection and isolation of *R. japonicum*. In the 1980 growing season soybean nodules were collected randomly from commerical fields at various locations by cooperators in the following states: Arkansas, Delaware, Florida, Kansas, Louisiana, Minnesota, Mississippi, New Jersey, North Carolina, Pennsylvania, South Carolina, and South Dakota. The procedure used for the isolation of *R. japonicum* from nodules is described elsewhere (22). A total of 972 isolates were obtained from 323 nodules from 65 locations in the 12 states.

Hydrogenase determination. Single-colony isolates were selected, and free-living cultures were tested for hydrogenase activity as described by Lim (17). These cloned isolates were sent to our laboratory in Beltsville, Md., for serological testing.

Serogroup determination. Isolates were grown in yeast-salts-mannitol broth (23) to a density of 10^8 to 10^9 cells per ml and heated at 100°C for 30 min to destroy the heat-labile flagellar antigens. Rabbit antisera prepared against 15 different serotype strains of *R. japonicum* were used in agglutination tests at a 1/100 final concentration. The short agglutination test (23) was performed, and each isolate was tested against all antisera.

RESULTS

The overall frequency of *R. japonicum* serogroups and the percentage of Hup$^+$ isolates within each serogroup are summarized in Table 1. Serogroups representing less than 1% of the isolates were not listed, whereas the nine serogroups listed in Table 1 accounted for 78.3% of the 972 isolates. The remainder were either unreactive with any antiserum (9.8%) or reactive with more than one antiserum. Only 20.1% of all isolates were Hup$^+$. Also, 10.8% of the nodules contained different isolates representing more than one serogroup, and 9.3% of the nodules contained isolates within the same serogroup but with different Hup phenotypes. Serogroup 31 was recovered with the highest frequency, containing only 11% Hup$^+$ isolates. Serogroups 135 and 122 had the most contrasting Hup$^+$ representation, 0 and 92.7%, respectively.

Table 2 summarizes the occurrence by state of *R. japonicum* serogroups and respective Hup phenotypes. Serogroup 31 was predominant in five states (Arkansas, Delaware, Florida, Kansas, and Louisiana) and was well represented in two other states (North Carolina and Mississippi). Serogroups 123, 76, and 46 were also well represented in several states. South Dakota had a high occurrence of isolates in serogroup 135.

DISCUSSION

Of the 12 states surveyed, 10 contained *R. japonicum* isolates which were at least 75% Hup$^-$. This result is similar to that of the previous survey from 28 states (22). The comparatively low percentage of Hup$^-$ isolates from New Jersey may be a reflection of the low number of locations and nodules sampled (Table 2) or of samples taken from areas of recent soybean plantings that were inoculated with an inoculum containing Hup$^+$ rhizobia.

Isolates belonging to serogroup 31 occurred with the

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TABLE 1. Serogroup and hydrogenase phenotype frequencies of *R. japonicum* isolates in 1980 survey

Serogroup[a]	No. of isolates	% of total	% Hup⁺
31[b]	209	21.5	11.0
123	132	13.6	32.6
76	99	10.2	21.2
46[b]	84	8.6	9.5
6[b]	74	7.6	12.2
135	59	6.1	0.0
94	49	5.0	20.4
122	41	4.2	92.7
110	15	1.5	40.0
Unknown	95	9.8	6.3

[a] The number indicates the USDA strain of *R. japonicum* against which antiserum was prepared.

[b] Serotype strains 6, 46, and 31 are also referenced as belonging in the composite serogroups e1, e2, and e3, respectively (5). These serogroups contain many strains with similar serological properties, and in this paper we use the serotype strain designation for the serogroup.

greatest frequency in this survey (Table 1). Previous surveys of *R. japonicum* serogroups in the United States also showed serogroup 31 to be frequent in certain states (3, 5, 12). Serogroup 123 has been reported to be a major component of indigenous *R. japonicum* in Iowa, Illinois, Ohio, and Minnesota (8, 11, 13, 15, 20, 21), and our data show that this serogroup is found in other regions as well. Serogroup 76 was the third most frequent, and its relatively high representation in Mississippi, North Carolina, and Florida confirms an earlier report of its prevalence in southeastern states (3).

Although most serogroups contained a small portion of Hup⁻ isolates, none of the isolates in serogroup 135 were Hup⁻, but 92.7% of serogroup 122 isolates were Hup⁻. All USDA *Rhizobium* Culture Collection strains in serogroup 122 that have been tested (USDA 122, USDA 136, USDA 142, and USDA 143) are also Hup⁻ (1, 6, 14, 16, 17). Although the serogroup 122 survey isolates are undoubtedly a heterogeneous group for any number of microbiological and symbiotic characteristics, almost all share the Hup⁻ phenotype.

The existence of different Hup phenotypes in most serogroups is not surprising, as variation for several characteristics within a given serogroup is known (10). Vincent (24) cautions against associating serogroup affinity with any other property, any such relationship being incomplete or no better than chance. However, certain symbiotic characteristics of *R. japonicum* strains do appear to be associated with certain serogroups, such as the ineffective N_2-fixing symbiosis formed by strains in serogroup 122 and most strains in the e1 serogroup with *G. max* cv. Hardee (2) and the ability of strains in serogroups 76 and 94 to nodulate the rj_1rj_1 soybean genotypes (so-called "nonnodulating") (9). The contrasting frequencies of the Hup⁻ phenotype in serogroups 135 and 122 may indicate an association between these two characteristics, but more extensive research may be needed to confirm this observation.

Information provided by cooperators in each state enabled us to identify 75.2% of the isolates to the cultivar (16 altogether) from which they were obtained. Although the soybean genotype is known to influence serogroup recovery in nodules (4), such a correlation was not possible in this survey due to widely varying representation. However, no particular serogroup-cultivar trend was seen, and *G. max* cv. Williams, which occurs on more soybean acreage than any

TABLE 2. Summary of *R. japonicum* serogroup and hydrogenase phenotype distribution by state

State[a]	No. of isolates of each Hup phenotype in serogroup:																								No. of sample locations	No. of nodules	No. of isolates	% of isolates HUP	Predominant serogroups
	6		46		31		76		94		110		122		123		135												
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−											
AK	5		1	31	2	56		9		9		5		9		10		4							18	66	164	75.0	31, 46
DE		5			4	12	9		5		1		3		3		11								5	28	81	75.3	31, 123
FL					1	21		12							12										3	17	57	77.2	31, 76
KS			3		9	15							4		8		7								4	21	67	49.3	31, 123
LA			1	13	1	25	3		3										22						5	26	86	87.2	31, 135
MN		4		3		6									3		17	25							4	20	69	[illegible]	123
MS	4	5		7	2	21	2	22	1						2			9							6	35	102	90.2	76, 31
NJ		3				3	4								3		9	5							2	9	27	40.7	123
NC		19	1	9	5	17	2	18	1	14								8							6	31	104	89.4	31, 76, 6
SC		14	2	6	1	9	1	7			1							14							5	29	93	86.0	6, 123
PA		13						8		23				3											3	18	60	90.0	94
SD		2		7		1		2								6			37						4	23	62	100	135

[a] AK, Arkansas; DE, Delaware; FL, Florida; KS, Kansas; LA, Louisiana; MN, Minnesota; MS, Mississippi; NJ, New Jersey; NC, North Carolina; SC, South Carolina; PA, Pennsylvania; SD, South Dakota.

other cultivar (7), yielded isolates from 11 different serogroups.

The possession of a hydrogenase uptake enzyme in *R. japonicum* does increase the efficiency of N_2 fixation, with a concomitant increase in plant weight and nitrogen content (1, 16, 25). Most of the *R. japonicum* population in field soils is Hup^-, and the serogroups with a relatively high frequency of Hup^+ isolates (122 and 110) make up only a small part of that population. Recently, Merberg and Maier (19) reported *R. japonicum* mutants, derived from 122DES, a small-colony isolate of USDA 122, with hydrogenase activity higher than that of their parent cultures. If such mutants can be shown at the field level to improve N_2 fixation, yield, or N content in soybeans, they would be valuable as candidates for commercial inocula. Their introduction and competitive establishment would be a worthwhile challenge for microbiologists studying the ecology of *R. japonicum*.

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LITERATURE CITED

1. **Albrecht, S. L., R. J. Maier, F. J. Hanus, S. A. Russell, D. W. Emerich, and H. J. Evans.** 1979. Hydrogenase in Rhizobium japonicum increases nitrogen fixation by nodulated soybeans. Science **203**:1255–1257.
2. **Caldwell, B. E.** 1966. Inheritance of a strain-specific ineffective nodulation in soybeans. Crop Sci. **6**:427–428.
3. **Caldwell, B. E., and E. E. Hartwig.** 1970. Serological distribution of soybean root nodule bacteria in soils of southeastern U.S.A. Agron. J. **62**:621–622.
4. **Caldwell, B. E., and G. Vest.** 1968. Nodulation interactions between soybean genotypes and serogroups of Rhizobium japonicum. Crop Sci. **8**:680–682.
5. **Caldwell, B. E., and D. F. Weber.** 1970. Distribution of Rhizobium japonicum serogroups in soybean nodules as affected by planting dates. Agron. J. **62**:12–14.
6. **Carter, K. R., N. T. Jennings, J. Hanus, and H. J. Evans.** 1977. Hydrogen evolution and uptake by nodules inoculated with different strains of Rhizobium japonicum. Can. J. Microbiol. **24**:307–311.
7. **Crop Reporting Board, Statistical Reporting Service, U.S. Department of Agriculture.** 1980. Crop production. CrPt 2-2. November U.S. Department of Agriculture, Washington, D.C.
8. **Damirgi, S. M., L. R. Frederick, and I. C. Anderson.** 1967. Serogroups of Rhizobium japonicum in soybean nodules as affected by soil types. Agron. J. **59**:10–12.
9. **Devine, T. E., and D. F. Weber.** 1977. Genetic specificity of nodulation. Euphytica **26**:527–535.
10. **Gibson, A. H., W. F. Dudman, R. W. Weaver, J. C. Horton, and I. C. Anderson.** 1971. Variation within serogroup 123 of Rhizobium japonicum. Plant Soil, spec. vol., p. 33–37.
11. **Ham, G. E., L. R. Frederick, and I. C. Anderson.** 1971. Serogroups of Rhizobium japonicum in soybean nodules sampled in Iowa. Agron. J. **63**:69–72.
12. **Johnson, H. W., and U. M. Means.** 1963. Serological groups of Rhizobium japonicum recovered from nodules of soybeans (Glycine max) in field soils. Agron. J. **55**:269–271.
13. **Kapusta, G., and D. L. Rouwenhorst.** 1973. Influence of inoculum size on Rhizobium japonicum serogroup distribution frequency in soybean nodules. Agron. J. **65**:916–919.
14. **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 atropurpureum. Plant Physiol. **70**:1626–1630.
15. **Kvien, C. S., G. E. Ham, and J. W. Lambert.** 1981. Recovery of introduced Rhizobium japonicum strains by soybean genotypes. Agron. J. **73**:900–905.
16. **Lepo, J. E., R. E. Hickok, M. A. Cantrell, S. A. Russell, and H. J. Evans.** 1981. Revertible hydrogen uptake-deficient mutants of *Rhizobium japonicum*. J. Bacteriol. **146**:614–620.
17. **Lim, S. T.** 1978. Determination of hydrogenase in free-living cultures of Rhizobium japonicum and energy efficiency of soybean nodules. Plant Physiol. **62**:609–611.
18. **Lim, S. T., S. L. Uratsu, D. F. Weber, and H. H. Keyser.** 1981. Hydrogen uptake (hydrogenase) activity of Rhizobium Japonicum strains forming nodules in soybean production areas of the U.S.A., p. 159–171. *In* J. M. Lyons, R. C. Valentine, D. A. Phillips, D. W. Rains, and R. C. Huffaker (ed.), Genetic engineering of symbiotic nitrogen fixation. Plenum Publishing Corp., New York.
19. **Merberg, D., and R. J. Maier.** 1983. Mutants of Rhizobium japonicum with increased hydrogenase activity. Science **220**:1064–1065.
20. **Miller, R. H.** 1979. Field survey of the distribution of Rhizobium japonicum serogroup in Ohio, p. 2. *In* R. W. Weaver and D. A. Zuberer (ed.), Proceedings of the 7th North American Rhizobium Conference. College Station, Tex.
21. **Reyes, V. G., and E. L. Schmidt.** 1979. Population densities of *Rhizobium japonicum* strain 123 estimated directly in soil and rhizospheres. Appl. Environ. Microbiol. **37**:854–858.
22. **Uratsu, S. L., H. H. Keyser, D. F. Weber, and S. T. Lim.** 1982. Hydrogen uptake (Hup) activity of Rhizobium japonicum from major U.S. soybean production areas. Crop Sci. **22**:600–602.
23. **Vincent, J. M.** 1970. A manual for the practical study of root-nodule bacteria. IBP handbook no. 15. Blackwell Scientific Publications, Oxford.
24. **Vincent, J. M.** 1982. Serology, p. 235–273. *In* W. J. Broughton (ed.), Nitrogen fixation, vol. 2: Rhizobium. Oxford University Press, New York.
25. **Zablotowicz, R. M., S. A. Russell, and H. J. Evans.** 1980. Effect of hydrogenase system in Rhizobium japonicum on the nitrogen fixation and growth of soybeans at different stages of development. Agron. J. **72**:555–559.

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Biochemical Characterization of Fast- and Slow-Growing Rhizobia That Nodulate Soybeans

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Fast-growing, acid-producing soybean rhizobia were examined to determine their biochemical relatedness to each other, to typical slow-growing *Rhizobium japonicum* strains, and to other fast-growing species of *Rhizobium*. Although both the fast- and slow-growing soybean rhizobia were positive for catalase, urease, oxidase, nitrate reductase, and penicillinase, the fast-growing strains grouped with other fast-growing species of *Rhizobium* in that they tolerated 2% NaCl, were capable of growth at pH 9.5, utilized a large variety of carbohydrates (notably disaccharides), and produced serum zones in litmus milk. In addition, these fast-growing strains were similar to other fast-growing species of *Rhizobium* in that they produced appreciable levels of β-galactosidase and nicotinamide adenine dinucleotide phosphate-linked 6-phosphogluconate dehydrogenase but had no detectable hydrogenase activity. The fast-growing soybean rhizobia share symbiotic host specificity with *Bradyrhizobium japonicum*, but appear to be related biochemically to the other fast-growing species of *Rhizobium*.

Members of the genus *Rhizobium* are symbiotic nitrogen-fixing bacteria which are able to invade and form nodules on the roots of leguminous plants. The species in this genus have been divided into two groups (17, 35) on the basis of growth rate and effect on the pH of yeast extract-mannitol (YEM) medium under standard laboratory conditions. The fast-growing rhizobia have mean generation times of 2 to 4 h and produce a net decrease in the pH of YEM culture medium, whereas the rhizobia referred to as slow growing have mean generation times of 6 h and longer and do not lower the pH of this medium (35).

In the past, the typical slow growers that form nodules on the roots of soybeans (*Glycine max*) have been referred to as *Rhizobium japonicum* (17). Recently, these rhizobia have been classified in a new genus, *Bradyrhizobium*, on the basis of their slow growth rate and other characteristics (16) to distinguish them from the fast-growing, acid-producing root nodule bacteria which now comprise the genus *Rhizobium* (15).

Recently, Keyser et al. (20) reported the isolation of fast-growing soybean rhizobia from root nodules and soil collected in the provinces of Shansi, Honan, Shandong, and Shanghai, Peoples' Republic of China (PRC). These isolates reportedly (20) have mean doubling times of 2 to 4 h and lower the pH of YEM culture medium (final pH range, 4.7 to 6.7). All strains tested were reported to form effective nitrogen-fixing nodules on wild perennial soybeans (*Glyine soja*) and on an unbred soybean cultivar from the PRC (*G. max* cv. Peking), but formed ineffective symbioses with most commercial soybean cultivars (20). In this study we used a series of diagnostic tests to examine the fast-growing, acid-producing PRC isolates for their relatedness to each other, to typical slow-growing *R. japonicum* strains, and to other fast-growing rhizobia.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Fast-growing soybean *Rhizobium* strains USDA 191, USDA 192, USDA 193, USDA 194, USDA 201, USDA 205, USDA 206, USDA 208, USDA 214, USDA 217, and USDA 257 were isolated from soil or nodules collected in the PRC (20). Slow-growing soybean rhizobium strains PRC 005, PRC 74, PRC 113-2, PRC 121-6, PRC 2031, and PRC B15, were obtained from T. S. Hu, Institute of Soils and Fertilizers, Chinese Academy of Agricultural Sciences, Beijing, PRC. *Bradyrhizobium japonicum* strains USDA 6, USDA 31, USDA 34, USDA 74, USDA 94, USDA 110, USDA 122, USDA 123, USDA 136, USDA 138, USDA 142, and Y1, Y1a, Y2, Y2a, Y3, K2, K2a, S1, and S1a, were from the United States Department of Agriculture Culture Collection, Beitsville, Md. *Rhizobium lupini* CC814s and NZP 2037, *Rhizobium leguminosarum* Nitragin 92A3, *Rhizobium phaseoli* NZP 5097, and *Rhizobium trifolii* NZP 560 were obtained from R. M. Greenwood, Department of Scientific and Industrial Research, Palmerston North, New Zealand. *R. leguminosarum* HI 5-0 was isolated in Hawaii (30). *R. leguminosarum* 6015(pJB5JI) was obtained from P. Hirsch, Max Planck Institut, Köln, Federal Republic of Germany; *R. phaseoli* Bel 7.1 was obtained from E. L. Schmidt, University of Minnesota, Minneapolis; *Rhizobium* sp.

strain Tal-82 (which nodulates *Leucaena*) was obtained from the Niftal Project, Paia, Hawaii; and *Rhizobium* sp. strain UMKL 19 (which nodulates *Leucaena*) from W. J. Broughton, Max Planck Institut, Köln, Federal Republic of Germany. *Agrobacterium tumefaciens* 79 and 101 and *Rhizobium* sp. strain USDA 3F4a4 (which nodulates *Sesbania*) were from the U.S. Department of Agriculture, Beltsville, Md. All *Rhizobium* cultures were maintained on YEM agar slants (34). The agar slants used for maintenance of fast-growing rhizobia contained 0.05% $CaCO_3$. *Agrobacterium* cultures were maintained on nutrient agar slants. All cultures were incubated at 28 to 30°C, stored at 4°C, and subcultured at least once every month.

Staining, morphology, and cultural characteristics. Cultures were examined for cell morphology and Gram reaction after 3 days of growth in YEM liquid medium. Colony morphology was examined by using cultures that were grown for 6 days on YEM agar containing 0.25 mg of bromthymol blue per liter. Motility was estimated from both YEM liquid and YEM agar cultures and in B5 medium (9). Isolates were identified by using strain-specific fluorescent antibodies prepared by the method of Schmidt et al. (32).

Biochemical tests. Tolerance to pH extremes was determined by inoculating 10^7 cells per ml from exponentially growing YEM liquid cultures into tubes containing 10-ml portions of YEM liquid media which were adjusted to pH 4.5, 9.0, and 9.5. The tubes were incubated at 30°C for 14 days and scored for growth. Tests were performed in triplicate.

Tolerance to sodium chloride was determined on YEM agar plates containing 2.0% NaCl. The plates were inoculated with 10^8 cells, and the growth was scored after 14 days at 30°C. Tests were done in triplicate.

For growth reactions in litmus milk (Difco Laboratories, Detroit, Mich.), tubes (10 ml/tube) were incubated in quadruplicate for 6 weeks at 30°C and examined for pH changes, reduction of litmus, and peptonization (serum zone formation).

Production of 3-ketolactose was determined by the method of Bernaerts and De Ley (1). *A. tumefaciens* was used as a positive control for this test.

To examine gelatinase activity, exponential-phase cultures from YEM liquid medium were swabbed onto the surfaces of tryptone-yeast extract agar plates (12) containing 0.4% (wt/vol) gelatin (Difco). The plates were incubated at 28°C for 7 days. A positive reaction was indicated by a clearing zone surrounding the growth of the organism.

The pH reactions of isolates on agar plates were determined by using YEM medium containing 0.25 mg of bromthymol blue per liter.

Urease activity was determined on urea agar slants (4) that were incubated for 7 days at 28°C.

Citrate utilization was determined on the solid medium of Koser (22). Plates were inoculated with 10^8 cells, incubated at 30°C for 14 days, and examined for growth.

Penicillinase (β-lactamase) was detected by the method of Foley and Perret (6).

Oxidase was determined by using the method of Kovaks (23); catalase was determined by the method of Graham and Parker (11).

Hydrogen sulfide production was determined on agar slants (13). The slants were inoculated and examined for H_2S after incubation for 14 days at 28°C.

Nitrate reduction was tested as described in the *Manual of Methods for General Bacteriology* (33) by using the medium of Graham and Parker (11).

For carbohydrate utilization tests, the basal medium used was that of Bishop et al. (2); different carbohydrates were substituted for mannitol, and KNO_3 (0.6 g/liter) was used as the nitrogen source. The medium was solidified with purified agar (Difco). All carbohydrates except dextrin and starch were filter sterilized (pore size, 0.4 μm; Nuclepore Corp., Pleasanton, Calif.) before they were added to cooled, melted agar medium. Dextrin and starch were added to the medium before it was sterilized at 100°C for 15 min. Each carbohydrate was added to a final concentration of 1.0% (wt/vol). Inocula were prepared by removing cells from YEM agar slants with a cotton swab and suspending the cells to a density of approximately 10^7 cells per ml in sterile distilled water. A 10-fold dilution of each cell suspension was added to the wells of a multiple inoculator plate (18) and inoculated onto the surfaces of carbohydrate-containing agar plates. Bishop agar plates without carbohydrate served as controls. Duplicate plates of each carbohydrate were incubated at 28°C for 7 days and scored for growth.

Enzyme assays. 6-Phosphogluconate dehydrogenase (6 PGD) activity was determined in cultures that were grown for 72 h at 26°C in yeast extract-glucose medium (19). The cultures were centrifuged at 6,000 × *g* for 10 min at 4°C, and the cells were washed twice in 0.05 M sodium phosphate buffer (pH 7.4). The cell pellets were suspended in the same buffer containing 2×10^{-4} M 2-mercaptoethanol (27) to a final concentration of 1.0 g (wet weight) of cells per 1.5 ml of buffer and then disrupted by two passages through a French pressure cell at 15,000 16 lb/in^2. The cell debris was removed by centrifugation at 14,000 × *g* for 30 min at 4°C. The clear supernatant was stored at −20°C until it was used. The activity of nicotinamide adenine dinucleotide phosphate (NADP)-linked 6PGD (EC 1.1.1.43) was measured by following the reduction of NADP, using the method of Martinez-de Drets and Arias (28). The apparent endogenous enzyme activity was subtracted from the results. Specific activities were expressed as nanomoles of reduced NADP formed per minute per milligram of protein at 25°C. Protein was determined by the method of Lowry et al. (26), using bovine serum albumin as the standard.

β-Galactosidase activity was determined in cultures that were grown for 3 days at 26°C in tryptone-yeast extract medium containing 0.5% (wt/vol) lactose. The cells were centrifuged and washed as described above and then suspended in 0.05 M sodium phosphate buffer (pH 7.2) to a final concentration of 1.0 g (wet weight) per 3.0 ml of buffer and broken by passage through a French pressure cell at 15,000 lb/in^2. The cell debris was removed as described above, and the supernatant fractions were stored at −20°C until they were used. Enzyme activity was measured by following the appearance of *o*-nitrophenol at 420 nm. The incubation mixture (4.8 ml) contained 2.7 ml of enzyme reaction buffer (0.1 M sodium phosphate buffer [pH 7.0], 10^{-3} M $MgSO_4 \cdot 7H_2O$, 2×10^{-4} M $MnSO_4$, 0.05 M 2-mercaptoethanol), 1.8 ml of an *o*-nitrophenyl-β-D-galactopyranoside solution (0.1 M sodium phosphate

buffer [pH 7.0], 1.3×10^{-2} M *o*-nitrophenyl-β-D-galactopyranoside [Sigma Chemical Co., St. Louis, Mo.]), and 0.3 ml of cell-free extract. Enzyme assays were carried out at 37°C, and the reactions were stopped by adding 1.3 ml of reaction stop buffer (8.0 M urea, 1.0 M Na_2CO_3, pH 12.0). Enzyme activities were expressed as micromoles of *o*-nitrophenol produced per minute per milligram of protein at 37°C. Corrections were made for absorbance values obtained in controls without substrate.

Hydrogen evolution and the hydrogenase (hydrogen uptake) activities of the fast-growing strains were measured in the bacteroid state as described by Keyser et al. (21). Soybean plants (*G. max* cv. Peking) were inoculated with cultures of rhizobia in Leonard jar containers (24) and grown in a plant growth chamber. Plant root nodules were harvested after 28 days. All treatments were sampled during peak nitrogenase activity (range 19 to 32 mol of C_2H_2 reduced per g [fresh weight] of nodules per h). Hydrogen evolution was expressed as micromoles of H_2 evolved per gram (fresh weight) of nodules per hour. Hydrogenase activity was expressed as micromoles of H_2 oxidized per gram (fresh weight) of nodules per hour.

RESULTS

Morphological and cultural characteristics. Both the fast- and slow-growing soybean rhizobia were gram-negative, nonsporeforming, rod-shaped bacteria. The cells from late-log-phase to stationary-phase YEM cultures of fast growers tended to become enlarged and exhibited marked pleomorphism. Only 1 to 5% of the cells of the fast-growing soybean rhizobia were motile in young (1- to 2-day-old) YEM cultures, whereas 80 to 90% of the slow-growing rhizobia were motile. However, when the organisms were grown on a moist B5 agar medium surface (9), a greater percentage (up to 25%) of the fast-growing soybean rhizobia were motile. On YEM agar plates containing bromthymol blue, both fast- and slow-growing rhizobia formed circular, convex, colonies. After 6 to 7 days, the colonies of the fast growers were between 1.0 and 5.0 mm in diameter and produced acid reactions, whereas the colonies of the slow growers were approximately 0.5 to 1.0 mm in diameter and produced alkaline reactions. In contrast to a large number of other fast-growing rhizobia, the fast-growing soybean strains did not a produce much extracellular polysaccharide on YEM agar. Two of the strains (USDA 191 and USDA 192) produced watery colonies. Upon repeated restreaking, some of the fast growers produced a second type of colonies which were somewhat smaller than the colonies produced by the parent cultures. These colonial variants were not always stable. Several of the variants that did appear to be stable were isolated from seven strains of fast growers (USDA 191, USDA 192, USDA 193, USDA 208, USDA 214, USDA 217, and USDA 257) and tested for serological affinity by using strain-specific fluorescent antibodies and for effectiveness on soybeans (*G. max* cv. Peking). These variants were found to be identical to the parental strains.

Growth responses. Both fast- and slow-growing soybean rhizobia were examined for the ability to grow in the presence of 2.0% NaCl and at pH extremes of 4.5 and 9.5. As Table 1 shows, the fast growers were uniformly sensitive to the low pH and tolerant of the high pH, whereas the reverse was found for the slow growers. The slow growers grew poorly at pH 9.0 and not at all at pH 9.5, whereas the fast growers grew well at pH 9.5. Conversely, the slow growers grew well at pH 4.5, whereas the fast growers did not grow at this pH. The fast- and slow-growing rhizobia also demonstrated a clear difference in tolerance to 2% NaCl. Although all of the fast-growing isolates examined were able to grow in the presence of this salt, there was variation among the strains in the extent of growth. A few strains (USDA 193, USDA 194, USDA 201, and USDA 257) produced a confluent lawn of growth on the medium, whereas others (USDA 191, USDA 192, USDA 205, USDA 206, USDA

TABLE 1. Biochemical characteristics of fast- and slow-growing soybean rhizobia

Characteristics	% of isolates giving positive reaction[a]	
	Slow-growing rhizobia	Fast-growing rhizobia
Catalase production	100	100
Oxidase production	100	100
Urease production	100	100
Penicillinase production	71	100
Nitrate reduction	100	100
3-Ketolactose production[b]	0	0
H_2S production	0	0
Citrate utilization[b]	0	0
Gelatinase production[b]	0	100
2% NaCl tolerance[c]	0	100
pH 4.5 tolerance[c]	100	0
pH 9.0 tolerance[c]	0	100
pH 9.5 tolerance[c]	0	100

[a] Unless otherwise indicated below, seven slow-growing strains (PRC 005, PRC 113-2, PRC 121-6, PRC 2031, PRC B15, USDA 110, and USDA 136) and eight fast-growing strains (USDA 192, USDA 193, USDA 194, USDA 201, USDA 205, USDA 206, USDA 208, and USDA 214) of soybean rhizobia were examined.

[b] These tests also included fast-growing soybean rhizobium strains USDA 191, USDA 217, and USDA 257.

[c] Fast-growing soybean rhizobium strains USDA 191, USDA 192, USDA 193, USDA 194, USDA 201, USDA 205, USDA 206, USDA 208, USDA 214, USDA 217, and USDA 257 and slow-growing soybean rhizobium strains USDA 34, USDA 110, USDA 122, and USDA 138 were examined.

208, USDA 214, USDA 217) yielded individually tolerant colonies. In contrast, none of the slow-growing *R. japonicum* strains grew in 2% NaCl.

Biochemical attributes. Table 1 shows that both the fast- and slow-growing rhizobia were catalase, oxidase, and urease positive. All of the strains examined except slow-growing strains PRC 2031 and PRC B15 produced penicillinase, and all strains reduced nitrate. None of the strains produced 3-ketolactose from lactose, produced hydrogen sulfide, or utilized citrate as a sole source of carbon.

The two groups exhibited a marked difference in gelatinase activity. The fast-growing soybean strains produced a clearing zone on tryptone-yeast extract-gelatin agar, whereas the slow growers were negative for gelatinase activity. No gelatinase activity was detected in *R. leguminosarum* HI 5-0, *R. leguminosarum* 92A3, *R. phaseoli* Bel 7.1, *R. trifolii* NZP 560, or *Rhizobium* spp. strains USDA 3F4a4 and UMKL 19 (which nodulate *Sesbania* and *Leucaena*, respectively) (data not shown). However, a strain which nodulates *Leucaena*, strain Tal-82, was positive for gelatinase activity.

The litmus milk reactions of the fast- and slow-growing rhizobia were quite varied. With the exception of strain PRC 121-6, the slow-growing soybean rhizobia (strains USDA 6, USDA 31, USDA 34, USDA 74, USDA 94, USDA 110, USDA 122, USDA 123, USDA 136, USDA 138, USDA 142, Y1, Y1a, Y2, Y2a, Y3, K2, K2a, S1, and S1a) exhibited no peptonization (serum zone formation) but did exhibit alkaline pH changes. The fast growers gave a variety of litmus milk reactions; these included acid (strains USDA 191, USDA 192, USDA 214, and USDA 257) and alkaline (strains USDA 193, USDA 194, and USDA 201) pH changes accompanied by peptonization, an alkaline pH change with a lack of peptonization (strain USDA 208), and no pH change with peptonization (strains USDA 205, USDA 206, and USDA 217). The fast-growing rhizobia tended to reduce litmus (strains USDA 191, USDA 192, USDA 194, USDA 201, USDA 214, USDA 217, and USDA 257), whereas the slow growers did not. Two fast-growing strains from the PRC, strains USDA 193 and USDA 206, peptonized litmus milk only weakly. The other fast-growing rhizobia examined, *R. phaseoli* Bel 7.1, *R. phaseoli* NZP 5097, and *R. leguminosarum* 92A3 and 6015(pJB5JI), all peptonized litmus milk, had neutral pH reactions, and were variable for litmus reduction (data not shown).

Carbohydrate utilization. Our carbohydrate utilization tests (Table 2) showed that the fast growers utilized a greater assortment of carbohydrates than the slow growers. All of the organisms examined utilized L-arabinose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, L-rhamnose, and D-xylose. Although the fast-growing soybean rhizobia were capable of utilizing arabinose, they grew more slowly on this carbon source than the slow growers. Only the fast-growing soybean rhizobia utilized cellobiose, i-inositol, lactose, maltose, raffinose, D-glucitol, sucrose, and trehalose. None of the organisms examined utilized dextrin, galactitol, inulin, or starch as a sole source of carbon for growth.

TABLE 2. Carbohydrate utilization by fast- and slow-growing soybean rhizobia

Carbohydrates	% of isolates showing growth[a]	
	Slow-growing rhizobia	Fast-growing rhizobia
L-Arabinose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, L-rhamnose, D-xylose	100	100
Cellobiose, i-inositol, lactose, maltose, raffinose, D-glucitol, sucrose, trehalose	0	100
Dextrin, galactitol, inulin, starch	0	0

[a] Eight slow-growing strains (USDA 110, USDA 123, USDA 136, PRC 005, PRC 113-2, PRC 121-6, PRC 2031, and PRC B15) and ten fast-growing strains (USDA 191, USDA 192, USDA 193, USDA 194, USDA 201, USDA 205, USDA 206, USDA 208, USDA 214, and USDA 217) of soybean rhizobia were examined.

NADP-linked 6PGD activity. Table 3 shows that NADP-linked 6PGD activity was detected only in the fast-growing rhizobia. The fast-growing soybean rhizobia had NADP-linked 6PGD activities that ranged from 50 to 181 nmol of NADP reduced per min per mg of protein. No activity was detected in the nine typical slow-growing *R. japonicum* strains examined. The five other fast-growing rhizobia examined (*R. leguminosarum* 92A3, *R. lupini* NZP 2037, *R. trifolii* NZP 560, *Rhizobium* sp. strain Tal-82 and *Rhizobium* sp. strain USDA 3F4a4) had similarly high specific activities, ranging from 26 to 156 nmol of NADP reduced per min per mg of protein.

β-Galactosidase activity. Table 3 shows that only the fast-growing rhizobia had appreciable levels of β-galactosidase activity. In general, the fast-growing soybean rhizobia had β-galactosidase activities 55 times greater than the slow growers. These levels were comparable to the levels found in the other fast-growing rhizobia examined (*R. leguminosarum* 92A3, *R. phaseoli* Bel 7.1, *R. trifolii* NZP 560, and *Rhizobium* sp. strain Tal-82) (data not shown).

TABLE 3. Enzyme activities of fast- and slow-growing soybean rhizobia

Strain	Enzyme activities			
	6PGD[a]	β-Galactosidase[b]	Hydrogenase[c]	H_2 evolution[d]
Slow-growing rhizobia				
USDA 31	<0.5	7.1		
USDA 110	<0.5	3.8		
USDA 122			2.5	0
USDA 123	<0.5	3.0		
USDA 136	<0.5	7.6		
USDA 138	<0.5	3.4		
PRC 005	<0.5	4.2		
PRC 113-2	<0.5	3.3		
PRC 121-6	<0.5	4.8		
PRC B15	<0.5	4.8		
Fast-growing rhizobia				
USDA 191	97		0	7.3
USDA 192	181	459	0	11.4
USDA 193	140	183		
USDA 194	126	101	0	8.6
USDA 201	50	345	0	7.6
USDA 205	159	97	0	8.4
USDA 206	59	261	0	7.6
USDA 208	147	383	0	7.6
USDA 214	160	245	0	7.5
USDA 217			0	9.1

[a] Expressed as nanomoles of reduced NADP produced per minute per milligram of protein.

[b] Expressed as micromoles of *o*-nitrophenol produced per minute per milligram of protein.

[c] Expressed as micromoles of H_2 oxidized per gram (fresh weight) of nodules per hour.

[d] Expressed as micromoles of H_2 evolved per gram (fresh weight) of nodules per hour. One slow-growing strain, USDA 122, was included for comparison.

Hydrogenase activity. Table 3 shows that none of the fast growers in the bacteroid state had detectable hydrogenase activity, whereas the one slow grower examined, strain USDA 122, expressed this enzyme. Conversely, slow-growing strain *B. japonicum* USDA 122 did not evolve H_2, whereas the fast growers had H_2 evolution levels that ranged from 7.3 to 11.3 μmol of H_2 evolved per g (fresh weight) of nodules per h.

DISCUSSION

Several diagnostic tests were performed to compare the fast- and slow-growing soybean rhizobia. Our results clearly indicate that the fast growers are quite different biochemically from the slow growers. Although the fast growers share host specificity with the slow growers (both nodulate soybeans, as well as other hosts [20]), they appear to be most similar to the fast-growing *Rhizobium* species in their microbiological and biochemical characteristics. However, some characteristics are shared between the two groups of organisms.

All of the fast- and slow-growing soybean rhizobia examined were positive for catalase, oxidase, urease, penicillinase, and nitrate reductase. Both groups were unable to utilize citrate as a sole source of carbon, to produce H_2S, or to produce 3-ketolactose. Graham and Parker (11) found that most of the *R. japonicum* strains which they tested also possessed several of these attributes. These authors reported that the production of H_2S and the utilization of citrate were restricted to some isolates of *Rhizobium meliloti*. Bernaerts and De Ley (1) found that the production of 3-ketolactose from lactose is limited to species of *Agrobacterium*, a genus that is closely related to *Rhizobium*. It is interesting that Graham and Parker (11) found that the production of penicillinase seemed to be restricted mainly to the slow-growing rhizobia, *R. japonicum*, *R. lupini*, and *Rhizobium* spp. of the cowpea miscellany group. However, these authors indicated that some *R. leguminosarum* strains also possess this attribute.

The results of the pH and salt tolerance tests place the fast-growing soybean rhizobia with other fast-growing rhizobia. Previous studies of rhizobia (3, 8, 11, 31) in pure culture and in soil have shown that the fast growers are relatively more alkali tolerant and acid sensitive than the slow growers. Also, Graham and Parker (11) have shown that among the rhizobia, tolerance to 2% NaCl is restricted to the fast-growing species *R. meliloti*. In addition, the responses of the fast-growing soybean rhizobia in litmus milk were typical of the fast-growing *Rhizobium* species (e.g., *R. trifolii*, *R. leguminosarum*, and *R. meliloti*) (7, 11).

Graham and Parker (11) and Fred et al. (7) showed that the fast-growing rhizobia tend to use a wider variety of carbohydrates than the slow growers. This is shown clearly in Table 2; the fast growers used the majority of the carbohydrates examined, and the slow growers used relatively few. It is interesting that the types of carbohydrates utilized also vary among the soybean rhizobia. As pointed out by Glenn and Dilworth (10), slow-growing rhizobia tend to lack both uptake systems and catabolic enzymes for disaccharides. Our results are in agreement with the results of these authors in that the disaccharides cellobiose, lactose, maltose, trehalose, and sucrose and the trisaccharide raffinose were metabolized by fast-growing strains but not by slow-growing rhizobia. Table 3 shows that only the fast-growing soybean rhizobia have appreciable β-galactosidase activities similar to the activities of the other fast growers examined (data not shown).

Consistent with the division of the rhizobia

into fast- and slow-growing groups on the basis of carbohydrate utilization is the division of these organisms based on the presence and absence of enzymes of the pentose phosphate pathway. As pointed out by Martinz-de Drets and Arias (26, 29), although both fast- and slow-growing rhizobia have nicotinamide adenine dinucleotide-linked 6PGD activity, only the fast growers have NADP-linked 6PGD activity. Table 3 shows that the fast-growing soybean rhizobia have levels of NADP-linked 6PGD activity that are comparable to the levels of the other fast-growing rhizobia. These results are consistent with those of Martinez-de Drets and Arias (27, 28) and Keele et al. (19).

The hydrogenase activities of the fast-growing soybean rhizobia (Table 3) are similar to those of other fast-growing species of *Rhizobium*, which reportedly have low bacteroid hydrogenase activities (5). Furthermore, the hydrogenase activities of culture-grown fast-growing soybean rhizobia (strains USDA 192, USDA 193, USDA 194, USDA 201, USDA 205, and USDA 206) were also found to be very low by the tritium exchange assay of Lim (25; S. Uratsu, personal communication).

In summary, although the fast-growing soybean rhizobia share symbiotic host specificity with the typical slow growers, they appear more closely related, on biochemical bases, to other fast-growing rhizobia. In a series of papers (14), Ishizawa noted the occurrence of fast-growing rhizobia in soybean nodules. We examined one of the isolates of Ishizawa, which was provided by D. C. Jordan. We found (data not shown) that although this isolate is somewhat faster growing than typical slow-growing soybean bacteria, it does not acidify YEM medium and is not at all serologically related to the fast-growing isolates from the PRC. The slow-growing soybean rhizobia are now classified as *Bradyrhizobium japonicum* (16). Several of the diagnostic tests that were used to describe the fast-growing soybean rhizobia indicated that these newly described organisms do not fit into the genus *Bradyrhizobium*. Therefore, the taxonomic position of these isolates must logically be in the new genus *Rhizobium* sensu stricto (15).

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LITERATURE CITED

1. Bernaerts, M. J., and J. De Ley. 1963. A biochemical test for crown gall bacteria. Nature (London) 197:406–407.
2. Bishop, P. E., J. G. Guevara, J. A. Engelke, and H. J. Evans. 1976. Relation between glutamine synthetase and nitrogenase activities in the symbiotic association between *Rhizobium japonicum* and *Glycine max*. Plant Physiol. 57:542–546.
3. Bryan, U. C. 1923. Effects of acid soils on nodule-forming bacteria. Soil Sci. 15:542–546.
4. Christensen, W. B. 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon organisms from each other and from *Salmonella* and *Shigella* types. J. Bacteriol. 52:461–466.
5. Evans, H. J., K. Purohit, M. A. Cantrell, G. Eisbrenner, S. A. Russel, F. J. Hanus, and J. E. Lepo. 1981. Hydrogen losses and hydrogenase in nitrogen-fixing organisms. p. 84–96. *In* A. H. Gibson and W. E. Newton (ed.), Current perspectives in nitrogen fixation. Australian Academy of Science, Canberra.
6. Foley, J. M., and C. J. Perret. 1962. Screening bacterial colonies for penicillinase production. Nature (London) 195:287–288.
7. Fred, E. B., I. L. Baldwin, and E. McCoy. 1932. Root nodule bacteria and leguminous plants. University of Wisconsin Studies in Science, no. 5. University of Wisconsin, Madison.
8. Fred, E. B., and A. Davenport. 1918. Influence of reaction on nitrogen-assimilating bacteria. J. Agric. Res. (Washington, D.C.) 14:317–336.
9. Gamborg, O. L. 1975. Callus and cell culture. p. 1–9. *In* O. L. Gamborg and L. R. Wetter (ed.), Plant tissue culture methods. National Research Council of Canada, Saskatoon, Saskatchewan.
10. Glenn, A. R., and M. J. Dilworth. 1981. The uptake and hydrolysis of disaccharides by fast- and slow-growing species of *Rhizobium*. Arch. Microbiol. 129:233–239.
11. Graham, P. H., and C. A. Parker. 1964. Diagnostic features in the characterization of the root-nodule bacteria of legumes. Plant Soil 20:383–396.
12. Hirsch, P. R., J. von Montagu, A. W. B. Johnston, N. J. Brewin, and J. Schell. 1980. Physical identification of bacteriocinogenic, nodulation, and other plasmids in strains of *Rhizobium leguminosarum*. J. Gen. Microbiol. 120:403–412.
13. Hunter, C. A., and H. G. Crecellus. 1938. Detection of hydrogen sulfide in cultures. J. Bacteriol. 35:185–196.
14. Ishizawa, S. 1953. Studies on the root nodule bacteria of leguminous plants. I. Characters in artificial media. J. Sci. Soil Manure 23:169–172.
15. Jarvis, B. D. W., C. E. Pankhurst, and J. J. Patel. 1982. *Rhizobium loti*, a new species of legume root nodule bacteria. Int. J. Syst. Bacteriol. 32:378–380.
16. Jordan, D. C. 1982. Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growing root nodule bacteria from leguminous plants. Int. J. Syst. Bacteriol. 32:136–139.
17. Jordan, D. C., and O. N. Allen. 1974. Genus II. *Rhizobium*. p. 262–264. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology. 8th ed. The Williams & Wilkins Co., Baltimore.
18. Josey, D. P., J. L. Beynon, A. W. B. Johnston, and J. E. Beringer. 1979. Strain identification in *Rhizobium* using intrinsic antibiotic resistance. J. Appl. Bacteriol. 46:343–350.
19. Keele, B. B., Jr., P. B. Hamilton, and G. H. Elkan. 1969. Glucose catabolism in *Rhizobium japonicum* J. Bacteriol. 97:1184–1191.
20. Keyser, H. H., B. B. Bohlool, T. S. Hu, and D. F. Weber. 1982. Fast-growing rhizobia isolated from root nodules of soybean. Science 215:1631–1632.
21. Keyser, H. H., P. van Berkum, and D. F. Weber. 1982. A comparative study of the physiology of symbiosis formed by *Rhizobium japonicum* with *Glycine max*, *Vigna unguiculata* and *Macroptilium atropurpureum*. Plant Physiol. 70:1626–1630.
22. Koser, S. A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bacteriol. 8:493–520.
23. Kovacs, N. 1956. Identification of *Pseudomonas pyo-*

29

cyanea by the oxidase reaction. Nature (London) 178:703.

24. **Leonard, L. T.** 1943. A simple assembly for use in the testing of cultures of rhizobia. J. Bacteriol. 45:523–527.
25. **Liu, S. T.** 1978. Determination of hydrogenase in free-living cultures of *Rhizobium japonicum* and energy efficiency of soybean nodules. Plant Physiol. 62:609–611.
26. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
27. **Martinez-de Drets, G., and A. Arias.** 1970. Metabolism of some polyols by *Rhizobium meliloti*. J. Bacteriol. 103:97–103.
28. **Martinez-de Drets, G., and A. Arias.** 1972. Enzymatic basis for differentiation of *Rhizobium* into fast- and slow-growing groups. J. Bacteriol. 109:467–470.
29. **Martinez-de Drets, G., and A. Arias.** 1977. 6-Phospho-D-gluconate:NAD$^+$ 2-oxidoreductase (decarboxylating) from slow-growing rhizobia. J. Bacteriol. 130:1139–1143.
30. **May, S. N., and B. B. Bohlool.** 1983. Competition among *Rhizobium leguminosarum* strains for nodulation of lentils (*Lens esculenta*). Appl. Environ. Microbiol. 45:960–965.
31. **Norris, D. O.** 1965. Acid production by *Rhizobium*, a unifying concept. Plant Soil 22:143–166.
32. **Schmidt, E. L., R. O. Bankole, and B. B. Bohlool.** 1968. Fluorescent antibody approach to the study of rhizobia in soil. J. Bacteriol. 95:1987–1992.
33. **Smibert, R. M., and N. R. Krieg.** 1981. General characterizations, p. 409–443. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
34. **Vincent, J. M.** 1970. A manual for the practical study of root nodule bacteria. IBP handbook 15. Blackwell Scientific Publications, Oxford.
35. **Vincent, J. M.** 1974. Root-nodule symbiosis with *Rhizobium*, p. 265–347. *In* A. Quispel (ed.), Biology of nitrogen fixation. North-Holland Publishing Co., Amsterdam.