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CHARACTERISATION OF SOYBEAN RHIZOBIAL STRAINS FROM JAVA AND SUMATRA

Setiyo Hadi Waluyo[a], Tek An Lie[b], Leendert't Mannetje[c], and Willem M. de Vos[b]

[a]*Agriculture Division, Center for Research and Development of Isotopes and Radiation Technology, National Nuclear Energy Agency, Jakarta, Indonesia; E-mail: shwaluyo@yahoo.com*

[b]*Laboratory of Microbiology, Department of Agrotechnology and Food Sciences, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT, Wageningen, The Netherlands*

[c]*Department of Plant Sciences, Wageningen University, Haarweg 333, 6709 RZ, Wageningen, The Netherlands*

ABSTRACT

To get insight in the structure of soybean rhizobial population native to Indonesian soils, a thorough survey of the occurrence of the soybean rhizobia were conducted in several locations in Java and Sumatra. A total of 51 different isolates of rhizobial strains were characterised phenotypically based on their symbiotic properties, and genetically using amplified ribosomal DNA restriction analysis (ARDRA). Based on their nodulation capacity on both soybean and the native legume mungbean, these rhizobial strains could be divided into a group of 16 strains specific for soybean only and another group of 35 promiscuous strains that nodulated both leguminous plants. Based on ARDRA of PCR-amplified 16S rDNA and 16S-23S rDNA spacer fragments, the rhizobial strains isolated from Java differed with those from Sumatra. Six Java isolates and only one Sumatra isolate were classified as *Bradyrhizobium japonicum* and these similar to that of *B. japonicum* strain USDA 110. All these *B. japonicum* strains were highly specific for soybean. One isolate from Java showed a rather unique position. The remaining strains from Java (20), which were symbiotically promiscuous strains, were clustered in another group. This group and another group containing most Sumatra isolates were distinct from *B. japonicum* USDA 110 and therefore it is tempting to speculate that these represent indigenous soybean rhizobial bacteria. Application of agricultural practices, such as enhancement of rhizobial population, to increase soybean production is still essential and noteworthy in Sumatra.

[***Keywords***: *Glycine max*, ARDRA, *Bradyrhizobium japonicum*, Java, Sumatra]

INTRODUCTION

Soybean (*Glycine max* Merrill) is an important food crop in Indonesia. The crop has already been cultivated for several centuries, notably in Java that has fertile soils. To meet the increasing food demands of the last decades, soybean is also grown in Sumatra, where soil conditions are low nutrient content and high acidity.

Soybean is a legume that can fix nitrogen through nodulating rhizobial bacteria belonging to the genera *Bradyrhizobium* or *Sinorhizobium* (Somasegaran and Hoben 1995). The importance of rhizobial strains for soybean cultivation in Indonesia was already shown by Toxopeus (1938). It was observed that indigenous rhizobial strains, capable of nodulating soybean, are mainly present in soils cultivated for a long period with soybean. This is especially the case in soils that favour the growth of soybean such as Java, where the numbers of indigenous and adapted soybean rhizobial bacteria may reach high levels ($>10^4$ cells per gram of soil) (Simanungkalit *et al.* 1995). The presence of high numbers of competitive but ineffective indigenous rhizobial strains may interfere with the inoculation practice and has been considered as the main reason for ambiguous results on soybean inoculation in Indonesia (Saono 1988).

Under unfavourable conditions, such as those present in poor acid soils in Sumatra, the number of indigenous rhizobial strains are lower or absent (S.H. Waluyo, unpublished data). However, there are indications that improvement of acid soils by liming or other treatments such as growing leguminous crops, may increase number of indigenous rhizobial strains such as *Rhizobium leguminosarum* bv. *trifolii* (Mulder and van Veen 1961; Richardson and Simpson 1988) and *Rhizobium meliloti* (Danso 1977). Planting of clover crop on acid soils doubles the number of rhizobial cells. This effect pronounced in limed soils (Mulder and van Veen 1961). In the North Carolina soils with a history of soybean cropping, the population of *Bradyrhizobium japonicum* increased almost 200-fold compared to control (Mahler and Wollum II 1982). Symbiotic legumes increased the level of rhizobial strains in soil almost hundred-fold (Thies *et al.* 1995). In pot experiments, it has been reported that nodules were occasionally found on uninoculated soybean plants grown on limed acid soils collected from Sitiung, West Sumatra (Adiningsih and Prihatini

1981; Mahmud and Rumawas 1983) and Jasinga, West Java (Ismunadji and Makarim 1989). This is in agreement with results from a field experiment in Sitiung, West Sumatra (S.H. Waluyo, unpublished data). It was observed that only occasionally a nodule was present on soybean plants that had not been inoculated and were grown on soils that had been neutralised with lime. These nodules were not located on the main root near the crown but on deep lateral roots, which is unusual for soybean and suggests an uneven distribution of rhizobial bacteria in this soil.

Growing of leguminous plants in acid soils followed with appropriate agronomic treatment such as liming increased population of indigenous nodulating strains. It is assumed that after several years of cultivation of the acid soils, the number of indigenous rhizobia doubled and therefore could interfere with rhizobia introduced as an inoculant (Weaver *et al.* 1972). The success of inoculation practices on soybean plant grown on acid soils reported by Kang *et al.* (1977) and Danso (1988) was due to the initial low numbers of indigenous nodulating strains in the soils.

An important aspect for the success of biological nitrogen fixation (BNF) is insight in the structure of indigenous rhizobial population. Whilst there is a general lack of information about the population structure of soybean rhizobia native to Indonesian soils. Hence, it is relevant to survey thoroughly the presence of indigenous rhizobial strains in soils in different locations in Indonesia prior to agricultural practice. Furthermore, it is essential to characterise the isolates using reliable molecular methods. This opens the possibility to select elite indigenous soybean rhizobia under favourable conditions, or to select rhizobia adapted to stress conditions as acid soils.

MATERIALS AND METHODS

Experiments and molecular analysis were conducted at Laboratory of Microbiology, Wageningen University, The Netherlands in 1998–2000.

Soil Samples, Rhizobial Strains, and Media

Soil samples collected from different areas, 27 located in Java (a soybean traditional area) and 63 in Sumatra (a new growing area of soybean) in 1997 were stored in a climate room at 4°C. These soil samples and two other soil samples, collected from Java 20 years ago and stored in a climate room at 4°C, were used to isolate rhizobial strains.

Soybean cv. Tidar and mungbean (*Vigna radiata*) cv. Manyar seeds used for testing were obtained from the Indonesian Center for Food Crops Research and Development, Bogor and from the Center for Research and Development of Isotopes and Radiation Technology, National Nuclear Energy Agency, Jakarta, respectively, and stored in a climate room at 4°C. *B. japonicum* strains USDA 110 and CB 1809 and *Bradyrhizobium* sp. strain CB756 (obtained from the Laboratory of Microbiology, Wageningen University) were used as reference strains. These rhizobial strains were maintained on Yeast Extract Mannitol Agar (YEMA) containing mannitol 10.0 g, yeast extract 0.4 g, K_2HPO_4 0.5 g, $MgSO_4.7\,H_2O$ 0.2 g, NaCl 0.1 g, agar 15 g and demineralised water 1.0 l. For inoculant preparation, the strains were grown in Yeast Extract Mannitol Broth (YEMB) at 30°C for 4-7 days (Somasegaran and Hoben 1995).

Isolation of Rhizobial Strains

Approximately 1.0 g of each soil sample was added to the sterile soybean plant grown in a modified Leonards jar on a modified Hoagland medium free of N (Winarno and Lie 1979), and incubated at a controlled climate room. The plants were harvested 3 weeks after inoculation, followed by the determination of the number of nodules. At this stage, the difference in plant growth due to BNF activity was obvious (Fig. 1). When many (>10) nodules per plant were present, 5 nodules were used to independently isolate rhizobial strains on YEMA, but if only a few nodules

Fig 1. Soybean and mungbean plants inoculated with soil samples from Sumatra (left) and Java (right), and uninoculated control (center).

(<5) produced, all of the nodules were isolated. If a small and white nodule was obtained, the chance of isolating rhizobium was improved by chrused the nodule and reinoculated to sterile soybean plant, and then isolates were taken from the resulting nodulation.

Rhizobial isolate from each nodule was purified by subculturing a single colony on YEMA. The pure cultures were maintained on a slant of YEMA at 4°C for further use. The isolates were coded by first letter S (Sumatra) or J (Jakarta) which reflects their origin, following letters indicate their locations, i.e. ST for Sitiung, BT for Bukittinggi, YG for Yogyakarta, NG for Ngawi, WG for Wangon, WK for Wangkal, PLR for Palur, KH for Kraksaan, DLG for Delanggu, SRG for Sragen, MDN for Madiun, KLT for Klaten, MJ for Mojosari, BSK for Besuki, TGS for Tongas, MLD for Mlandingan, PSR for Pasuruan, TM for Tasikmalaya, PN for Pusakanegara, JKT for Jakarta, BGR for Bogor, and CTM for Citayam.

Symbiotic Analysis

To characterise the symbiotic properties, a modified Leonard jars experiment was conducted in a controlled condition room, i.e. temperature at 26°C, 16 hours light (200 lux)/18 hours dark cycle, and relative humidity 70%, at Laboratory of Microbiology, Wageningen University. Completely randomised design was performed with three replications.

The rhizobial isolates were inoculated to soybean and mungbean plants grown on a modified Hoagland-N free medium as described previously by Winarno and Lie (1979). Mungbean was chosen because this is a leguminous cowpea plant known to be native to Indonesia (Summerfield and Lawn 1987). Three reference strains, *B. japonicum* strain USDA 110, strain CB 1809 and *Bradyrhizobium* sp. strain CB 756, were used as positive control. Uninoculated plants were used as negative control.

Plants were harvested 20 days after inoculation. Colour of the shoots was observed, and weight of shoots and number of nodules were determined. The capacity to fix N was deduced from a comparison of the colour (qualitatively) and the weight of the plants between control and inoculated plants that all were grown in a N-free medium (Winarno and Lie 1979). Hence, a small and yellow coloured plant is suffering from N-deficiency while a large and green coloured plant is assumed to have capacity to fix N (Fig. 1). The efficiency of the N fixation of the rhizobial strains was determined by weighing dry matter of the inoculated plants as described by Haydock *et al.* (1980). Data were calculated and analysed with computer program statistic MSTAT-C (1988).

Genetic Analysis

Genomic DNA to be used as template for PCR amplification of the 16S rRNA gene and the 16S-23S rRNA intergenic spacer regions was extracted from YEMB-grown bacterial cells in the following way. Four ml of bacterial culture containing 10^9 cfu ml^{-1} was harvested by centrifugation and suspended in 450 µl of a buffer solution consisting of 1 mM Tris-HCl and 0.1 mM EDTA, pH 7.4 containing 0.5% SDS, followed by incubation at 37°C with gently shaking for 30 minutes. DNA was extracted by the addition of an equal volume of phenol buffered in 10 mM Tris-HCl and 1 mM EDTA, pH 7.0 to the mixture. This extraction was repeated once with an equal volume of a mixture of phenol and chloroform. Subsequently, nucleic acids were precipitated by the addition of 0.1 volume of sodium acetate (3 M, pH 5.2) and 1.0 volume of ethanol 90% (-20°C), collected by centrifugation, dried and dissolved in 50 µl of sterile water. The amount of the isolated DNA was determined by electrophoresis on a 1% agarose gel containing ethidium bromide with 0.1 µg of λDNA digested with *Hin*dIII (GibcoBRL LifeTechnology, Breda, The Netherlands) as a reference. Ribosomal DNA fragments were amplified using a set of primers as described by Lane (1991) and Massol-Deya *et al.* (1995) as shown on Table 1. DNA amplification using approximately 50 ng of genomic DNA in a 100 µl PCR reaction volume was done in a UNOII Thermocycler, Biometra, Gottingen, Germany as described by Massol-Deya *et al.* (1995).

The size and amount of the amplified DNA was examined by electrophoresis of an aliquot of 5.0 µl on a 0.7% agarose gel containing ethidium bromide and λDNA digested with *Hin*dIII as a reference. Approximately 400 ng of the amplified DNA was digested with the restriction endonucleases *Cfo*I, *Dde*I, *Hae*III, and *Msp*I (5 units per 25 µl reaction) following instruction of the manufacture (GibcoBRL LifeTechnology, Breda, The Netherlands) and the generated fragments were separated by electrophoresis on 3% agarose gels containing ethidium bromide at 100 V for 2 hours. ARDRA fingerprints of 16S rDNA and 16S-23S rDNA PCR-products were recorded as TIFF files, analysed and used to prepare a dendogram based on predictions by Unweighted Pair Group Method using Arithmetic averages (UPGMA) by Molecular Analysis Software (BioRad 1995).

Table 1. The sequence of the PCR primers used in characterising rhizobial isolates from Java and Sumatra soils.

Primer	Sequence	PCR reaction	Location	Reference
8	5'-CAC GGA TCC AGA GTT TGAT (C/T) (A/C) TAG TCC AG-3'	16S rDNA	8-27 (16S rDNA)	Lane (1991)
1510H	5'-GTT AA GTT ACTG (C/T) TAC GTT GTT ACG ACTT-3'	16S rDNA	1100-1115 (16S rDNA)	Lane (1991)
pHr	5'-TGCGGCTGGATCACCTCCTT-3'	16S-23S rDNA	1518-1541 (16S rDNA)	Massol-Deya *et al.* (1995)
p23SROI	5'-GGCTGCTTCTAAGCCAAC-3'	16S-23S rDNA	1069-1052 (23S rDNA)	Massol-Deya *et al.* (1995)

RESULTS

Plant Growth and Nodulation

Growth of the soybean plants inoculated with soil samples from Java was different with those inoculated with soil samples from Sumatra. Soybean plants inoculated with soil samples from Java appeared healthy and carried nodules that all fixed N. Average number of nodules was more than 20. However, most of the plants inoculated with soil samples from Sumatra were not healthy and number of root nodules varied from 1 to 10 (Fig. 2). Ability of the soil samples from Java and Sumatra to form root nodules on the soybean plants was shown on Table 2.

Rhizobial Isolation and Symbiotic Properties

Fifty-one rhizobial strains that nodulated soybean were isolated from effective (fixing N) and ineffective (less or not fixing N) crushed nodules obtained with soils from Java (27) and Sumatra (24). The nodulation efficiency of the tested rhizobial strains and the reference strains were shown on Table 3. In general, rhizobial strains obtained from Java were as effective as the reference strains *B. japonicum* USDA 110 and CB 1809. The number and weight of nodules, as well as the weight of shoots from the plants inoculated with the rhizobial strains from Java, were significantly higher than those from Sumatra, especially in the weight of shoots ($\alpha = 0.01$). The first two leaves of the soybean plants inoculated with rhizobial strains from Sumatra were yellow and in most cases were lost quickly, indicating poor N fixing capacity (Fig. 3).

Table 2. Nodulation efficiency and nitrogen fixation capacity of soybean plants inoculated with soil samples from Java and Sumatra.

Source	Number of soil samples	Nodulation (%)	N fixation (%)
Java	29	100	100
Sumatra	63	67	37

Fig. 2. Soybean plants inoculated with soil samples from Sumatra shows effectively fixing N (left) and ineffective one (right).

Fig. 3. Shoot performance of soybean plants inoculated with isolates from Sumatra (left) and Java (right).

Table 3. Efficiency of rhizobial strains isolated from Java and Sumatra soils compared to the reference strains in inducing nodules, nodule weight and shoot weight of soybean.

Strain tested	Nodule number per plant	Nodule weight per plant (g)	Shoot weight per plant (g)
None (control)	0	0	0.57 ± 0.32
Bradyrhizobium sp.[1] CB 756	0	0	0.30 ± 0.03
B. japonicum[1] USDA 110	43 ± 10	0.288 ± 0.07	3.36 ± 0.80
B. japonicum[1] CB 1809	29 ± 5	0.250 ± 0.06	3.70 ± 0.28
Java[2]	46 ± 10a*	0.263 ± 0.07a**	3.75 ± 0.62a***
Sumatra[2]	37 ± 14b	0.225 ± 0.070b	2.43 ± 0.80b
CV[3] (%)	33	29	25

[1]For these reference strains means were calculated from three replicates.
[2]A total of 24 randomly choosen bacterial strains were used for each set of soil samples.
*, **, *** Values followed by a different letter in same column are statistically different, revealed by T-test with confidence levels at α = 0.10, 0.05 and 0.01 respectively.
[3]CV = coefficient of variation (MSTAT-C 1988).

The capacity of the isolated rhizobial strains to additionally nodulate mungbean was shown on Fig. 4. This analysis revealed that most of the strains from both Java and Sumatra showed promiscuous nodulation properties that were not observed with the well-known inoculant strains *B. japonicum* USDA 110 and CB 1809.

Amplified Ribosomal (16S and 16S-23S) DNA Restriction Analysis

ARDRA analysis showed that all of the amplified 16S rDNA have the size of approximately 1.6 kb. Upon digestion with four different restriction enzymes (*Cfo*I, *Dde* I, *Hae*III and *Msp*I), a variety (up to 19) of restriction length polymorphism patterns were obtained. This ARDRA approach allowed the grouping of the rhizobial isolates into two main clusters; while one Java isolate J-TGS50 and two Sumatra isolates S-ST123 and S- ST414 showed rather unique position (Fig. 4). One cluster consists of most (25) rhizobial isolates from Java, four Sumatra isolates, and also *B. japonicum* USDA 110. The other cluster consists of most (18) rhizobial isolates from Sumatra and only one strain isolated from Java (J-YG49). Both clusters contained isolates that showed only nodulation of soybean as well as ones with promiscuous nodulation properties. The PCR products of the 16S-23S spacer rDNA were all of the same size of approximately 2 kb. Differentiation of these 16S-23S rDNA amplicons by ARDRA using the same restriction enzymes as used for the 16S rDNA amplicons revealed that these large DNA fragments showed significant sequence variation (Fig. 5).

Twenty-seven composite restriction pattern types were obtained by combining data from the digestion result (Table 4). *Dde*I was the most discriminating enzyme with 21 genotypes detected among the rhizobial isolates. Based on these complex ARDRA fingerprints, the rhizobial isolates were grouped into four main clusters (Fig. 6). All strains isolated from Java, except for J-YG49, could be grouped into two clusters, one including *B. japonicum* USDA 110. Again the Java isolate J-TGS50 showed a unique position. Similarly, all strains isolated from Sumatra, except for S-ST224, S-ST325 and S-ST123, could be grouped in one large cluster, while two strains (S-BT221 and S-BT322) formed a cluster that was distantly related to that of *B. japonicum* USDA 110.

DISCUSSION

Rhizobial strains capable of nodulating soybean were isolated from soil samples obtained from traditional soybean areas in Java and various new soybean growing areas in Sumatra. Java soil was found to be rich with effective soybean rhizobial strains since all of the tested soil samples (29) produced effectively fixing N nodules on inoculated soybean plant. This is likely to be a consequence of extensive cultivation of soybean in Java for over several centuries (De Vries 1932) as well as the introduction of many *Bradyrhizobium* strains from abroad (Newton 1962). Inoculation of rhizobial strains in these areas not necessary, unless competitive and high fixing N rhizobial strains are used as inoculant. This finding explains the ambiguous results on soybean inoculation in Indonesia reported by Saono (1988). In

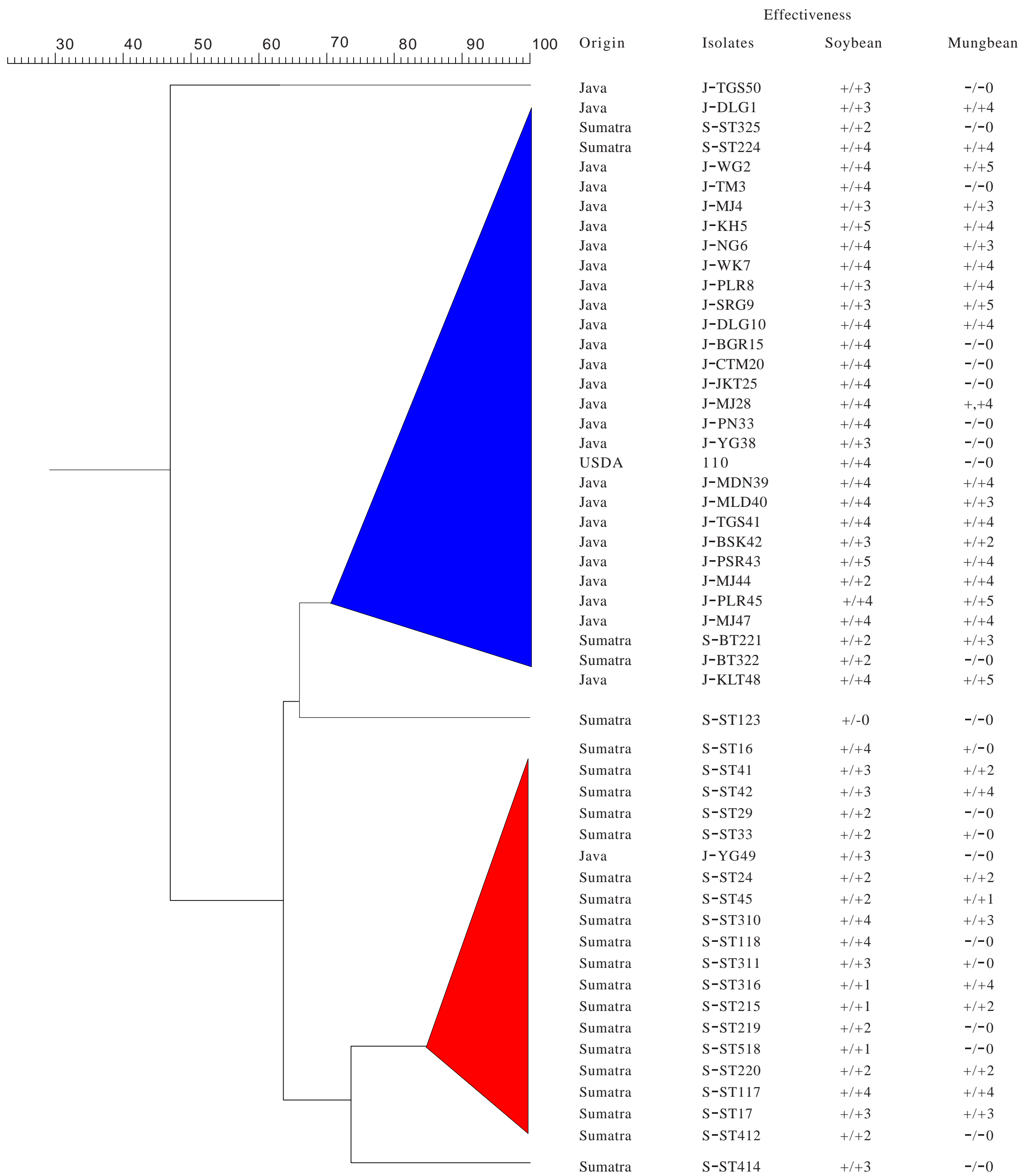

Fig. 4. Dendogram derived from 16S rDNA ARDRA fingerprints and symbiotic properties of rhizobial strains isolated from Java and Sumatra soil samples as well as *B. japonicum* USDA 110. +/+ = nodulated and N-fixed; +/- = nodulated and no N-fixed; -/- = not nodulated and no N-fixed. The effectiveness of N fixation is indicated by values ranging from 1 (ineffective) to 5 (effective).

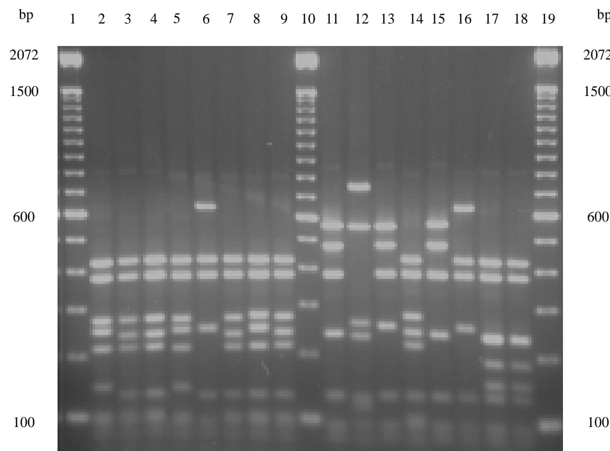

Fig. 5. Restriction patterns of PCR amplified fragments of 16S-23S rDNA digested with *Msp*I. Lanes 1, 10, 19 and 20 are 100 bp DNA ladder (Life Technology). Lanes 2-9 (S-ST216; S-ST41; S-ST42; S-ST29; S-ST224; S-ST33; S-ST24; S-ST45) were derived from rhizobial isolates from Sumatra, and lanes 11-18 (J-MDN39; J-TGS50; J-BSK42; J-YG49; J-KLT48; J-KH5; J-MLD40; J-PSR43) were from Java.

contrast, in Sumatra where soybean cultivation has been introduced quite recently, several locations were found to be devoid of soybean rhizobial strains. Only 42 of 63 (67%) tested soil samples were found to nodulate soybean, and from these only 23 (37%) appeared to fix N. This implies that the chance of agricultural practices to increase soybean production by enhancing BNF is necessary in Sumatra.

A total of 51 different rhizobial strains (27 from Java and 24 from Sumatra) were isolated from soybean nodules. Based on their nodulation capacity on both soybean and mungbean, these rhizobial isolates could be classified as promiscuous strains (35) and strains that show a narrow host-range (16) which only nodulate soybean (Fig. 4). Saono (1988) reported that the native soybean-rhizobium population in Java were dominated by promiscuous strains, and this is supported here with quantitative data. All soybean plants inoculated with rhizobial isolates from Java (except J-MJ44) grew vigorously in a N-free medium. In contrast, several ineffective bradyrhizobial isolates were found in Sumatra. It is likely that the ineffective isolates would have a negative impact on the inoculation by effective *Bradyrhizobium* sp. inoculants. Repeated cultivation of soybean in Sumatra soils will increase population of the in effective rhizobial strains. As the result they will more adapt and compate with the introduced strains.

ARDRA of PCR-amplified 16S rDNA and 16S-23S rDNA spacer fragments was used to differentiate soybean-nodulating rhizobial strains. Based on ARDRA of 16S rDNA, nearly all the soybean-nodulating bacteria could be grouped into two separate large clusters comprising either the Java or the Sumatra isolates. ARDRA of 16S-23S rDNA spacer fragments was successfully used for subclassification of bacteria (Jensen *et al*. 1993; Masol-Deya *et al*. 1995; Gurtler and Stanisich, 1996; Scheinert *et al*. 1996), because this region exhibits a high degree of sequence and size variation at the level of the genus and species. The sequence variation of the 16S-23S rDNA spacer region of the soybean-nodulating bacteria confirmed the clustering of most Sumatra isolates, and allowed a further classification of the Java isolates into two large distinct clusters. One of these clusters accommodated strains that were closely related to *B. japonicum* USDA 110, a highly specific for soybean. This means that most of Java isolates and one isolate from Sumatra (S-ST123) are *B. japonicum*. Although clustered at one group, the isolate S-ST123 showed distinct in its symbiotic property to *B. japonicum* USDA 110. It formed many but ineffective soybean nodules.

One unique isolate from Java, J-TGS50, showed an unique position in comparison with the clustered isolates from Java and Sumatra. This and the group containing most Sumatra isolates which were also shown distinct from *B. japonicum* USDA 110, are all nodulated both soybean and the indigenous mungbean. Hence these strains may represent the indigenous bacterial population capable of nodulating soybean. Further characterisation of the isolated rhizobial strains using more powerful and descriptive molecular technique, such as sequencing of 16S rDNA gene, is required. This technique could differentiate *B. japonicum*, *B. elkanii*, and *Sinorhizobium fredii* strains, which are well known as soybean nodulating rhizobia.

Efforts to increase soybean production via enhancement of BNF (rhizobial inoculation) in Sumatra are still essential and noteworthy. The differences in the capacity to fix N of the isolated soybean rhizobial strains offer the selection of native and adapted soybean rhizobial strains. However, a care should be addressed to the presence of ineffective rhizobial strains as well. The abundance of these strains at certain level become more competitive and may influence the success of inoculation of new rhizobial strains.

CONCLUSION

Bradyrhizobium strains specific for soybean as well as promiscuous soybean rhizobial strains were

Table 4. 16S-23S rDNA genotypes and restriction patterns of the rhizobial isolates from Java and Sumatra soils revealed by ARDRA.

Isolate	16S-23S r DNA genotype	Restriction pattern of amplified 16S-23S rDNA digested with			
		*Msp*I	*Dde*I	*Hae*III	*Cfo*I
S-ST16	I	a	a	a	a
S-ST29	I	a	a	a	a
S-ST18	II	a	h	a	a
S-ST215	III	a	q	a	h
S-ST41	IV	b	b	b	a
S-ST42	IV	b	b	b	a
S-ST45	IV	b	b	b	a
S-ST33	IV	b	b	b	a
S-ST311	IV	b	b	b	a
S-ST117	IV	b	b	b	a
S-ST17	IV	b	b	b	a
J-YG49	V	b	a	b	a
S-ST414	VI	b	j	b	a
S-ST224	VII	c	c	c	c
J-WG2	VII	c	c	c	c
J-NG6	VII	c	c	c	c
J-WK7	VII	c	c	c	c
J-PLR8	VII	c	c	c	c
J-KH5	VII	c	c	c	c
J-DLG10	VII	c	c	c	c
J-DLG1	VII	c	c	c	c
J-SRG9	VII	c	c	c	c
S-ST24	VIII	d	d	a	d
S-ST310	IX	d	k	b	i
S-ST412	X	d	k	b	p
J-MDN39	XI	e	e	c	e
J-KLT48	XI	e	e	c	e
J-MJ44	XI	e	e	c	e
J-PLR45	XI	e	e	c	e
J-BSK42	XII	e	n	c	g
S-ST220	XIII	e	p	h	m
J-TGS50	XIV	f	f	d	f
J-MLD40	XV	g	g	c	g
J-TGS41	XV	g	g	c	g
J-PSR43	XV	g	g	c	g
S-ST518	XVI	h	i	e	h
J-TM3	XVII	i	l	f	j
J-PN33	XVII	i	l	f	j
J-YG38	XVII	i	l	f	j
J-JKT25	XVII	i	l	f	j
J-BGR15	XVIII	o	l	f	j
J-CTM20	XVIII	o	l	f	j
S-ST123	XIX	l	o	g	k
S-ST219	XX	m	p	h	l
S-ST325	XXI	n	k	b	o
J-MJ4	XXII	j	m	c	g
S-ST316	XXIII	k	d	a	n
S-BT221	XXIV	p	r	i	q
S-BT322	XXV	q	s	j	r
J-MJ28	XXVI	r	t	k	c
J-MJ47	XXVII	s	u	e	e

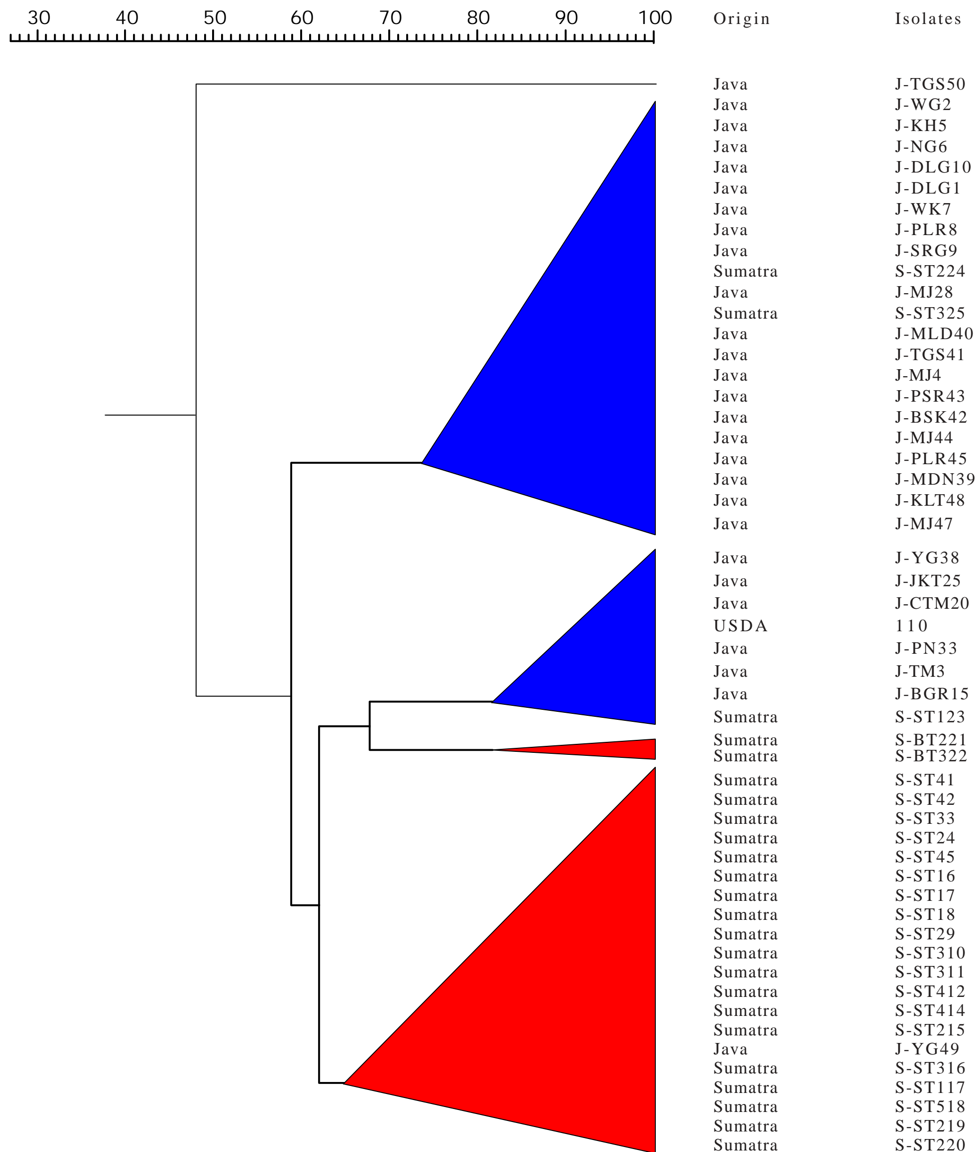

Fig 6. Dendogram derived from 16S-23S rDNA ARDRA fingerprints of soybean rhizobial strains isolated from Java and Sumatra soil samples as well as *B. japonicum* USDA 110.

isolated both in Java and Sumatra soils. Symbiotically, soybean rhizobial strains from Java were more effective in fixing N than those Sumatra strains. Genetically, soybean bradyrhizobia from Java were closely related to *B. japonicum* USDA 110, a reference strain for soybean, than that of Sumatra strains. The Sumatra strains are likely to be indigenous bradyrhizobia.

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