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ISOLATION AND CHARACTERIZATION OF ENDOPHYTIC BACTERIA IN SOYBEAN (*GLYCINE* SP.)

Pham Quang Hung[1] and K. Annapurna[2]

ABSTRACT

Plant-associated bacteria that live inside plant tissues without causing any harm to plants are defined as endophytic bacteria. The present investigation was carried out to analyse the phenotypic and genotypic diversity in the bacterial endophytes of two species of soybean viz. Glycine max and G. soja. A total of 65 bacterial endophytes were isolated from three tissues: stem, root and nodule. All the isolates were screened for Gram reaction, secretion of hydrolytic enzymes (pectinase and cellulase), fluorescent pigment production, and motility, resistance to streptomycin @ 100 µg/ml, capsule formation and IAA production. Genotypic variation was studied using PCR-based 16S rDNA-RFLP. Preliminary characterization of the 65 endophytes showed that approximately equal percentages of gram positive (49%) and gram negative (51%) bacteria were present. Approximately 80% were motile, 33% and 70% secreted pectinase and cellulase, respectively and 17% did not produce IAA in vitro. Phenotypically the 65 isolates were found to show less closeness among themselves for the characters studied. Molecular characterization of selected 35 endophytic bacteria was carried out by PCR amplification of 16S rDNA gene, and its restriction analysis using three tetra cutters, HaeIII, MboI and MspI. Two main clusters were observed at 48% and 43% similarity coefficients in which most of the endophytes belonged. Six of the total isolates (I-8, I-15, I-25, I-68, I-121 and I-137) did not come into these clusters, showing their divergence from the rest. The genetic variation was more among endophytes isolated from G. max tissues than G. soja.

Keyword: characterization, endophytic bacteria, phyllosphere, rhizosphere, soybean.

INTRODUCTION

Plants are constantly involved in interactions with a wide range of bacteria. These plant-associated bacteria colonize the rhizosphere (rhizobacteria), the phyllosphere (epiphytes) and the inside of plant tissues (endophytes). Endophytes are sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo, 2000). Some endophytic bacteria exert several beneficial effects on host plants, such as stimulation of plant growth (Sturz *et al.*, 1997), nitrogen fixation (Kirchhorf *et al*., 1997; Stoltzfus *et al*., 1997; Reinhold-Hurek and Hurek, 1998) and induction of resistance to plant pathogens (Chen *et al*., 1995; Liu *et al*., 1995; Sturz and Matheson, 1996).

Rhizobia are perhaps the best known beneficial plant-associated bacteria because of the importance of the nitrogen fixation that occurs during the *Rhizobium*-legume symbiosis. However, endophytic bacteria have been isolated from legume plants such as alfalfa (Gagne *et al.,* 1987), clover (Sturz *et al*., 1997) and pea (Elvira-Recuenco and van Vuurde, 2000). Bacteria of several genera have been isolated from legume tissues, including *Aerobacter, Aeromonas, Agrobacterium, Bacillus, Chryseomonas, Curtobacterium, Enterobacter, Erwinia, Flavimonas, Pseudomonas* and *Sphingomonas*

[1] Cuu Long Delta Rice Research Institute, Can Tho, Vietnam
[2] Indian Agricultural Research Institute, New Delhi, India

(Gagne *et al.,* 1987; Sturz *et al*., 1997). The legume shows a remarkable diversity because of its long history of cultivation and its selection under various agroclimatic conditions. The present investigation was taken up to isolate and characterize endophytic bacteria in the two soybean species *Glycine max* and *G. soja*.

MATERIALS AND METHODS

Host plants used in the experiment were two species of soybean, one cultivated (*Glycine max* L. var. 9720) and one wild species (*G. soja* Sieb and Zucc). These plants were grown in IARI fields in the Summer - Autumn season from August to December, 2002.

Isolation of endophytic bacteria

The soybean tissue was collected at the flowering stage. Five healthy plants were carefully removed, washed under tap water to remove soil and separated into stems, roots and nodules. Stems and roots were cut into sections 2-3 cm long. The tissue was put in beaker, soaked in distilled water and drained. It was rinsed in 70% ethanol for 30 seconds and then sterilized with 0.1% $HgCl_2$ for 3 minutes for roots and nodules, 5 minutes for stems. The tissue was then washed ten times with sterile water (Gagne *et al*., 1987). Surface-disinfected tissue was aseptically macerated with homogenizers. Macerated tissue was diluted into 10^{-1} dilution by adding 9 volumes of sterile distilled water. Serial dilution was made up to 10^{-6} dilution by taking 1 ml of well-shaken suspension and adding into 9 ml water blank tubes. 100 µl from appropriate dilutions were spread plated on two different media, *viz.* PDA and TSA.

Potato Dextrose Agar (PDA): Potato (peeled) 200 g, dextrose 20 g, agar 18 g, distilled water (DW) 1000 ml. ***Trypticase Soy Agar (TSA)*** Trypticase Soy Broth (Becton Dickinson Co.) 30.0 g containing: pancreatic digest of casein 17.0 g, pancreatic digest of soybean mea l3.0 g, NaCl 5.0 g, K_2HPO_4 2.5 g, dextrose 2.5 g; agar 18.0 g, DW 1000 ml.

Morphological and physiological characterization

Gram staining and capsule staining were carried out followed standard staining protocols.

Cellulase activity test The test isolates were spot-inoculated on the swollen cellulose agar plates and incubated for one week at 30°C. Bacterial growth was observed (Rautela and Cowling 1966). ***Cellulase activity test medium*** *O*-phosphoric acid swollen cellulose 10.0 g, KH_2PO_4 2.0 g, $(NH_4)_2SO_4$ 1.4 g, urea 0.3 g, $MgSO_4.7H_2O$ 0.3 g, $FeSO_4$ 8.0 mg, $MnSO_4$ 1.6 mg, $CoCl_2$ 2.0 mg, agar 18.0 g, DW 1000 ml.

Pectinase activity test The test isolates were spot-inoculated on the pectin agar plates and incubated for one week at 30°C. The plates then were flooded with 0.1% aqueous Red ruthenium solution for one hour, drained, rinsed with water and observed. Red ruthenium is bound to unhydrolysed pectine and gives the red color. Halo zone around isolate's colony was observed (Cotty *et al.*, 1990). ***Pectinase activity test medium*** Pectin 5.0 g, KH_2PO_4 4.0 g, Na_2HPO_4 6.0 g, yeast extract 1.0 g, agar 18.0 g, DW 1000 ml.

Motility test Each isolate was spot-inoculated on the centre of semi-solid nutrient agar plates (0.2% agar) and incubated at 30°C. The diffusion of colony was observed and recorded at 24 hours (Elbeltagy *et al.*, 2000).

IAA production test 5µl of log phase culture was inoculated in 5ml of LB (Luria Bertani) broth alone and LB broth medium amended with L-tryptophan at the rate of 100 µg/ml. The test tubes were covered with brown paper and incubated at 28°C for 24 hours on a rotary shaker. The broth was centrifuged at 10,000 rpm for 15 minutes. 2 ml of supernatant was collected and 2 – 3 drops of *o*-phosphoric acid were added. The aliquots were shaken, added 4ml of reagent (1 ml of 0.5 M $FeCl_3$ in 49 ml of 35% perchloric acid ($HClO_4$)) and votexed thoroughly. The samples were incubated at room temperature for 25 minutes and their absorbance was read at 530 nm. Auxin quantification value was recorded by extrapolating calibration curve made by using IAA as standard (10 - 100µg/ml) (Gordon and Weber, 1951). ***Luria Bertani Broth*** Bacto-Tryptone 10.0 g, yeast extract 5.0 g, NaCl 5.0 g, DW 1000 ml.

Antibiotic resistance test The test isolates were spot-inoculated on the nutrient agar plates incorporated with filter sterilized

streptomycin at the rate of 100µg/ml and incubated for 48 hours at 30°C. The antibiotics resistance was recorded as positive if the test colony appeared on the plates, as compared to the control plate in which no antibiotic was added.

Fluorescence pigment production test The test isolates were spot-inoculated on the King's B medium agar plates and incubated at 30°C. The plates were exposed to UV light to examine the fluorescence ability after 24-48 h of incubation.

Identification with BIOLOG system Eleven Gram-positive bacterial isolates were tested for C-source utilization pattern and identified using Biolog System kits. Bacterial isolates were raised on Biolog Universal Growth (BUG) medium and 24 hour growing cultures were then suspended in Phosphate-buffered Saline (PBS), adjusted to required optical density and inoculated to the 96 well Biolog plates, 95 wells contain different C substrates. Plates were incubated at 30°C and observed for color development at intervals of 12 hours. Color development pattern was compared to the database and isolates were identified at species level.

Molecular characterization

Bacterial genomic DNA extraction Bacterial isolates were grown in 10 ml of nutrient broth. Genomic DNA was extracted when the bacterial growth was saturated and followed the protocol described by Masterson *et al.* (1985) with modifications. Bacterial cells were harvested by centrifuging at 8000 rpm for 5 min. Pellet was washed with TE pH 8.0 (*Tris* EDTA buffer) and centrifuged at 6000 rpm for 3 min in at least 3 times. Cells were lysed with 400 µl of TE plus 40 µl of 10% SDS under incubating at 37°C for 30 min. DNA containing supernatant was extracted with phenol and chloroform, precipitated with absolute ethanol plus CH_3COONa, washed with 70% ethanol and diluted in TE buffer.

Polymerase chain reaction (PCR) Amplification of 16S rDNA gene was carried out by polymerase chain reaction using a thermalcycler (M.J. Research PTC-100). The amplification reactions were performed in a 50 µl volume by mixing template DNA with polymerase reaction buffer (10X), 1.5 mM $MgCl_2$, 200 µM dNTPs, primers p13B and PCR-1 (10 pM each) and 1 U *Taq* Polymerase (Zinniel *et al.* 2002).

Primer sequences:

p13B(5'-AGGCCCGGGAAGGCGTATTCAC-3') (IDT, Inc., USA)
PCR-1 (5'-AGTTTGATCCTGGCTCAGGA-3') (IDT, Inc., USA)

The thermocycling conditions consisted of an initial denaturation at 94°C for 3 minutes, 30 amplification cycles of 94°C for 1 minute (denaturation), 57°C for 1 minute (annealling), and 72°C for 2 minute (extension) and final polymerization at 72°C for 4 minutes.

Restriction fragment length polymorphism (RFLP) analysis Aliquots of purified PCR products (3 – 5 µl) were digested with 1.5 U of restriction endonuclease in 25 µl reaction volume by using the manufacturer's recommended buffer (2.5 µl of 10X), final volume of reaction was adjusted by adding water. The following endonucleases were used *Hae*III, *Mbo*I and *Msp*I.

Data analysis The RFLP profile was analysed using GeneProfiler software and dendrograms were constructed using TreeCon software. Each band produced with a particular restriction enzyme was scored across all the samples. The data were entered in a matrix in which all observed bands were listed, and was used to calculate Jaccard's similarity coefficient for each pairwise comparison.

Jaccard coefficient = a/n

Where, a = number of matching bands for each pair of comparisons

n = total number of bands in two samples observed.

Dendrograms were constructed from the similarity matrix by unweighted pair group method with arithmetic mean (UPGMA).

RESULTS

1. Population size of endophytic bacteria

Serially diluted macerate was plated on TSA and PDA and colony forming units (cfu) were determined after appropriate incubation at 30°C. Population dynamics of the endophytic bacterial isolates in the various plant tissues of

the two hosts are given in Table 1. Nodule tissue of both hosts *G. soja* (8.4×10^5) and *G. max* (5.3×10^7) supported more number of bacterial isolates than either stem or root tissues. The root tissue of both hosts supported least number of bacteria on both media. In general, PDA supported more of endophytic bacterial growth than TSA.

Table 1. Endophytic bacterial population recovered at flowering stage (CFU g^{-1} FW)

Host plant	PDA			TSA		
	Nodule	Root	Stem	Nodule	Root	Stem
Glycine max	5.3×10^7	1.8×10^5	2.2×10^5	9.3×10^6	2.3×10^4	7.5×10^3
Glycine soja	8.4×10^5	1.3×10^4	2.1×10^5	4.1×10^6	2.1×10^5	3.6×10^5

PDA – Potato Dextrose Agar TSA – Tryptic Soy Agar FW – Fresh weight

2. Morphological and physiological characterization

There was a large variation in colony morphology – color, shape and size (data not shown). Equal number of Gram positive and Gram negative bacteria were found in the two hosts, however in individual tissue the number varied. Out of 21 Gram positive isolates of *G. max*, 5 were from nodule and root tissue each and 11 from stem. Thirteen of a total of 15 Gram negative bacteria of *G. max*, were from nodule and only one each from root and stem tissues. From *G. soja* out of 11 Gram positive isolates only one was from stem. Gram negative isolates were more or less equally distributed in *G. soja* though stem gave least recovery (data not shown).

Of the 65 isolates screened 13% and 12% from *G. max* and *G. soja*, respectively formed capsules (Table 2). When grown on 0.2% agar, 78% of the isolates were found to be motile. Seventy-five percent were endophytes from *G. max* and 81% were from *G. soja* (Table 2).

All the 65 endophytic bacteria were screened for growth on NA amended with Streptomycin @ 100 µg/ml (Table 2). Twenty-one of the endophytes were able to grow in the presence of the antibiotic. In total, 8 Gram negative and 7 Gram positive bacteria from *G. max* and 4 –negative and 2 – positive from *G. soja* showed resistance to Str_{100} (data not shown).

When grown on King's B medium (specific for fluorescent Pseudomonads), only seven were found to be putative fluorescent pseudomonads (Table 2).

Seventeen isolates from *G. max* and three from *G. soja* gave a clear zone of hydrolysis on pectin agar plate. About 89% of *G. soja* isolates did not display pectinase activity. When grown on cellulase medium, 28 of the endophytes from *G. max* and 16 of *G. soja* were able to grow, utilizing the C-source with the production of cellulase enzyme (Table 2).

Table 2. Number of isolates reacted in screening tests

Test	*G. max*		*G. soja*	
	Positive	Negative	Positive	Negative
Motility	29 (78)	9 (25)	22 (81)	5 (19)
Cellulase	28 (74)	10 (26)	16 (59)	11 (41)
Pectinase	17 (47)	19 (53)	3 (11)	24 (89)
Fluorescence	4 (11)	34 (89)	3 (12)	24 (88)
Antibiotic resistance	15 (42)	21 (58)	6 (22)	21 (78)
Capsule formation	13 (34)	25 (66)	12 (44)	15 (56)

Number in brackets is expressed in percentage.

BIOLOG identification

Differentiation and identification of 11 Gram positive bacterial endophytes were done using BIOLOG MicroStation System – an automated identification system. Test result yielded a characteristic pattern of substrate utilization of each endophyte, which was compared to a current database. Table 3 gives the identity of the 11 isolates. Isolates number 136, 72 and 32 gave a similarity index less than 0.5.

Table 3. Identification of gram-positive isolates based on BIOLOG system

Isolate	Host	Tissue	Name	Probability
5	*Glycine max*	Nodule	*Deinococcus radiophilus*	77%
72	*Glycine max*	Nodule	*Staphylococcus lentus*	-
32	*Glycine max*	Root	*Bacillus racemilacticus*	-
11	*Glycine max*	Stem	*Clavibacter michiganensis ss michiganensis*	88%
18	*Glycine max*	Stem	*Leuconostoc fallax*	88%
109	*Glycine soja*	Nodule	*Bacillus fastidiosus*	90%
113	*Glycine soja*	Nodule	*Tsukamurella inchonensis*	90%
121	*Glycine soja*	Nodule	*Tsukamurella inchonensis*	97%
106	*Glycine soja*	Root	*Bacillus fastidiosus*	90%
107	*Glycine soja*	Root	*Bacillus laevolacticus*	71%
136	*Glycine soja*	Root	*Bacillus laevolacticus*	-

IAA production

After 4-5 days of growth under dark and shaking conditions, the presence of IAA in the medium was detected. Out of 65 endophytes 15 produced IAA more than 25 µg/ml in the presence of the precursor tryptophan. Of these 15 isolates, 10 were Gram negative and 5 – positive bacteria. Nine isolates did not produce any IAA, even when amended with tryptophan (Fig. 1).

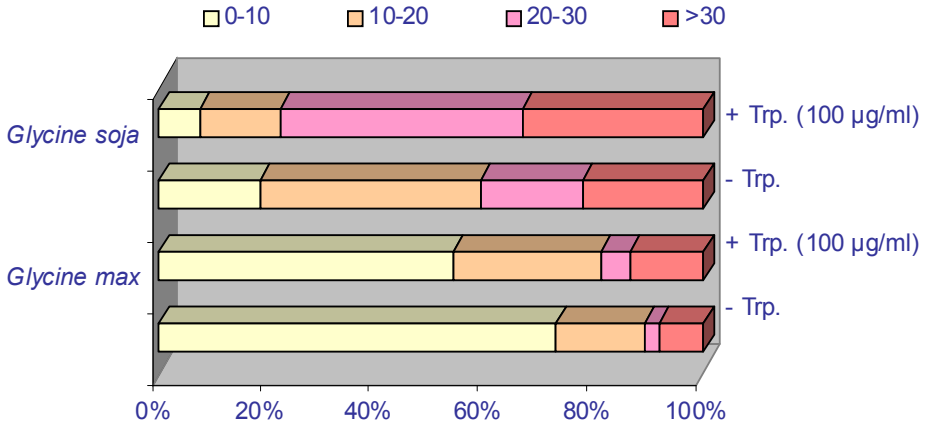

Fig. 1: *In vitro* IAA production (µg/ml) by endophytes from *G. max* and *G. soja*

3. Molecular characterization

RFLP analysis of 16S rDNA

The PCR amplified product had the size of 1.3 kb to 1.4 kb as produced by all isolates was restricted using three tetracutters. Nine to sixteen distinct restriction patterns with two to five restricted fragments per pattern were detected in endophytes from *G. max*. Similarly, eight to fifteen distinct restriction patterns with one to seven fragments per

pattern were detected in endophytes from *G. soja*.

The sum of the restricted products was approximately the same as the size of the amplified PCR product of 16S rDNA viz. 1.3 kb – 1.4 kb. Nine and eleven restriction patterns were obtained by these enzymes in *G. max* and *G. soja* endophytes, respectively. *Hae*III was least discriminatory of the three enzymes for *G. max* endophytes. Restriction enzyme *Mbo*I was least discriminatory among the 3 endonucleases used for *G. soja* isolates, and gave 8 restriction patterns. Among the 3 endonucleases, *Msp*I was the most discriminatory and could distinguish closely related isolates.

Dendrogram construction

The consolidated dendrogram generated by pooling all restriction products of the 35 isolates is presented in Fig. 2. Cluster analysis revealed two major clusters, I and II. Cluster I was heterogeneous consisting of two sub-clusters Ia and Ib. Cluster Ia members showed a genetic closeness ~62% (except I-113). Cluster Ib members showed genetic distances between 0.1 – 0.41. These isolates were similar to each other by 57%. Cluster II comprised of endophytes with genetic distances ranging 0.1 – 0.52. Three isolates in this cluster I-107, I-112 and I-129 showed 100% similarity. 16S rDNA PCR-RFLP separated these two clusters at 40% similarity coefficient value.

Six of the endophytes I-25, I-15, I-68, I-137, I-8 and I-121 did not fall in the two clusters. These were genetically divergence by more than 78% to either of the two clusters. The genetic distances among these 6 endophytes ranged among 0.78 – 0.90.

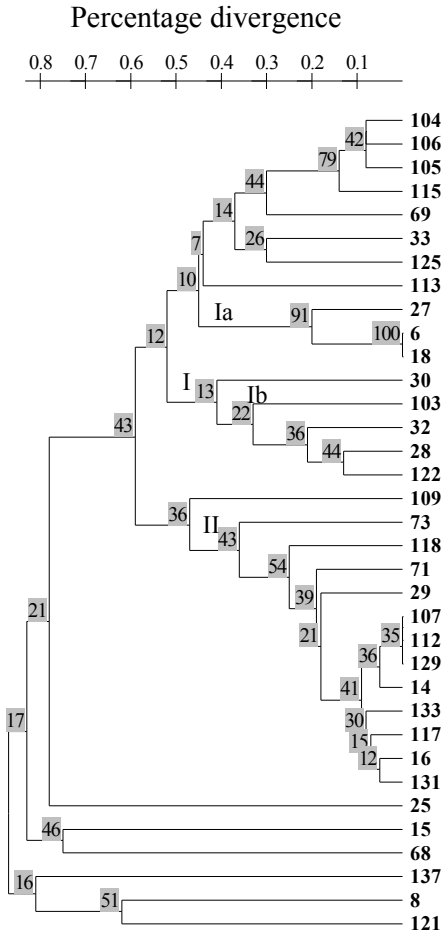

Fig. 2: Dendrogram constructed using cluster analysis of endophytes. The analysis was done using unweighted pair grouping method based on arithmetic averages (UPGMA)

DISCUSSION

Soybean shows a remarkable diversity because of its long history of cultivation and selection under various climatic, edaphic and biotic environments in geographically diverse areas. These two may harbour unique populations of endophytic bacteria. It is well established that plant bacterial endophytes are to be found in most healthy plant tissues (Frommel *et al*. 1993; McInroy and Kloepper 1995; Sturz 1995). This particular host-endophyte interaction has been variously defined as altruism, commensalisms, symbiosis or passivity to pathogenicity. Whatever the specific relationship(s) involved, internal plant colonization by bacteria constitutes a vast and, as yet, little mapped ecological niche.

This investigation is to describe indigenous bacterial endophytes isolated from two species one cultivated (*G. max*) and one wild (*G. soja*) of soybean. There was significant variation both phenotypic and genotypic in the types of indigenous bacteria. Several factors may explain these differences, including host specificity and tissue types.

The diversity of a collection of sixty five putative endophytic bacteria isolated from different tissues of the hosts was assessed using phenotypic and genotypic characterization methods. Colony morphology gave an indication of the variation among the endophytes. The isolates studied were chosen for their dominance as well as uniqueness or differences with other in colony morphology. Interestingly, Gram positive and Gram negative isolates were equally distributed between two species *G. max* and *G. soja*. Earlier workers have reported a predominance of Gram negative bacteria in the tissues of various plants (Stoltzfus *et al*. 1997; Elbeltagy *et al*. 2000). However, Zinniel *et al*. (2002) reported an equal presence of Gram negative and Gram positive bacteria.

All the isolates from surface-sterilized soybean nodules tested carried non-*B. japonicum* bacteria, most of these were morphologically distinct. Endophytic bacteria have been isolated from legume root nodules previously. For example, Sturz *et al.* (1997) characterized 15 bacterial species from red clover nodules and estimated endophyte population densities to be in the range of 10^4 viable bacteria per g fresh nodule. In plant tissue in general, endophytic bacterial populations have been reported between 10^2 to 10^4 viable bacteria per gram (Kobayashi and Palumbo 2000). In our study, endophytic population was highest in the nodule tissue. There is much debate as to how to define an endophyte (reviewed in Kobayashi and Palumbo 2000). For example, Hallmann *et al.* (1997) suggested that bacteria that are isolated from surface sterilized plant tissues, and that do no apparent harm to the plant, could be considered endophytes. Other definitions suggest that it is necessary to demonstrate that the bacterial colonization is of internal plant tissues.

Colony morphology, and BIOLOG tests indicated that five of the Gram positive nodule isolates to be *Deinococcus radiophilus, Staphylococcus lentus, Bacillus fastidiosus* and two *Tsukamurella inchonensis*. Bai *et al.* (2002) also reported *Bacillus* sp. from nodules of soybean. Our assay system to evaluate the functions and persistence of endophytic bacteria in soybean tissues generally showed common traits for pectinase, cellulase and motility. Hydrolytic enzymes, pectinases and cellulases may play a role in the mechanisms by which endophytic bacteria penetrate into and persist in the host plant (Hallmann *et al*., 1997; Reinhold-Hurek and Hurek, 1998). However, except for Elbeltagy *et al*. (2000), no survey on the secretion of these enzymes by endophytes has been conducted. Because they act as virulence factors for pathogenic bacteria of plants, these enzymes might be involved in the invasion of host plants by endophytes, as reported for *Azoarcus* sp. (Hurek *et al.*, 1994) and *Enterobacter asburiae* JM22 (Quadt-Hallmann and Kloepper, 1996). In our study, 33% of the isolates secreted pectinases, and 70% produced cellulases. More than 80% were motile. Our findings are in congruence to those of Elbeltagy *et al*. (2000). Due to the motility of these endophytes, the pectinolytic activity may confer an advantage for intercellular ingress and spreading of endophytes into the host plant, the cell wall of the host plant contain cellulose, whereas the

middle lamella between cell walls contain mainly pectin (Hallmann *et al*. 1997).

Genotyping of the endophytes was done by PCR-RFLP of 16S rDNA. Multiple restriction digests uncovered distinct genotypes. The 16S rDNA genes were subjected to standard PCR protocol that readily amplified large amounts of DNA approximately 1.3 – 1.4 kb in size, corresponding to the predicted size of the small subunit ribosomal genes. Based on preliminary screening using 5 tetrameric restriction enzymes available to us, three restriction enzymes *Hae*III, *Mbo*I and *Msp*I were selected for use in restriction fragment length polymorphism (RFLP). RFLP analysis detected 17 and 15 genotypes in *G. max* and *G. soja* respectively (data not shown). A majority of 29 endophytes from the two hosts were distributed in two well separated clusters of 46-54% internal homology (Fig. 2). The other 6 endophytes were found distinct to the members of these two clusters, showing genetic divergence between 0.6 to 0.9 (Fig. 2). Interestingly, I-137 from *G. soja* root tissue was found to be distinct from others genotypically as well as phenotypically. This Gram positive bacteria did not produce cellulase and pectinase enzymes, did not fluoresce and was non-motile. It showed resistance to Str_{100} and formed capsules. I-137 showed a genetic divergence of more than 0.85 distance unit to all others.

The genetic heterogeneity was more in the endophytes of *G. max* as compared to *G. soja*. RFLP profiles of isolates I-6 and I-18 were identical, so also for isolates I-107, I-112 and I-129. The three enzymes could not differentiate them. Among the three enzymes, *Msp*I was found most discriminatory. In few cases, sequence divergence of 16S rDNA was detected. The banding patterns compared were frequently found to contain surplus fragments of DNA, adding to the ~1.3-1.4 kb analyzed. As these were weak, these fragments were easy to recognize and eliminate from the analysis. Most importantly they were checked for partial digestion and were found not to be the case. Others have also expressed such intraspecies sequence divergence (Balakrishnan 2002).

This study demonstrated the occurrence and diversity of culturable endophytes in soybean species. This can be utilized in future application, such as delivery of degradative enzymes for controlling certain plant diseases or other useful products.

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SUMMARY IN VIETNAMESE

Phân lập và tìm hiểu đặc tính vi khuẩn trong đậu tương

Vi khuẩn trong đậu tương trồng và đậu tương hoang dại được phân lập trên môi trường không chuyên tính và tìm hiểu một số đặc điểm. Kết quả bước đầu cho thấy số nòi vi khuẩn gram âm và gram dương trong đậu tương có số lượng tương tự nhau (51% và 49%). Trên 80% số chủng vi khuẩn thử nghiệm có khả năng di chuyển được, 33% tiết ra men phân hủy pectin, 70% có khả năng tiết ra men phân giải xen lu lô và 17% không sản sinh ra auxin trong điều kiện *in vitrô*.

35 chủng vi khuẩn (17 từ *G. max* và 18 từ *G. soja*) được chọn ra để tìm hiểu đa dạng di truyền với phương pháp PCR-RFLP trên đoạn gen 16S rDNA. Phản ứng nhân bản khuyếch đại gen 16S rDNA sử dụng các đoạn mồi phổ rộng (PCR-1 và p13B) cho sản phẩm có độ dài 1,3 – 1,4 kb. Phân tích cắt tới hạn sử dụng 3 men giới hạn *Hae*III, *Mbo*I và *Msp*I có độ dài trình tự nhận dạng là 4 nu-clê-ô-tit. 17 kiểu gen (các chủng vi khuẩn trên đậu *G. max*) và 15 (trên *G. soja*) đã được xác định. Men *Msp*I cho kết quả đa hình cao nhất trong phân tích di truyền đối với vi khuẩn ở cả hai loài đậu tương. Đa số vi khuẩn được chia thành hai nhóm ở mức 48% và 43% về độ đồng đều di truyền, chỉ có sáu chủng không thuộc vào hai nhóm này thể hiện tính đa dạng cao. Tính đa dạng di truyền giữa các chủng vi khuẩn phân lập từ *G. max* cao hơn so với các chủng trên *G. soja*.