

出國報告（出國類別：會議及訪問考察）

參加 2015 東京生命科學與生物工程國際研討  
會，並至「日本東京大學土木工程學系」研  
討監測技術研究之合作事宜

服務機關：國立嘉義大學

姓名職稱：李嶸泰助理教授

派赴國家：日本東京

出國期間：104 年 11 月 29 日至 12 月 6 日

報告日期：105 年 1 月 5 日

## 摘要

本次赴日主要為參加 2015 東京生命科學與生物工程國際研討會，發表接受科技部補助研究計畫之研究成果。會議中，向他國與會學者專家說明本團隊之研究主要係由臺灣相思樹(*Acacia confuse*)分離出之根瘤菌 *Bradyrhizobium*，經試驗發現有接種根瘤菌之苗木除了其地上部之生長量有明顯較未接種苗木佳外，其地下根系之生長亦較佳於未接種組。未來可實際於崩塌地進行造林時應用，可增進保土固土之能力。

另近年臺灣對於防災避災的新技術與新方法之研究愈來愈受重視，所以順道參訪東京大學土木工程學系，對於邊坡滑動監測技術與方法進行交流與學習，期對於未來的研究和教學工作有所助益。

## 目次

一、目的 .....	1
二、過程 .....	4
三、心得及建議事項 .....	14

## 一、目的

本次出國主要目的為參加 2015 東京生命科學與生物工程國際研討會，發表接受科技部補助研究計畫之研究成果；另一則是參訪東京大學土木工程學系，對於邊坡滑動監測技術與方法進行交流與學習，期對於未來的研究和教學工作有所助益。茲分為目標、主題、緣起及預期效益等四項說明如下：

### (一)目標：

1. 完成科技部補助計畫之研究成果發表，希望藉由國際性會議的參與提升本研究之國際能見度並與同領域研究人之交流。
2. 研習交流邊坡監測技術，期可應用於臺灣森林坡地，並期可向政府單位申請防災研究計畫。

### (二)主題：

1. 2015東京生命科學與生物工程國際研討會，發表論文題目為「A *Bradyrhizobium* Starin AC1 Isolated from *Acacia confusa*」。
2. 訪問日本東京大學土木工程學系考察「邊坡監測」之新方法與科研成果，並討論未來合作交流事宜。

(三)緣起：

因申請科技部計畫時核準補助出席國際學術會議差旅費，且研究成果之時程與領域正好與「2015 東京生命科學與生物工程國際研討會」可互相配合，故就決定至日本參加此次會議，並發表論文。

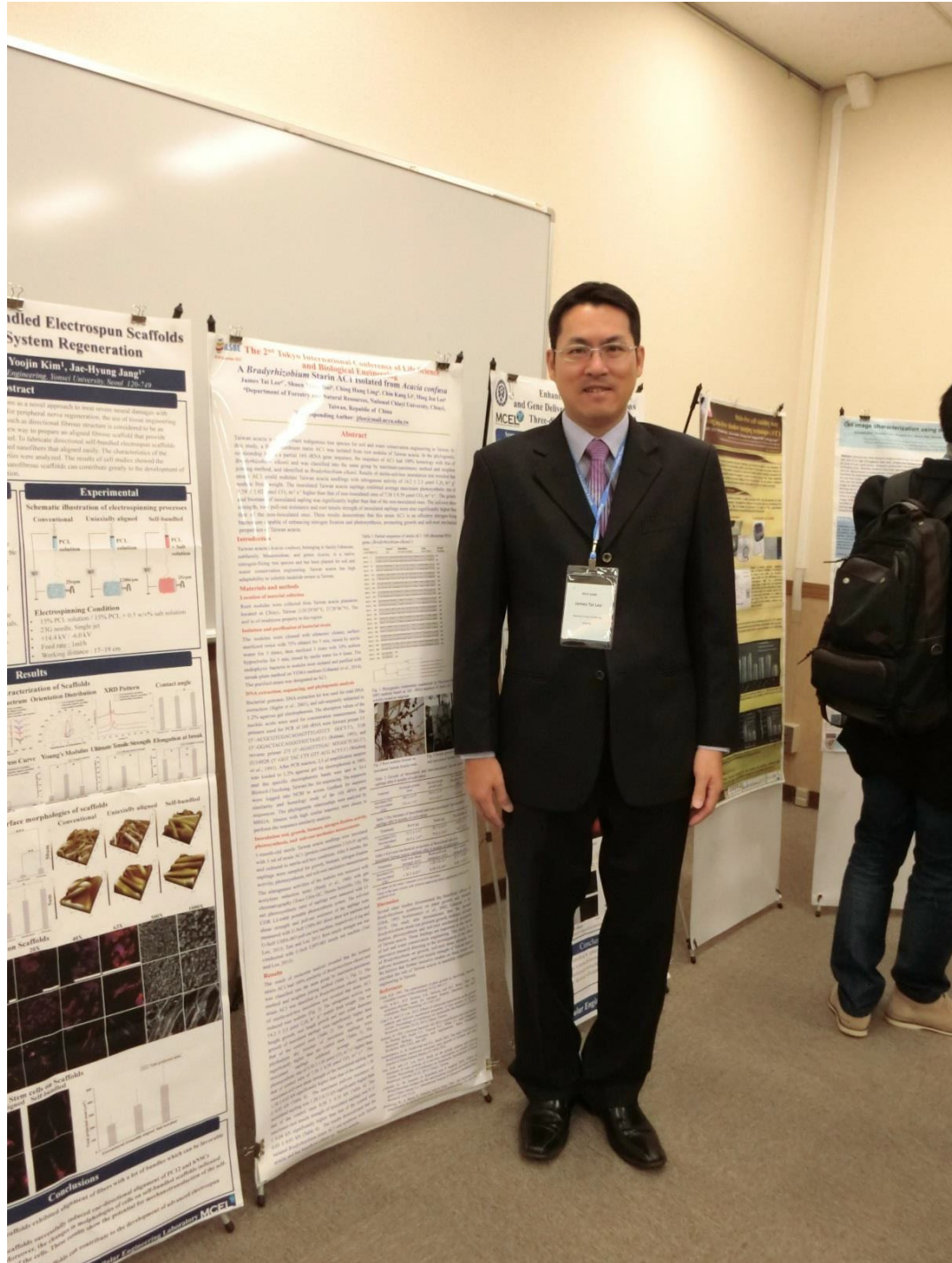
(四)預期效益：

1. 將研究成果發表於國際研討會中，可藉由國際性會議的參與提升本研究之國際能見度，同時增進計畫研究人員與同領域之國際研究人才之學術交流，強化本職學能。
2. 研習與交流邊坡監測新技術與研究方法，期可借鏡並推廣到臺灣山坡地高崩塌潛勢地區使用，以發揮經濟且有效之防災功能。同時，亦可做為申請國內相關機關之防災研究計畫之參考資料。

## 二、過程

本人目前正於阿里山森林鐵路沿線第一分道進行現地監測研究工作。主要係因近年來，該區域曾發生鐵軌不均勻沉陷及擋土牆開裂情形，為了解該邊坡滑動程度，以避免發生大規模崩塌滑動，而危及森林鐵路安全，故進行相關計畫。但因若僅採用傳統常用之測傾管進行監測，則可能因埋置深度未穿越滑動面固定於不動點(岩盤)，導致監測儀器與坡體同時發生滑動，而發生量測不到相對位移變化量或是量測數據有飄移之情形。因此，該研究計畫之監測工作除傳統測傾管量測外，同時採用精巧型監測儀器(含坡面傾斜感應計、土壤含水量水份計、現場資料記錄器及資料處理軟體等)，沿結構物開裂處或地表有明顯張力裂縫處設置，並進行24hr的自動數據蒐集記錄。再透過定期與不定期量測該邊坡上既有之測傾管，分析比對測傾管變位資料及精巧型監測儀器之成果。

(一) 研討會論文發表：



以海報型式進行發表

## Electrospun Scaffolds generation

**Sae-Hyung Jang<sup>1</sup>**  
*University Seoul 120-749*

To treat severe neural damages with generation, the use of tissue engineering scaffolds structure is considered to be an ideal fibrous scaffold that provide small self-banded electrospun scaffolds and easily. The characteristics of the scaffold structure was analyzed in a results of cell studies showed the an contribute greatly to the development of neural tissue.

### Experimental

Preparation of electrospinning processes

- Uniaxially aligned
- Self-banded

Electrospinning Solution: 15% PCL + 0.5 w/v salt solution in CHCl<sub>3</sub> solvent  
Single Jet  
Voltage: 15.0 kV  
Flow rate: 0.3 ml/h  
Distance: 17-19 cm

### Characterization

FTIR, XRD Pattern, Contact angle

### Cell Culture

Uniaxially aligned, Self-banded

### Conclusions

The uniaxially aligned PCL scaffold could be used for the reconstruction of neural tissue.

## The 2nd Tokyo International Conference of Life Sciences and Biological Engineering

### A Bradyrhizobium Starin AC1 Isolated from Acacia confusa

James Lai Lee<sup>a</sup>, Shuen-Ming Tsai<sup>b</sup>, Ching-Hung Ling<sup>a</sup>, Chin-Kang Li<sup>a</sup>, Ming-Jen Lee<sup>a</sup>  
<sup>a</sup>Department of Forestry and Natural Resources, National Chiayi University, Chiayi, Taiwan, Republic of China  
<sup>b</sup>Corresponding Author: hlee@mail.nyu.edu.tw

**Abstract**  
Taiwan acacia is an important indigenous tree species for soil and water conservation engineering in Taiwan. In this study, a Bradyrhizobium starin AC1 was isolated from root nodules of Taiwan acacia. In the phylogenetic relationship based a partial 16S rRNA gene sequence, the sequence of AC1 had 100% homology with that of Bradyrhizobium elkanii and was classified into the same group by maximum-likelihood parsimony method and neighbor joining method, and identified as Bradyrhizobium elkanii. Results of root-nodule assay inoculation test revealed that strain AC1 could nodulate Taiwan acacia seedlings with nitrogenase activity of 142.2 ± 2.3 μmol C<sub>2</sub>H<sub>4</sub> hr<sup>-1</sup> g<sup>-1</sup> nodule fresh weight. The inoculated Taiwan acacia saplings exhibited average maximum photosynthetic rate of 9.58 ± 1.02 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> higher than that of non-inoculated ones of 7.38 ± 0.39 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. The growth and biomass of inoculated sapling was significantly higher than that of the non-inoculated ones. The soil-root shear strength, root pull-out resistance and root tensile strength of inoculated saplings were 21% significantly higher than that of the non-inoculated ones. These results demonstrate that this strain AC1 is an effective nitrogen-fixing bacterium capable of enhancing nitrogen fixation and photosynthesis, promoting growth and soil-root mechanical properties of Taiwan acacia.

**Introduction**  
Taiwan acacia (*Acacia confusa*, belonging to Family Fabaceae, subfamily Mimosoideae, and genus *Acacia*, is a native nitrogen-fixing tree species and has been planted for soil and water conservation engineering. Taiwan acacia has high adaptability to infertile landless terrain in Taiwan.

**Materials and methods**  
**Location of material collection**  
Root nodules were collected from Taiwan acacia plantation located at Chiayi, Taiwan (207°29'09"E, 23°28'06"N). The soil of nodules property in this region.

**Isolation and purification of bacterial strain**  
The nodules were cleaned with 75% ethanol, clamped, surface sterilized twice with 75% ethanol for 5 min, rinsed by sterile water for 3 times, then sterilized 3 times with 10% sodium hypochlorite for 5 min, rinsed by sterile water for 4 times. The nodules were incubated and purified with streak plate method on YEMA medium (Ukeshen et al., 2014). The purified strain was designated as AC1.

**DNA extraction, sequencing, and phylogenetic analysis**  
Bacterial genomic DNA extraction was done for total DNA extraction (High et al., 2005), and subsequently subjected to 1.5% agarose gel electrophoresis. The absorption values of the nucleic acids were used for concentration measurement. The primer used for PCR of 16S rRNA were forward primer (1) 5'-GAGCTGACAGCAATTTGATCCT GGCCTCA-3', reverse primer (2) 5'-AGATTGTTTAC MTGGGCTAG-3', (3) 5'-GAGCTGAC CTT GTT ACC ACTCT-3' (Munishige et al., 1991). Also PCR reaction, 2.5 μl amplification mixture and the specific electrophoresis bands were used as TM1 were ligated into SMC1 to access GeneBank for sequence alignment and homology study of the 16S rRNA gene. Multiple sequence alignments were analyzed by Clustal W software with high similar sequence were chosen to perform the sequence similarity analysis.

**Nitrogenase test, growth, biomass, nitrogen fixation activity, photosynthesis, and soil-root mechanical measurements**  
Inoculated with Taiwan acacia seedlings were inoculated with 3 ml of strain AC1 genome concentration (2.5 × 10<sup>8</sup> cfu/ml) and cultured in sterile-soil-box conditions. After 6 months, the saplings were sampled for growth, biomass, nitrogen-fixation activity, photosynthesis, and soil-root mechanical measurements.

**The nitrogenase activity of the nodules was measured with acetylene reduction assay (Harley et al., 1968) with gas chromatography (Fluxus CEC, Fluke Scientific, USA). The rate of acetylene reduction was measured with 11.5% CH<sub>4</sub> (400 ppm) portable photosynthesis system. The soil-root shear strength and pull-out resistance of the saplings were measured with (1) Soil Shear Machine (Liang and Lee, 2015) and (2) Soil Pull-out Test Machine (Liang and Lee, 2015). Soil pull-out test machine, respectively (Liang and Lee, 2015). Root tensile strength test was conducted with (3) Soil USP1-003 tensile test machine (Tiao and Lee, 2015).**

**Results**  
The result of molecular analysis revealed that the isolated strain AC1 had 100% similarity of *Bradyrhizobium elkanii* and was classified into the same group by maximum-likelihood parsimony method and neighbor joining method. Results of root-nodule assay revealed that strain AC1 could nodulate Taiwan acacia seedlings with nitrogenase activity of 142.2 ± 2.3 μmol C<sub>2</sub>H<sub>4</sub> hr<sup>-1</sup> g<sup>-1</sup> nodule fresh weight. The soil-root shear strength, root pull-out resistance and root tensile strength of inoculated saplings were 21% significantly higher than that of the non-inoculated ones. The soil, stem and photosynthesis rates of inoculated saplings were significantly higher than those of the non-inoculated ones. The photosynthesis rates of 9.58 ± 1.02 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> higher than that of the non-inoculated ones of 7.38 ± 0.39 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. The soil-root shear strength of inoculated saplings was 1.84 ± 0.09 kN significantly higher than that of the non-inoculated ones of 1.50 ± 0.07 kN (Table 4). The root pull-out resistance of inoculated saplings was 2.28 ± 0.11 kN significantly higher than that of the non-inoculated ones of 1.87 ± 0.10 kN (Table 4). The root tensile strength of inoculated saplings was 0.13 ± 0.01 kN significantly higher than that of the non-inoculated ones of 0.11 ± 0.01 kN (Table 4). The results demonstrated that the strain AC1 is an effective nitrogen-fixing bacterium, promoting growth and soil-root mechanical properties of Taiwan acacia.

**References**  
Harley, J.R., 1968. The measurement of acetylene gas by thin-film electron capture detector. *Journal of Biological Chemistry*, 243, 1441-1443.  
High, R.A., 2005. *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc.  
Liang, K.H., Lee, M.J., Tsai, S.M., Ling, C.H., Lee, C.K., 2015. The growth and biomass of inoculated sapling was significantly higher than that of the non-inoculated ones. *Journal of Agricultural Science*, 155, 1-10.  
Liang, K.H., Lee, M.J., 2015. The soil-root shear strength and pull-out resistance of the saplings were measured with (1) Soil Shear Machine (Liang and Lee, 2015) and (2) Soil Pull-out Test Machine (Liang and Lee, 2015). *Journal of Agricultural Science*, 155, 1-10.  
Munishige, M., et al., 1991. *Journal of Biological Chemistry*, 266, 10885-10890.  
Tiao, C.H., Lee, M.J., 2015. The soil-root shear strength and pull-out resistance of the saplings were measured with (1) Soil Shear Machine (Liang and Lee, 2015) and (2) Soil Pull-out Test Machine (Liang and Lee, 2015). *Journal of Agricultural Science*, 155, 1-10.  
Ukeshen, D., 2014. *Journal of Biological Chemistry*, 289, 15551-15555.  
Liang, K.H., Lee, M.J., 2015. The soil-root shear strength and pull-out resistance of the saplings were measured with (1) Soil Shear Machine (Liang and Lee, 2015) and (2) Soil Pull-out Test Machine (Liang and Lee, 2015). *Journal of Agricultural Science*, 155, 1-10.  
Liang, K.H., Lee, M.J., 2015. The soil-root shear strength and pull-out resistance of the saplings were measured with (1) Soil Shear Machine (Liang and Lee, 2015) and (2) Soil Pull-out Test Machine (Liang and Lee, 2015). *Journal of Agricultural Science*, 155, 1-10.

## Enhanced and Gene Deletion

### MCEL Three-dimensional Culture System

Seung-Hyun Kim<sup>a</sup>, Mihyung Park<sup>b</sup>  
<sup>a</sup>Department of Chemical and Biomolecular Engineering  
<sup>b</sup>Department of Chemical Engineering  
\*Corresponding Author: seung-hyun.kim@yonsei.ac.kr

Stem cell therapies have been studied as a regenerative medicine for various tissues. For the effective therapeutic regeneration of damaged central nervous system, three-dimensional cell clusters-neurospheres are considered as a promising biological functions. Here, "Spheriform" which regulates formation of neurosphere[2]. In this study, we investigated the interactions led to fabrication of a single neurosphere diameter. A correlation with short exposure time in a high potential as a platform technology with a cell culture system.

### Introduction

#### Neural stem cells

- Capability in differentiating into central nervous system (CNS)
- Neurosphere formation in NSCs
- Migration of the NSCs
- Formation control of neurosphere
- The most important in stem cell research is to stimulate NSC's cell in vitro

S. Das et al., *Nat. Clin. Pract. Neurol.*, 2008

#### Catecholamine

- As a polyhydroxy-L-phenol (DOPA) has adhesive ability on any surfaces.
- Self-polymerization of catechol under basic pH condition (DOPA self-polymerization) can be used to change the cell surface wettability.

Hoskins Lee et al., *Science*, 2007

#### Adeno-associated virus

- Nonpathogenic human parvovirus
- Long-term gene expression
- Highly efficient *in vivo* and *in vitro*
- Replication incompetent
- The AAV viral vector could be used to stimulate neural stem cells.

Sloan et al., *PNAS*, 2002

### Enhanced Interfacial Cell Culture

0 min

### Neurosphere Formation

3D Culture, 2D Culture

- A single neurosphere formation in this 3D culture system enhanced cell viability by increasing cellular interactions.

### Transduction Efficiency

3D Culture, 2D Culture

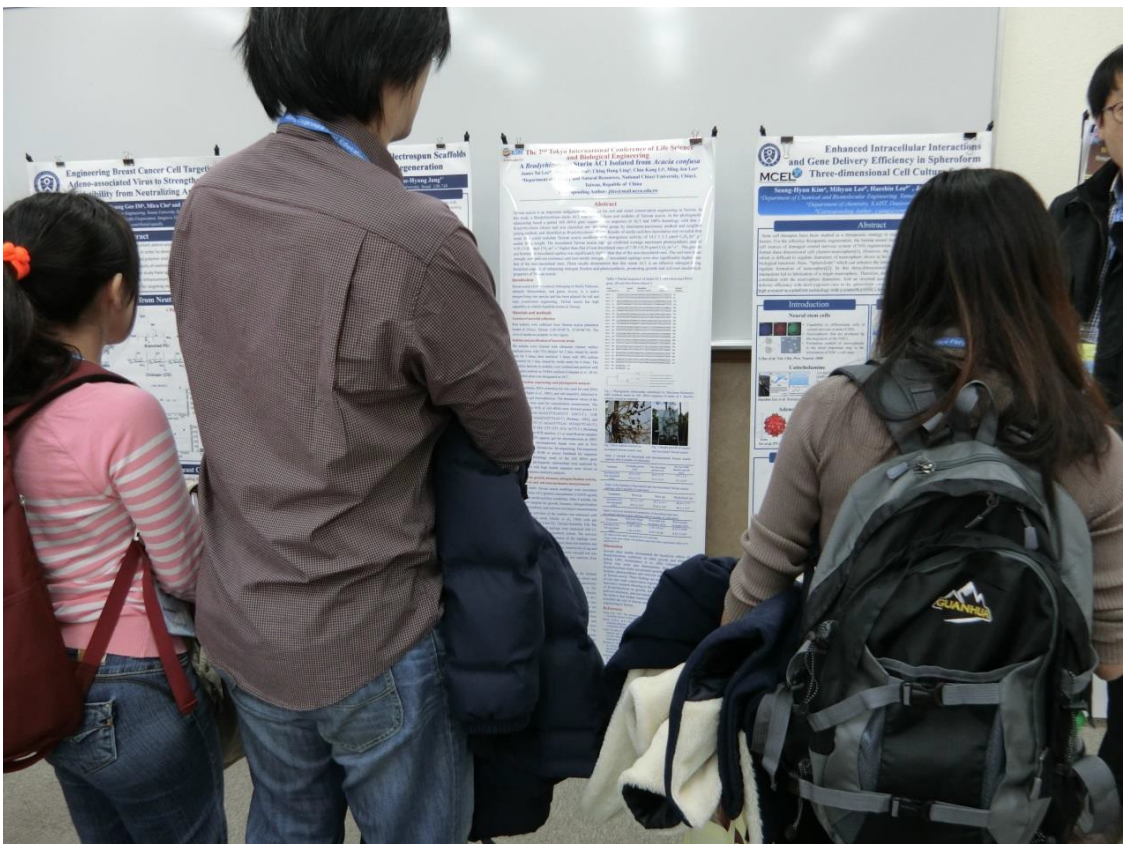
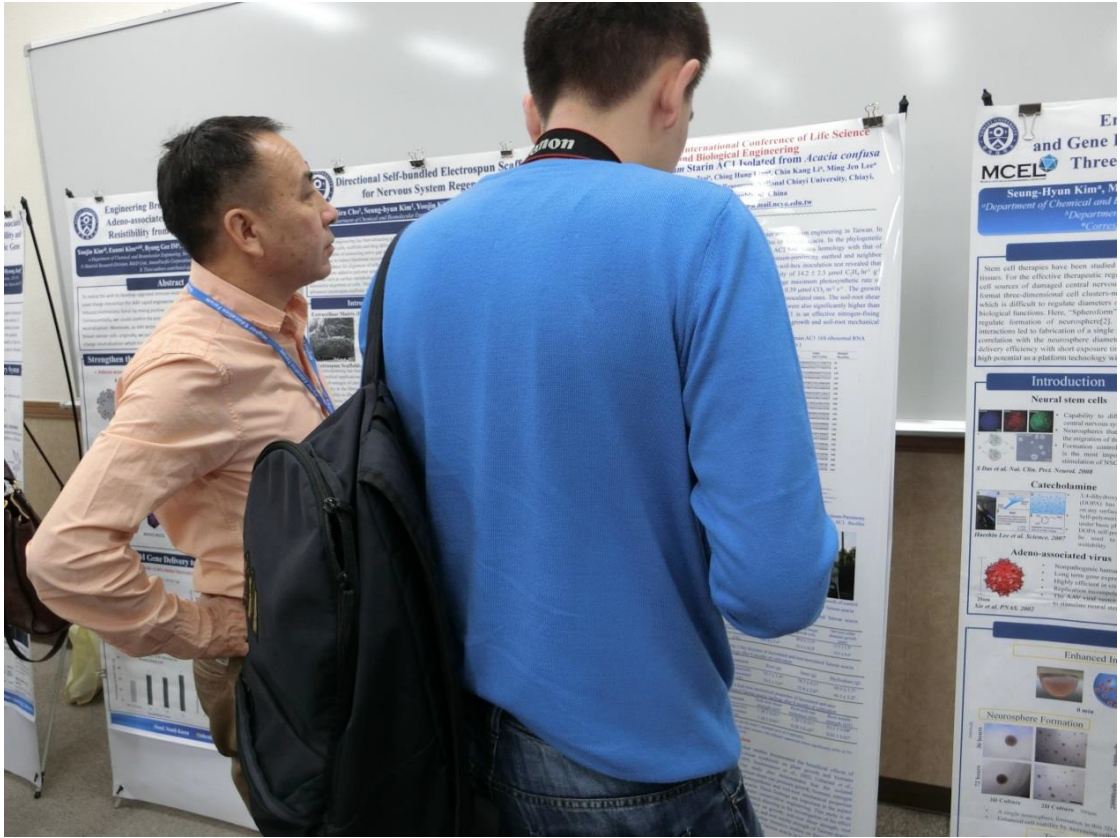
- Improved transduction efficiency by increased interaction between cells.

### Conclusions

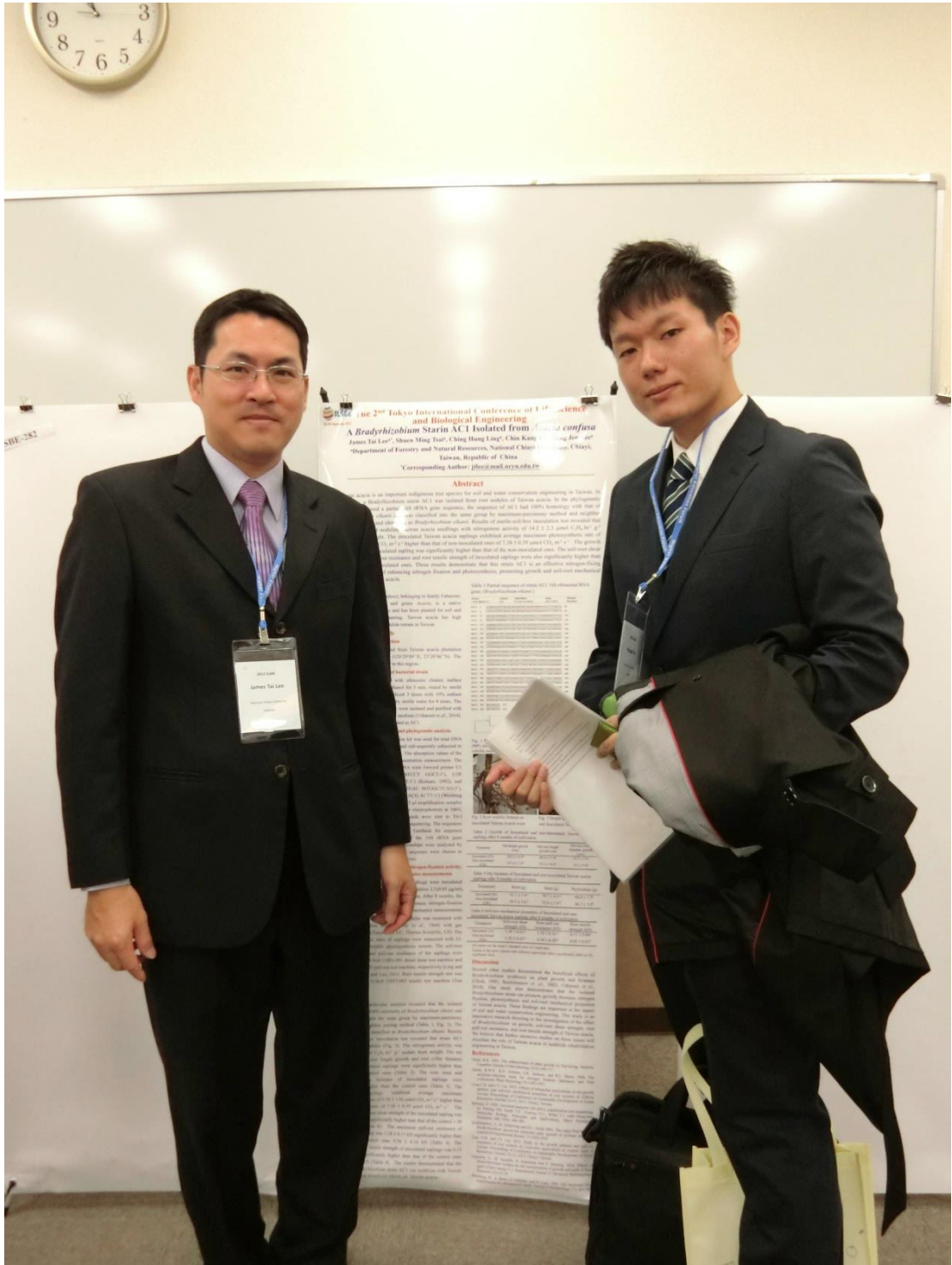
- The size of neurospheres can be controlled by spheriform.
- The three-dimensional cell culture system promotes neurosphere formation by producing reinforced interfacial neuronal stem cells by producing reinforced interfacial.
- The spheriform culture system showed efficient 3D two-dimensional culture system.

Molecular and Cellular Engineering





各國學者專家參訪並討論研究成果情形



與日本學者合影

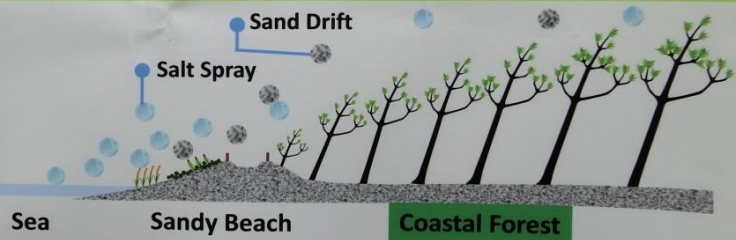
# A Study on the Salt Tolerance of Native Tree Species in Japan for the Establishment of Coastal Forest

## Introduction

The distribution of seedling of arboreous plants growing at the coastal area of Japan and soil environment in some coastal forests were surveyed.

Many of plants growing at the coastal region of Japan are exposed to hazards such as salt spray, high tide and tsunami. These hazards cause growth inhibition of most plant species. And these hazards has bad effect for people who living on coastal area.

In coastal region of Japan, black pine forest has been afforested by the people as the disaster prevention forest, because black pine has high tolerance to natural disaster of coastal region. And these forests are called **Coastal Forest**.



★ Disaster reduction function of coastal forest is attracted socially.

The 2011 off the Pacific coast of Tohoku Earthquake and subsequent huge tsunami occurred in 2011 heavily impacted coastal forest in Tohoku, Japan.



★ Multiple Functional roles of forests are required by people.

- ◆ Changing values of lifestyle in recent years
  - ◆ Rapid spread of pine wilt disease
- Function Required by people
- Biodiversity Conservation
  - Carbon Dioxide Fixation
  - Environmental Education



Scientist from various fields started argument about an improvement of disaster reduction function of coastal forest.

**Planting Broad leaved tree with Black Pine is especially suggested**

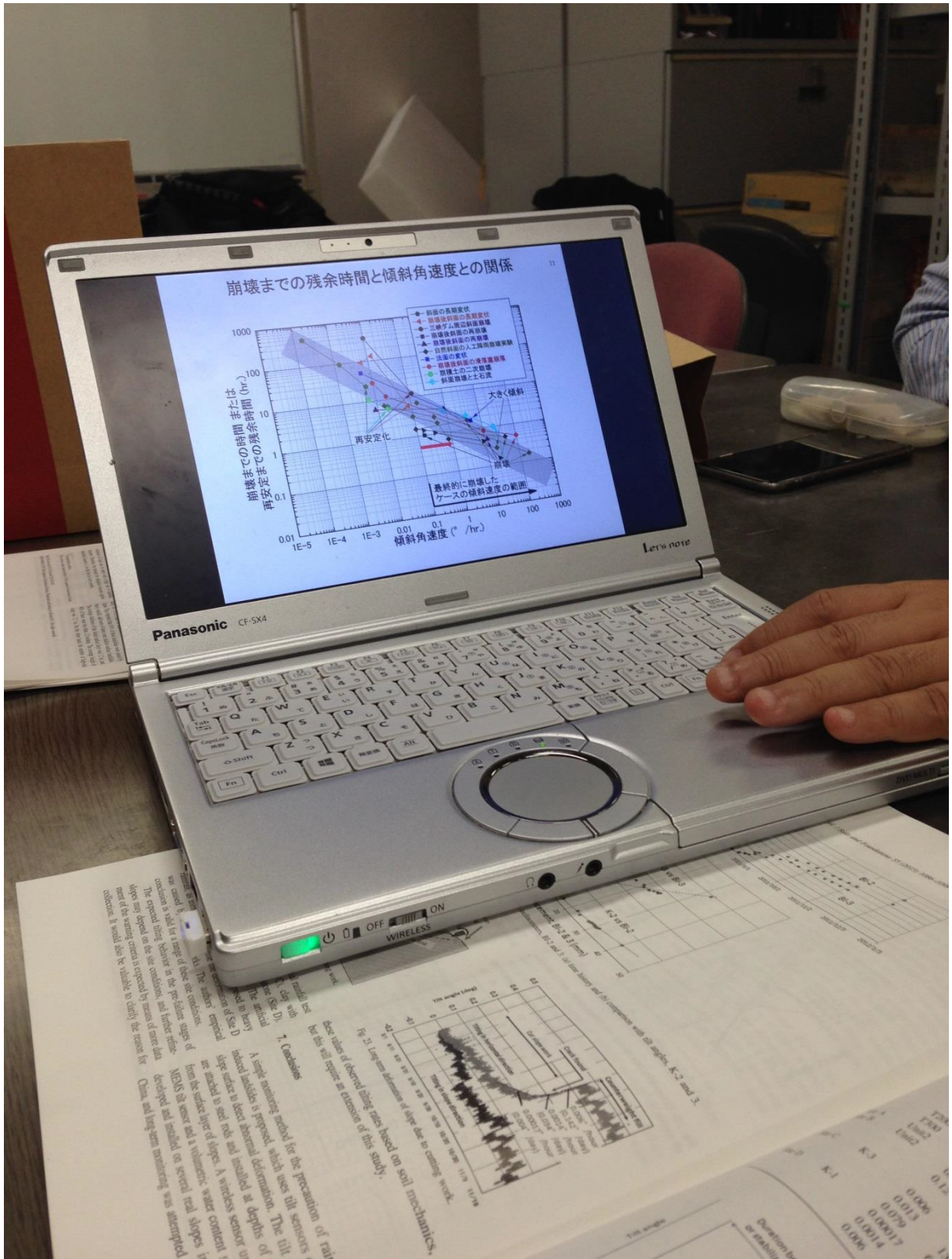
日本海岸林之本土樹種對鹽容忍度的研究

(二) 東京大學土木工程學系參訪：

此次使用到的精巧型監測系統是由日本東京大學內村准教授所研發，故此  
行另外安排與內村教授會面，討論有關警戒值與管理值之訂定標準與分析  
方法，參觀其試驗室及討論試驗內容，最後提到未來寒暑假可派學生至東大進  
行短期研究及交流。



與東大土木系內村教授及王博士討論監測技術



與東大土木系内村教授及王博士討論管理値之訂定方法



室內試驗之試體箱外觀



人工降雨模擬設備



傾斜計



土壤水份計



土壤試驗填築情形



### 三、心得及建議事項

#### (一) 心得

此行除了在國際研討會上有論文發表之外，也在幾天的會議中獲得許多國際新知，讓個人在未來的研究領域上可以持續的進步與成長。

在東京大學的幾天討論中，也是對於監測新技術在日本的應用與研究有了更進一步的認識，在未來研究計畫申請與研究上，也增加了許多寶貴的經驗可供參考；另外，也談到了未來可以互派學生於寒暑假前往交流，如果 105 學年度的暑假可以有學校經費的支持下，可以讓研究生赴日本東京大學交流二個月的話，我想對學生來說是一非常難得的機會。

#### (二) 建議事項

爾後，本系應積極爭取科技部、行政院農業委員會林務局、水土保持局補助進行區域性（如嘉義、雲林）森林水土保持及崩塌地監測及治理計畫，並與海外的大學進行學術交流，以增進水土保育之技術並與世界尖端接軌。