出國報告（出國類別：開會）

第 14 屆 ICCC 國際幾丁質與幾丁聚醣研討會

服務機關：台灣中油股份有限公司煉製研究所
姓名職稱：許明晃 化學工程師
派赴國家/地區：日本
出國期間：107 年 8 月 26 日~107 年 8 月 31 日
報告日期：107 年 9 月 28 日

摘要

ICCC, International Chitin and Chitosan Conference 國際幾丁質與幾丁聚醣研討會，每隔三年定期舉辦；APCCS, Asia-Pacific Chitin and Chitosan Symposium 亞太幾丁質與幾丁聚醣研討會，每隔二年定期舉辦。本次會議為第 14 屆國際幾丁質與幾丁聚醣研討會暨第 12 屆亞太幾丁質與幾丁聚醣研討會聯合舉辦，進行幾丁質與幾丁聚醣之學術交流。會議於 8 月 27 至 30 日在日本大阪府關西大學舉辦，本次會議議程涵蓋國際最新幾丁質幾丁聚醣相關研究，與應用於醫藥、環境、農業領域上等內容，活動包含邀請世界各專家學者演講、海報論文發表及廠商參展等三個部分，會議中包括 115 篇口頭論文發表及 119 篇海報論文，此行瞭解世界幾丁質幾丁聚醣研究與產業應用的最新國際動態，並發表研究論文海報「Adsorption with chitin as the key factor of N-acetylglucosamine production by *Chitinibacter tainanensis*」，會中獲得許多幾丁質相關研究之新知，將來能運用於中油生技原料-利用酵素生產幾丁寡糖製程的研究開發，以及 N-乙醯葡萄糖胺相關應用之研究。

目次

(一)目的

第14屆國際幾丁質與幾丁聚糖研討會(14th International Chitin and Chitosan Conference, ICCC)暨第 12 屆亞太幾丁質與幾丁聚醣研討會(12th Asia-Pacific Chitin and Chitosan Symposium, APCCS)議程涵蓋國際最新幾丁質幾丁聚醣相關研究與醫藥、環境、農業領域應用議題，與生技中心目前進行幾丁質研究相關。藉由參加此研討會，發表研究論文海報「Adsorption with chitin as the key factor of N-acetylglucosamine production by *Chitinibacter tainanensis*」，同時瞭解世界幾丁質幾丁聚醣研究與產業應用的最新國際動態，與國內外專家學者交流，將來能運用於中油生技原料-利用酵素生產幾丁寡糖製程的研究開發，以及 N-乙醯葡萄糖胺相關應用之研究。

(二)過程

ICCC, International Chitin and Chitosan Conference 國際幾丁質與幾丁聚醣研討會，每隔三年舉辦進行幾丁質與幾丁聚醣之學術交流，第 1 屆 ICCC 於 1977 年在美國 Bostan 舉辦，隨後分別於日本 Sapporo (1982)、義大利 Ancona (1985)、挪威 Trondheim (1988)、美國 Princeton (1991)、波蘭 Gdynia (1994)、法國 Lyon (1997)、日本 Yamaguchi (2000)、加拿大 Montreal (2003)、法國 Montperie (2006)、台灣 Taipei (2009)、巴西 Fortaleza (2012)、德國 Munster (2015)舉辦會議。APCCS, Asia-Pacific Chitin and Chitosan Symposium 亞太幾丁質與幾丁聚醣研討會，每隔二年舉辦，第 1 屆 APCCS 於 1994 年在馬來西亞 Bangi 舉辦，隨後分別於泰國 Bangkok (1996)、台灣 Keelung (1998)、日本 Yamaguchi (2000)、泰國 Bangkok (2002)、新加坡 Singapore (2004)、韓國 Busan (2006)、台灣 Taipei (2009)、越南 Nha Trang (2011)、日本 Yonago (2013)、印度 Kochi (2015)舉辦。本次會議為第 14 屆國際幾丁質與幾丁聚醣研討會暨第 12 屆亞太幾丁質與幾丁聚醣研討會聯合舉辦，會議於 8 月 27 至 30 日在日本大阪府關西大學舉辦，本次會議議程涵蓋國際最新幾丁質幾丁聚醣相關研究，與應用於醫藥、環境、農業領域上等內容，活動包含邀請世界各專家學者演講、海報論文發表及廠商參展等三個部分，議程共計四天時間，會議中包括 115 篇口頭論文發表及 119 篇海報論文，其中職參與之海報論文發表「Adsorption with chitin as the key factor of N-acetylglucosamine production by *Chitinibacter tainanensis*」於 8/29 日進行海報展示討論。會議詳細議程如圖 1，會場場地一覽圖如圖 2，會議過程中之照片如圖 3~6。參加此次研討會著重在 N-乙醯葡萄糖胺、幾丁寡醣及幾丁質酶的相關研究議題，下列針對 N-乙醯葡萄糖胺、幾丁寡醣及幾丁質酶的研究議題詳述。

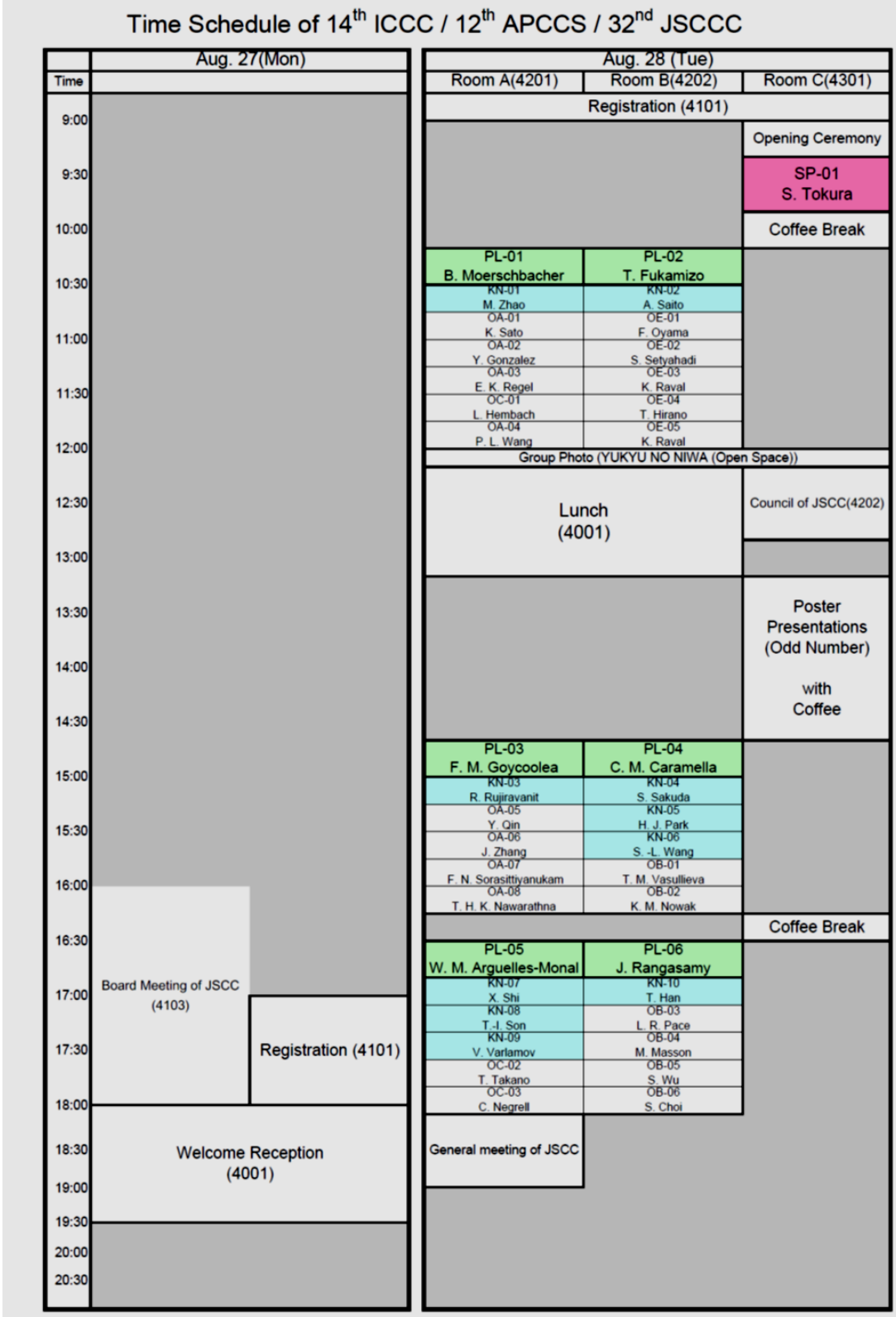


圖 1、14th ICCC 暨 12th APCCS 詳細議程。

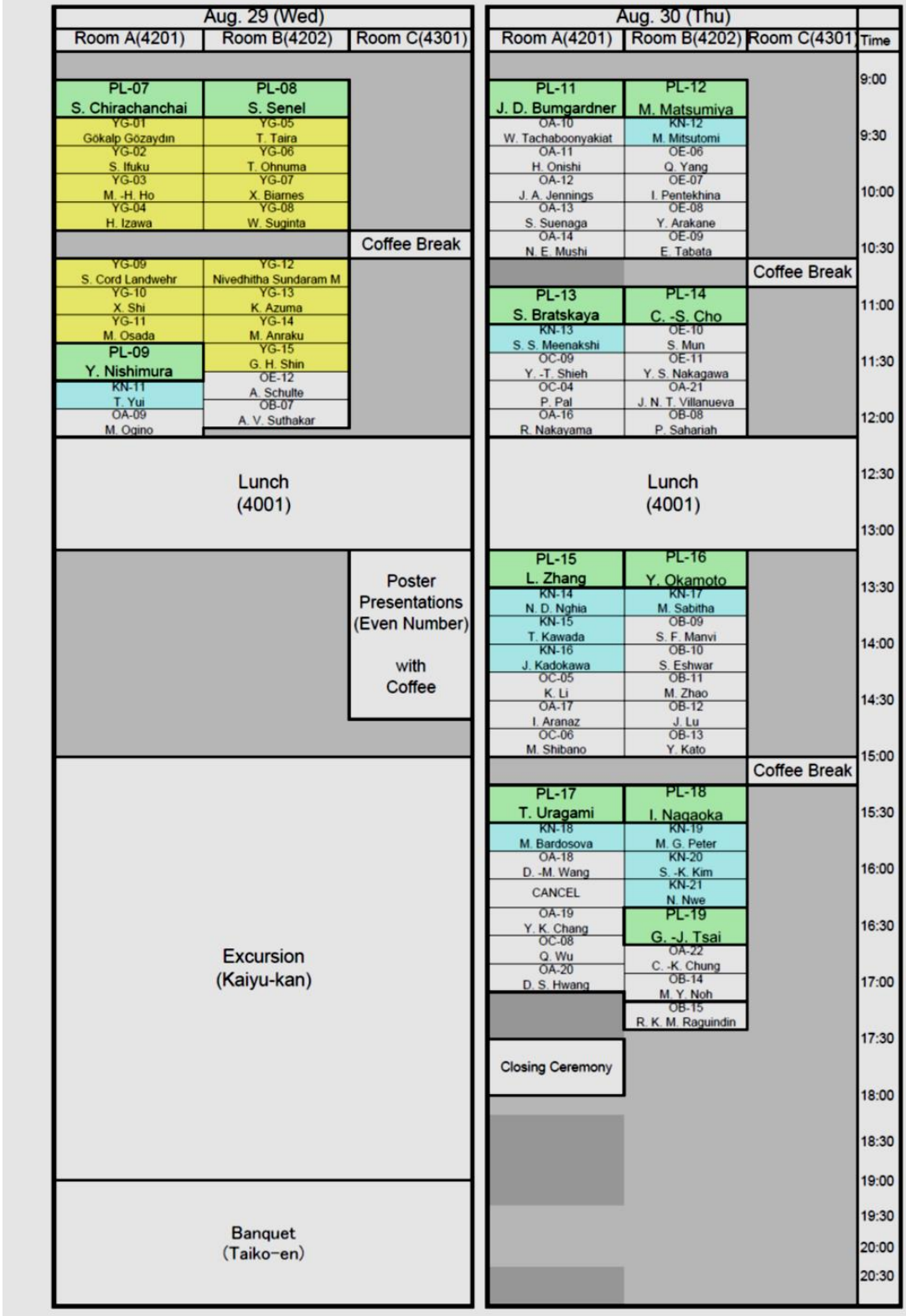


圖 1 續、14th ICCC 暨 12th APCCS 詳細議程。

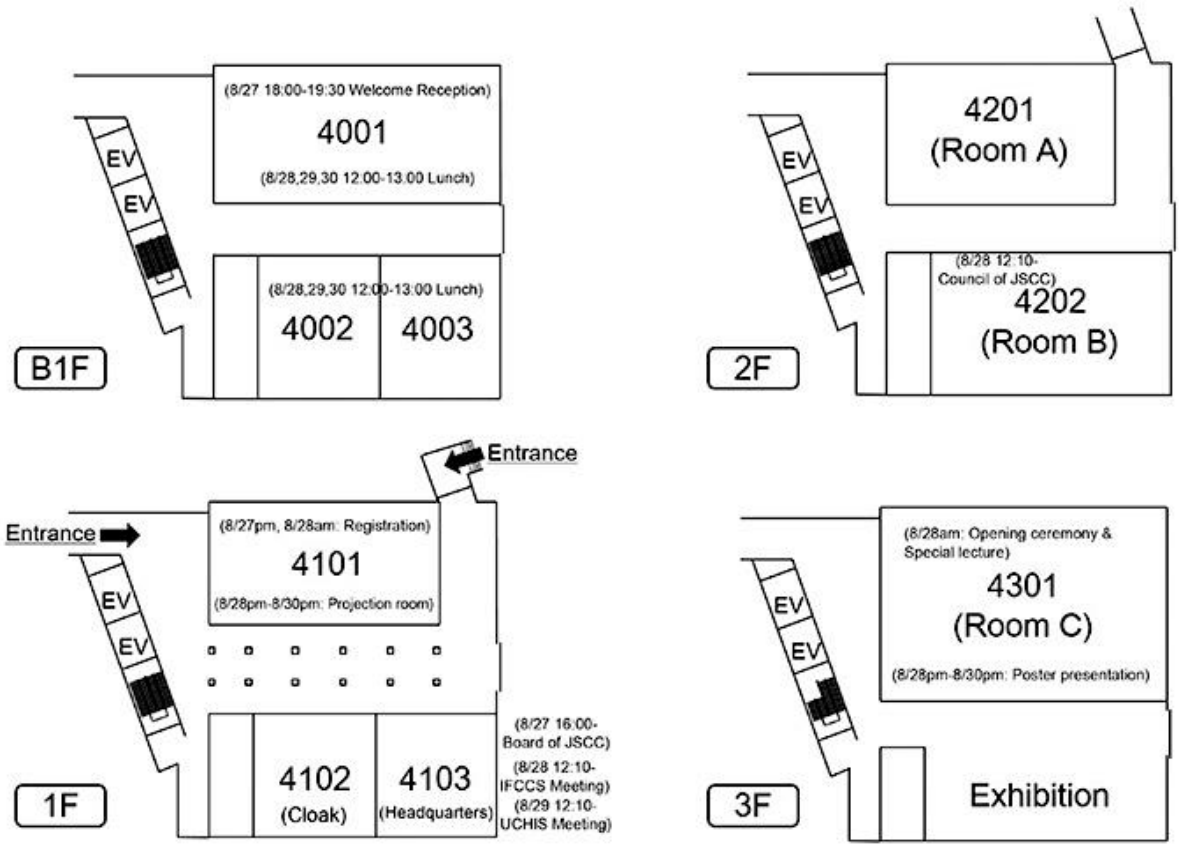

(8/27 18:00-19:30 Welcome Reception)
4001
(8/28,29,30 12:00-13:00 Lunch)
EV
EV
(8/28,29,30 12:00-13:00 Lunch)
4002
4003
B1F
4201
(Room A)
EV
EV
(8/28 12:10-
Council of JSCC)
4202
(Room B)
2F
Entrance
Entrance
(8/27pm, 8/28am: Registration)
4101
(8/28pm-8/30pm: Projection room)
EV
EV
4102
(Cloak)
4103
(Headquarters)
(8/27 16:00-
Board of JSCC)
(8/28 12:10-
IFCCS Meeting)
(8/29 12:10-
UCHIS Meeting)
1F
(8/28am: Opening ceremony &
Special lecture)
4301
(Room C)
(8/28pm-8/30pm: Poster presentation)
EV
EV
Exhibition
3F

圖 2、會場場地一覽圖。

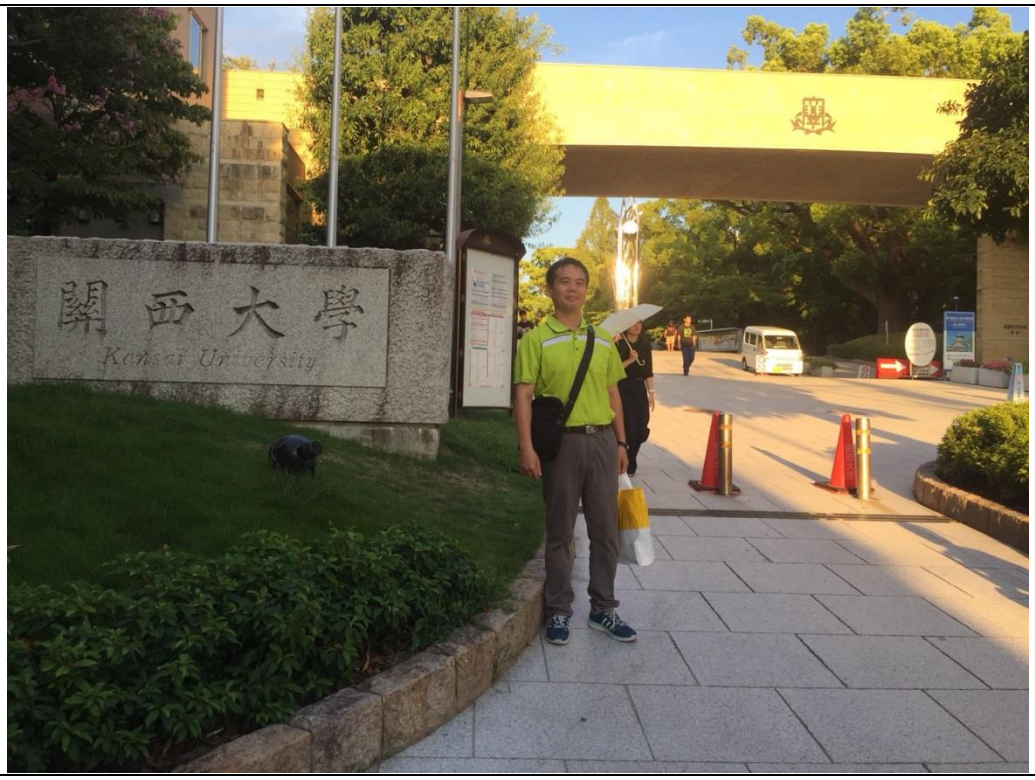


圖 3、研討會舉辦地點日本大阪府關西大學(上)及研討會歡迎茶會(下)。

圖 4、會議參加人員大合照(上)及口頭論文發表會場(下)。

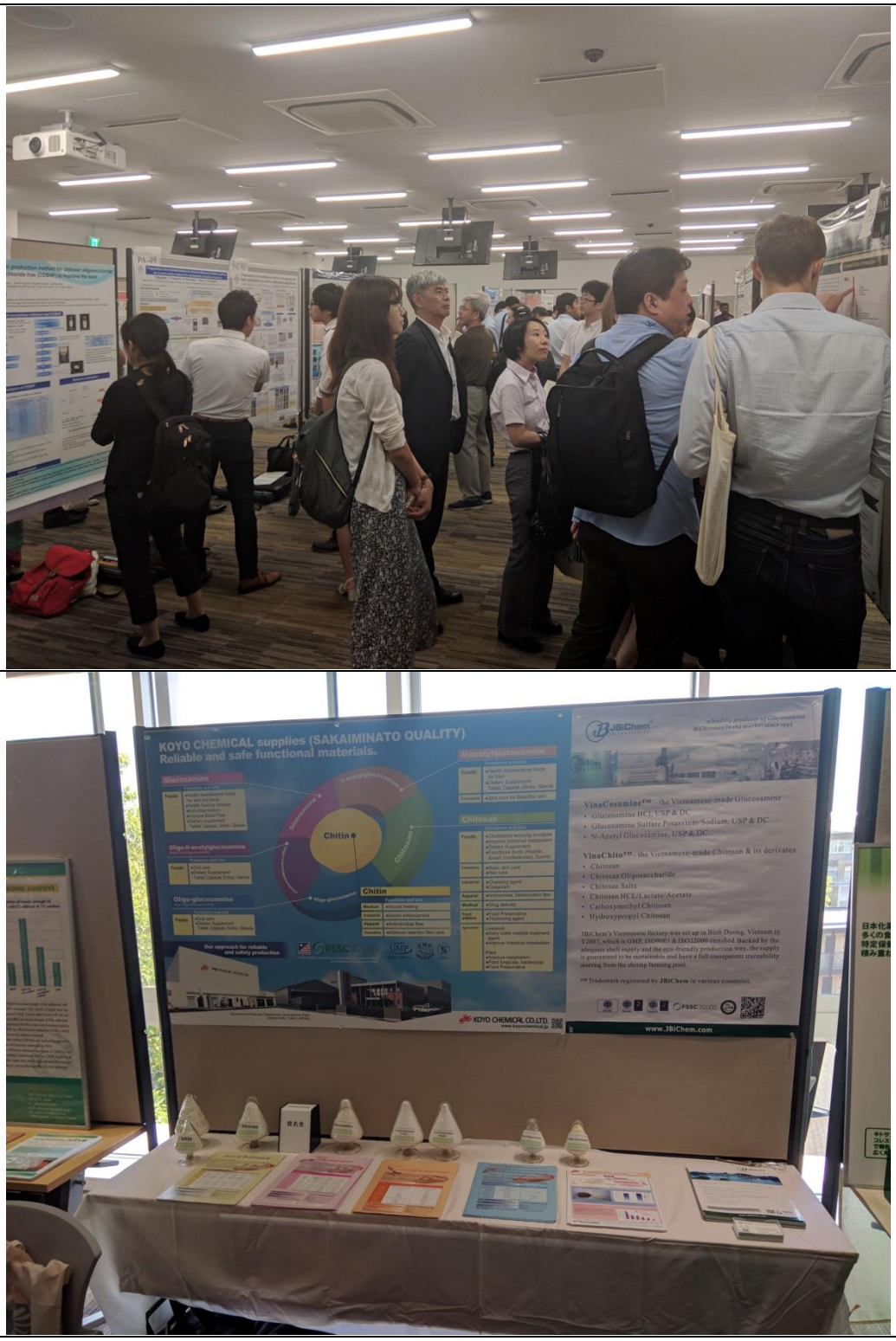


圖 5、海報論文發表會場(上)及廠商參展會場(下)。

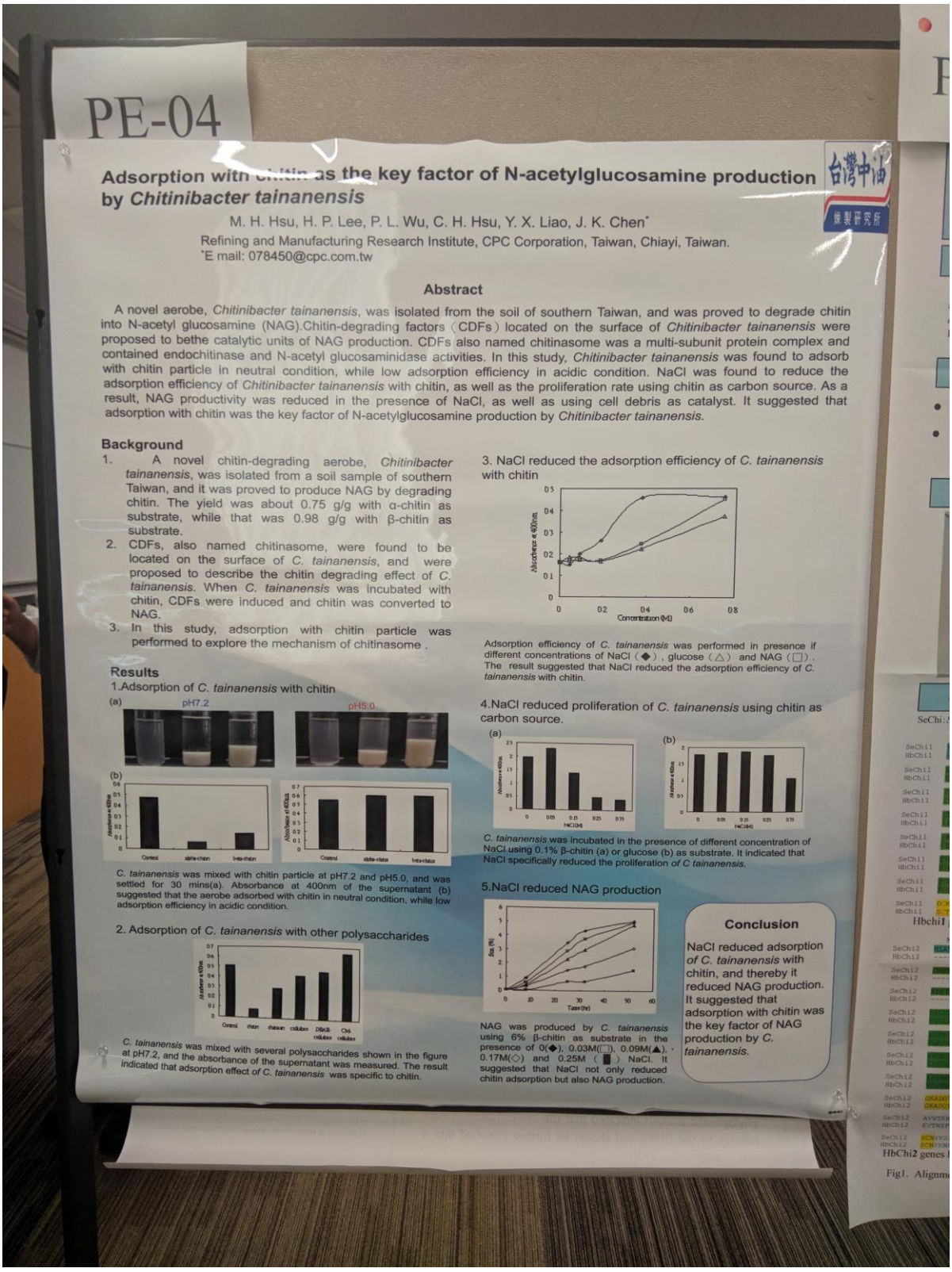
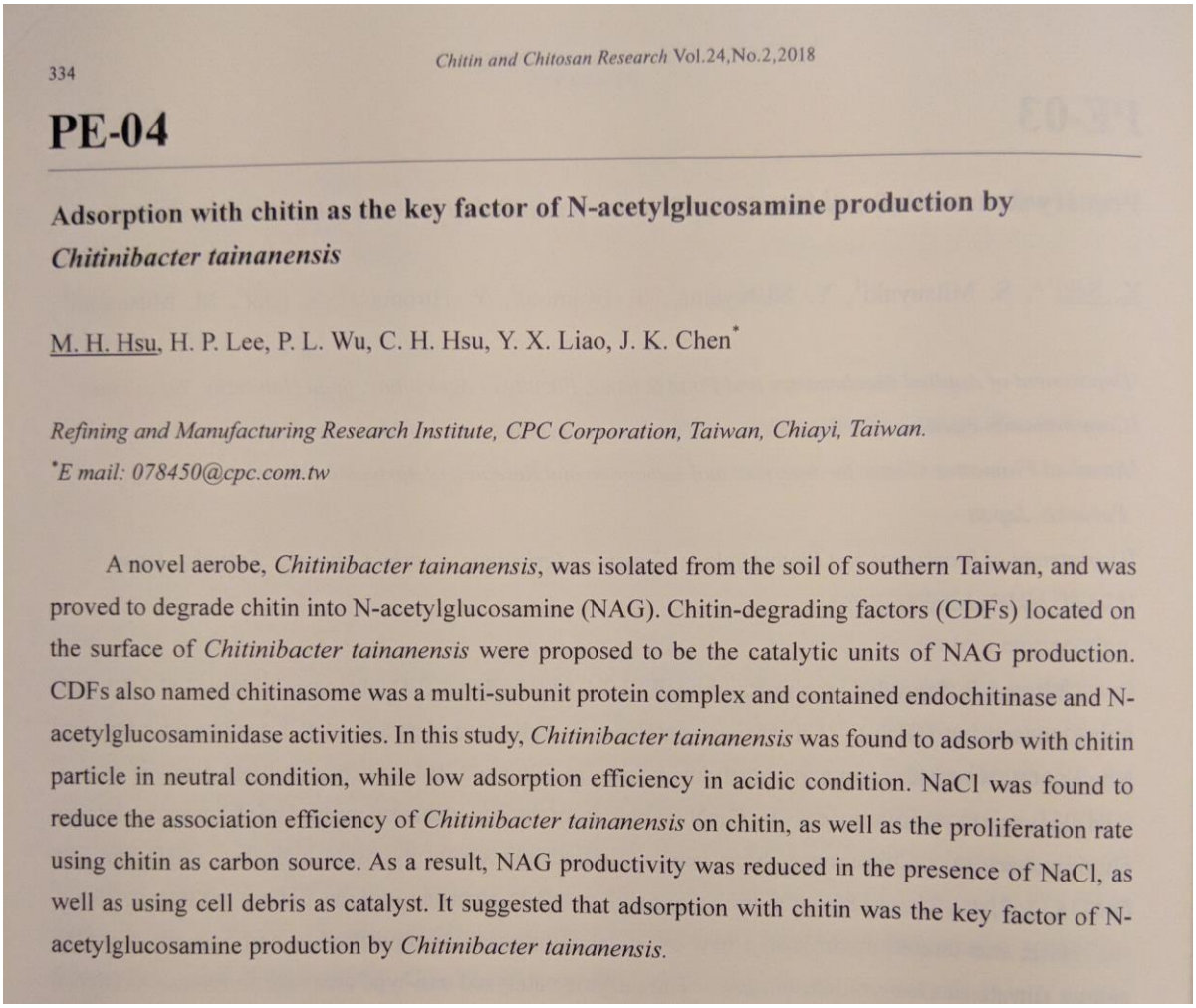


圖 6、發表海報論文 Adsorption with chitin as the key factor of N-acetylglucosamine production by *Chitinibacter tainanensis*。

1. N-乙醯葡萄糖胺生產製備

幾丁質(chitin)存在於節肢動物的外骨骼、烏賊軟骨及真菌的細胞壁中，是僅次於纖維素存量位居第二位的天然聚合物。幾丁質的化學結構是利用 N-乙醯葡萄糖胺(N-acetylglucosamine, NAG)為單體，以 β-1,4 糖苷鍵聚合而成，結構緊密，形成不溶於稀酸、稀鹼或水的特殊結構。台灣中油公司專利菌種 *Chitinibacter tainanensis* 是台灣本土篩選出之好氣菌，能直接將幾丁質顆粒分解成為 N-乙醯葡萄糖胺(NAG)，稱為生物轉化法。*C. tainanensis* 在中性環境下可以與幾丁質緊密結合，但在酸性環境則失去結合能力。NaCl 會降低菌體對幾丁質的吸附能力，進而影響培養前期菌體利用幾丁質為碳源的生長效率，NaCl 干擾菌體與幾丁質的結合進而影響幾丁質的分解效率。上述結果顯示對幾丁質的吸附能力是 *C. tainanensis* 分解 chitin 成為 NAG 的重要因子，一但此結合現象受到干擾，NAG 的產率便會降低。針對 *C. tainanensis* 分解幾丁質的現象，提出幾丁質分解因子(CDFs)假說，認為菌體表面存在一種 CDFs 來進行幾丁質的分解作用，且具有 endochitinase 與 NAGase 的活性。菌體表面的 CDFs 具有酵素活性與幾丁質的吸附能力，與纖維素分解菌的 cellulosome 類似，因此推測菌體表面有 chitinasome 存在。



PE-04

Adsorption with chitin as the key factor of N-acetylglucosamine production by *Chitinibacter tainanensis*

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A novel aerobe, *Chitinibacter tainanensis*, was isolated from the soil of southern Taiwan, and was proved to degrade chitin into N-acetylglucosamine (NAG). Chitin-degrading factors (CDFs) located on the surface of *Chitinibacter tainanensis* were proposed to be the catalytic units of NAG production. CDFs also named chitinasome was a multi-subunit protein complex and contained endochitinase and N-acetylglucosaminidase activities. In this study, *Chitinibacter tainanensis* was found to adsorb with chitin particle in neutral condition, while low adsorption efficiency in acidic condition. NaCl was found to reduce the association efficiency of *Chitinibacter tainanensis* on chitin, as well as the proliferation rate using chitin as carbon source. As a result, NAG productivity was reduced in the presence of NaCl, as well as using cell debris as catalyst. It suggested that adsorption with chitin was the key factor of N-acetylglucosamine production by *Chitinibacter tainanensis*.

PB-27

Identification of Chitinasome from *Chitinibacter tainanensis* with nanoparticle

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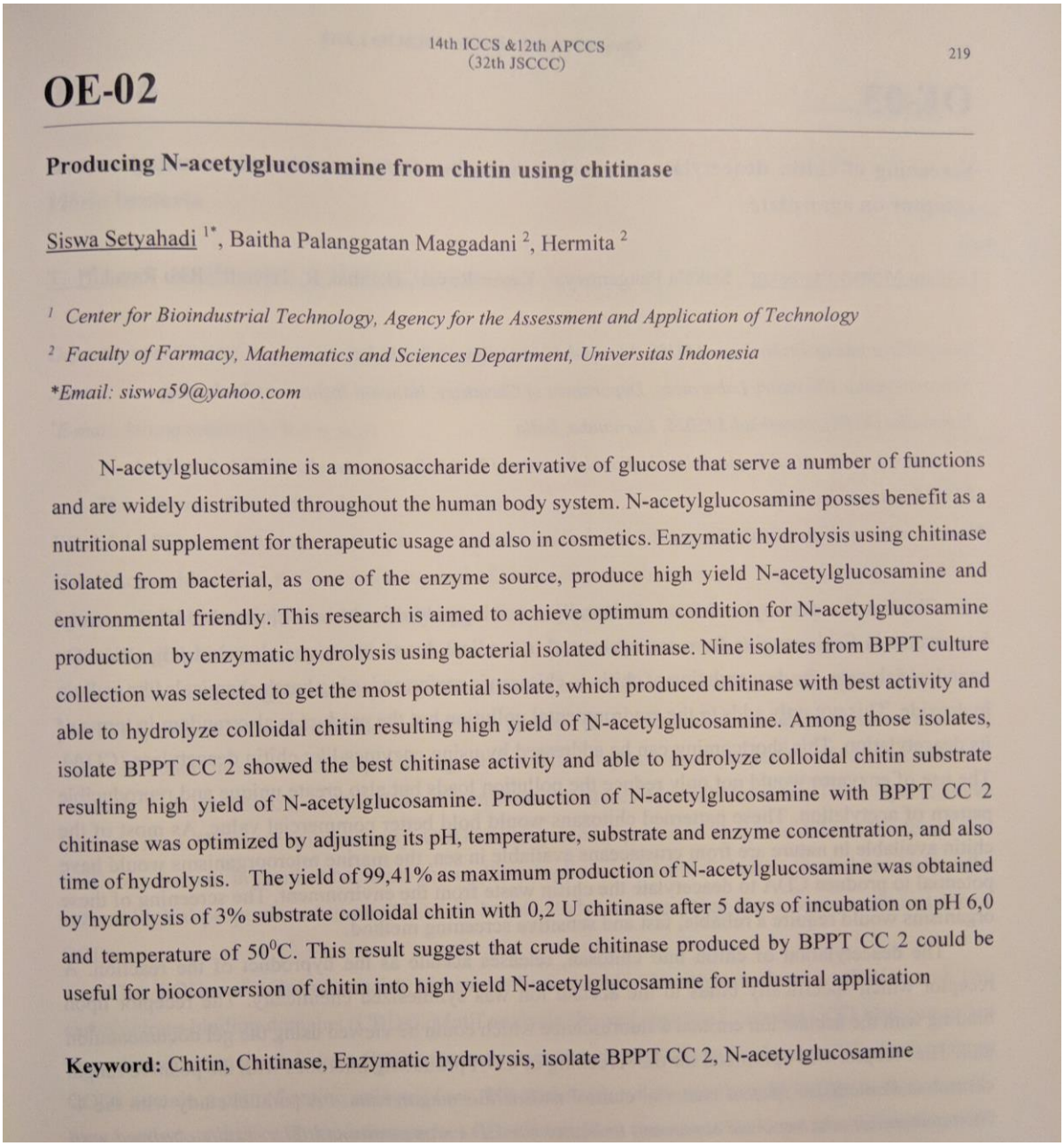
3. College of Engineering, Chang Gung University, Taoyuan, 333, Taiwan

Chitin is the major component of fungal cell walls, insect exoskeletons and the shells of crustaceans. Besides cellulose, chitin is the most abundant polysaccharide on the earth. Derivatives of chitin, inclusive of deacetylation polysaccharides, oligosaccharides and monosaccharides, have been applied for adsorption, enzyme-immobilization and biomedical materials because of the unique characteristics of being biodegradable, histo-compatible and nontoxic. The monomer of chitin, N-acetylglucosamine (NAG) has specific roles in physiology and cell biology as well as for its therapeutic uses in treating arthritis, pediatric enteritis and Crohn's disease. It also promotes the synthesis of hyaluronic acid to anti-wrinkle. In the market, the NAG is high industrial output value in raw material of pharmaceutics, food and agriculture.

Recently, a new species microorganism, *Chitinibacter tainanensis*, was isolated from the soil of southern Taiwan. The bacteria can produce NAG by degrading chitin, and the yield reaches almost 90%. The high purity (99.9%) of NAG can be conventionally obtained by recrystallization. The transformation of chitin is subjected on the surfave of membrane in *C. tainanensis*. For the identification degradation molecules as “chitinasome” with their unique characteristics according to the mechanism similar to cellulosome, a nonparticle was synthesized. The chitinasome was identified after culvaitation.

Setyahadi 等人的研究則以酵素法生產 NAG，由細菌篩選出 BPPT CC 2 幾丁質酶，在 pH 6.0，50℃條件下培養 5 天，可將 3%膠態幾丁質受質分解為 NAG，轉化率可達 99.41%，BPPT CC 2 可以做 NAG 商業化生產之應用。



OE-02

Producing N-acetylglucosamine from chitin using chitinase

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[2] *Faculty of Farmacy, Mathematics and Sciences Department, Universitas Indonesia*

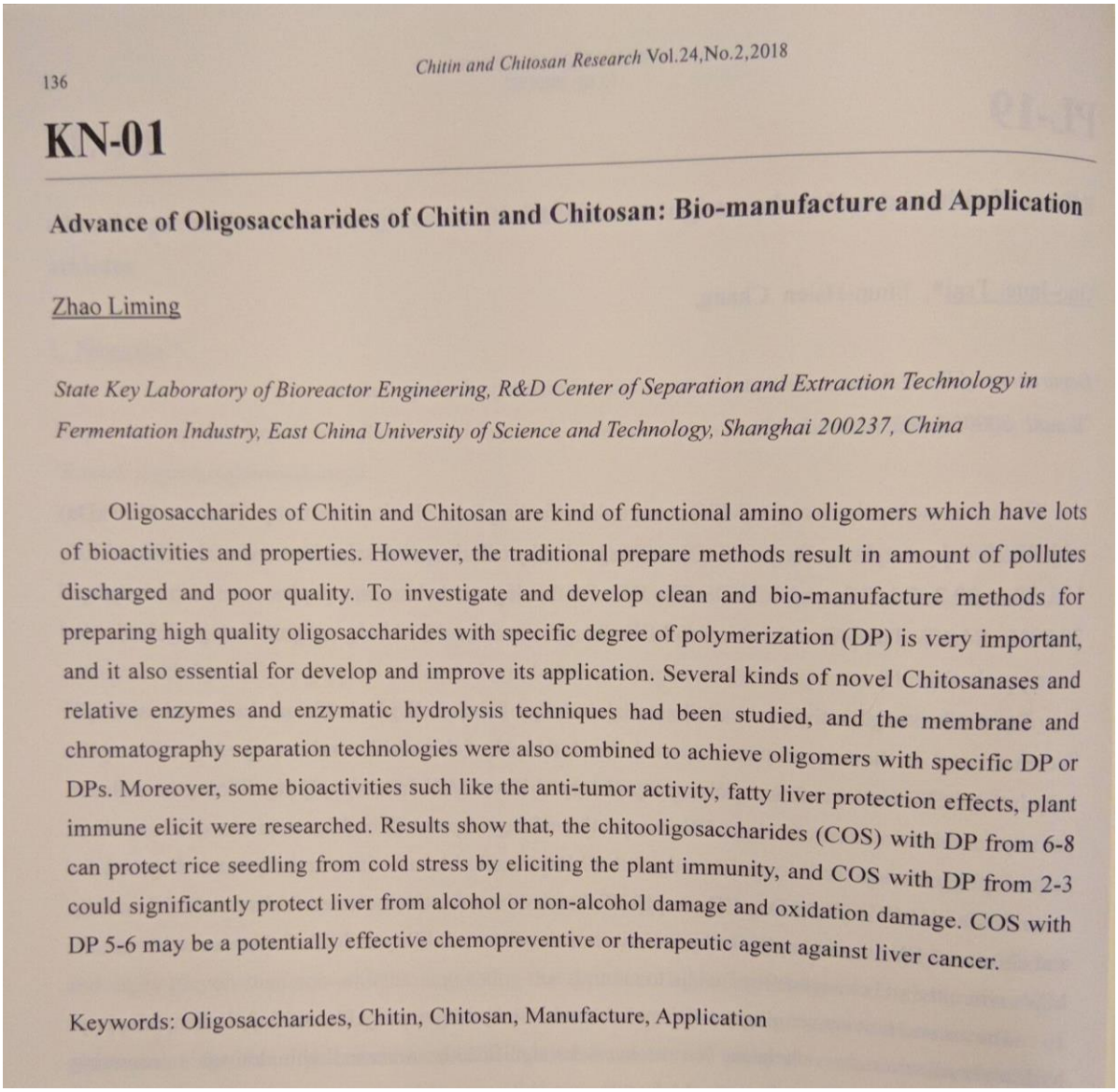
**Email: siswa59@yahoo.com*

N-acetylglucosamine is a monosaccharide derivative of glucose that serve a number of functions and are widely distributed throughout the human body system. N-acetylglucosamine posses benefit as a nutritional supplement for therapeutic usage and also in cosmetics. Enzymatic hydrolysis using chitinase isolated from bacterial, as one of the enzyme source, produce high yield N-acetylglucosamine and environmental friendly. This research is aimed to achieve optimum condition for N-acetylglucosamine production by enzymatic hydrolysis using bacterial isolated chitinase. Nine isolates from BPPT culture collection was selected to get the most potential isolate, which produced chitinase with best activity and able to hydrolyze colloidal chitin resulting high yield of N-acetylglucosamine. Among those isolates, isolate BPPT CC 2 showed the best chitinase activity and able to hydrolyze colloidal chitin substrate resulting high yield of N-acetylglucosamine. Production of N-acetylglucosamine with BPPT CC 2 chitinase was optimized by adjusting its pH, temperature, substrate and enzyme concentration, and also time of hydrolysis. The yield of 99,41% as maximum production of N-acetylglucosamine was obtained by hydrolysis of 3% substrate colloidal chitin with 0,2 U chitinase after 5 days of incubation on pH 6,0 and temperature of 50^0C. This result suggest that crude chitinase produced by BPPT CC 2 could be useful for bioconversion of chitin into high yield N-acetylglucosamine for industrial application

Keyword: Chitin, Chitinase, Enzymatic hydrolysis, isolate BPPT CC 2, N-acetylglucosamine

2. 幾丁寡醣之生產製備

在幾丁寡醣及幾丁聚寡醣的生產上可由化學法及酵素法來製備，化學法的缺點為品質控制不易，產生有毒副產物，在食用或藥用上有疑慮等。而酵素法利用幾丁質酶或幾丁聚醣酶水解生成短鏈的寡醣，高度專一性，低污染且產品穩定。學者 Zhao 利用酵素法製備幾丁幾丁(聚)寡醣，其中六－八醣具有植物保護功效，二－三醣可保護肝損傷，五－六醣則有抗肝癌之潛力。



136 *Chitin and Chitosan Research* Vol.24,No.2,2018

KN-01

Advance of Oligosaccharides of Chitin and Chitosan: Bio-manufacture and Application

Zhao Liming

State Key Laboratory of Bioreactor Engineering, R&D Center of Separation and Extraction Technology in Fermentation Industry, East China University of Science and Technology, Shanghai 200237, China

Oligosaccharides of Chitin and Chitosan are kind of functional amino oligomers which have lots of bioactivities and properties. However, the traditional prepare methods result in amount of pollutes discharged and poor quality. To investigate and develop clean and bio-manufacture methods for preparing high quality oligosaccharides with specific degree of polymerization (DP) is very important, and it also essential for develop and improve its application. Several kinds of novel Chitosanases and relative enzymes and enzymatic hydrolysis techniques had been studied, and the membrane and chromatography separation technologies were also combined to achieve oligomers with specific DP or DPs. Moreover, some bioactivities such like the anti-tumor activity, fatty liver protection effects, plant immune elicit were researched. Results show that, the chitooligosaccharides (COS) with DP from 6-8 can protect rice seedling from cold stress by eliciting the plant immunity, and COS with DP from 2-3 could significantly protect liver from alcohol or non-alcohol damage and oxidation damage. COS with DP 5-6 may be a potentially effective chemopreventive or therapeutic agent against liver cancer.

Keywords: Oligosaccharides, Chitin, Chitosan, Manufacture, Application

學者 Kim 則發展雙反應器系統(Dual reactor system)，包含固定化酵素反應器及超濾膜反應器，可大量連續生產固定分子量範圍之幾丁(聚)寡醣，這些幾丁(聚)寡醣具有不同的生物活性。

KN-20

Continuous production of chitooligosaccharides and their biological activities

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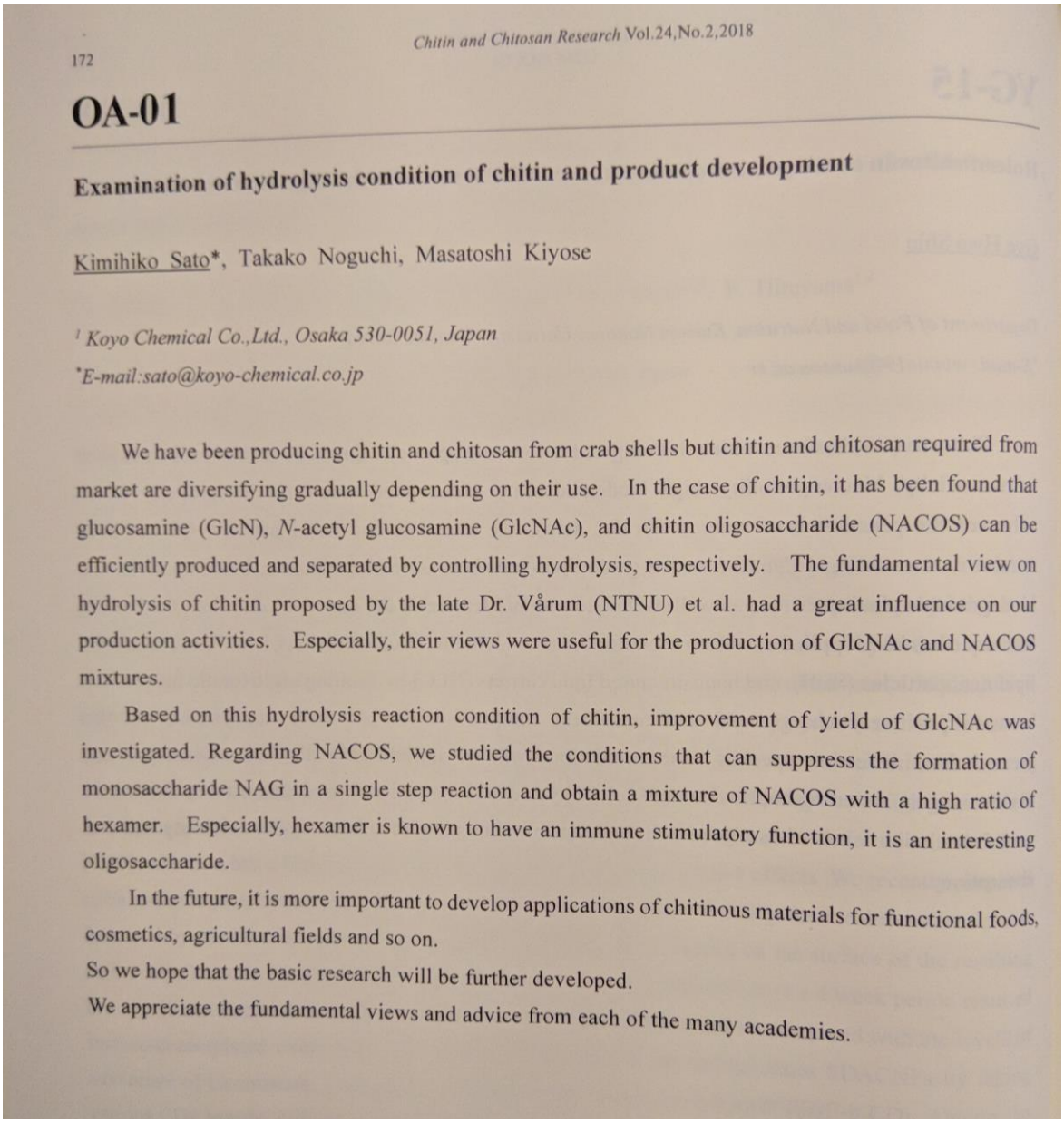
Crustacean shells are the source of chitosan and its partially hydrolyzed products; Chitooligosaccharides (COS) is subject of various studies for their potent notable biological utilization areas. In order to produce COS, there are several approaches including chemical and enzymatic methods which are two most common choices. Among them, chemical hydrolysis is preferred widely for the industrial-scale production of COS. However, chemical hydrolysis possess undeniable disadvantages for being commercialized such as development of toxic compounds, high risk of environmental pollution and relatively lower production yields. Therefore, past years it was difficult to produce large-scale COS with defined molecular weights for human utilization due to lack of proper technology. In this regard, several new methods were intended to be promoted which use the enzymatic hydrolysis with a lower cost and desired properties. Hence, the dual reactor system has gained more attention than other newly develops technologies. Dual reactor system is consisted of a column reactor packed with an immobilized enzyme and an ultrafiltration (UF) membrane reactor. The most prominent advantages which makes this method suitable for large-scale production can be counted as ease of maintaining a desired molecular weight range and continuous production availability. By this method, three kind of COS with high, medium and low molecular weights have been prepared. Studies with these three different COS revealed that they possess important biological activities such as antibacterial, antifungal, antiobesity, anti-HIV, cellular radical scavenging, angiotensin-I converting enzyme (ACE) inhibition, β-secretase inhibition and matrix metalloproteinase inhibition. In addition, biomedical application of COS have been explored for tissue engineering and drug delivery area. Collectively, all these results strongly suggest that properties of COS including degree of deacetylation and molecular weight are quite significant for the reported biological activities.

Keywords: Chitooligosaccharides, Ultrafiltration membrane reactor, Biological activities

Sato 等人則發展幾丁質水解程度控制技術，降低單醣 NAG 之產生，增加幾丁寡醣 (chitin oligosaccharide, NACOS)產生，得到之幾丁寡醣 NACOS 混合物含高比例的幾丁六醣，幾丁六糖在促進免疫功效及抗腫瘤上具有顯著效果。



OA-01

Examination of hydrolysis condition of chitin and product development

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We have been producing chitin and chitosan from crab shells but chitin and chitosan required from market are diversifying gradually depending on their use. In the case of chitin, it has been found that glucosamine (GlcN), *N*-acetyl glucosamine (GlcNAc), and chitin oligosaccharide (NACOS) can be efficiently produced and separated by controlling hydrolysis, respectively. The fundamental view on hydrolysis of chitin proposed by the late Dr. Vårum (NTNU) et al. had a great influence on our production activities. Especially, their views were useful for the production of GlcNAc and NACOS mixtures.

Based on this hydrolysis reaction condition of chitin, improvement of yield of GlcNAc was investigated. Regarding NACOS, we studied the conditions that can suppress the formation of monosaccharide NAG in a single step reaction and obtain a mixture of NACOS with a high ratio of hexamer. Especially, hexamer is known to have an immune stimulatory function, it is an interesting oligosaccharide.

In the future, it is more important to develop applications of chitinous materials for functional foods, cosmetics, agricultural fields and so on.

So we hope that the basic research will be further developed.

We appreciate the fundamental views and advice from each of the many academies.

除了化學法及酵素法外，幾丁聚醣以低溫電漿處後產生幾丁(聚)寡醣，此種技術可縮短生產時程，不會產生有毒副產物。此技術生產之幾丁(聚)寡醣可以促進植物生長、增產及抑制植物病源菌。

OB-01

Plasma chemistry for production of plant biostimulants from chitosan

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A novel approach to the bio-safe controlled production of low-molecular weight chitooligosaccharides (COS) with plant biostimulating activity by means of low-temperature non-equilibrium electron-beam plasma (EBP) is described.

Chitosan with viscosity-average molecular weight of 500 kDa, deacetylation degree of 85%, and polydispersity index of 1.5 was used for the further EBP-treatment. Chitosan powder was treated in a special mixer where EBP was excited by injecting an electron beam into chemically pure oxygen. To produce an adequate amount of COS for practical uses (up to 10 grams), the treatment conditions were optimized:

Water-soluble COS with weight-average molecular mass of 600 Da and polydispersity index of 1.5 were formed due to the EBP-stimulated destruction of β-1,4-glycosidic bonds from the original chitosan. While 85% yield of water-soluble COS was attained at treatment time τ ~10 min under optimized conditions, conventional chemical chitosan hydrolysis usually takes several days. In addition, no hazardous by-products and toxic wastes were generated during the EBP-processing of chitosan.

Plant biostimulating properties of the EBP-produced COS were studied using *Arabidopsis thaliana* (Col-0), a popular model organism in plant biology, and tomato plants (*Lycopersicon esculentum*, cv. Micro-Tom). After adding EBP-produced COS in a concentration range from 0.25 to 1% w/v, a strong stimulation of root growth in *Arabidopsis thaliana* seedlings was observed (up to 40% with respect to untreated plants). Foliar application of this COS formulation at 0.025% w/v on unstressed tomato plants resulted in increases up to 10% in a number of plant productivity indicators such as flower production or fruit yield compared to untreated control. The EBP-produced COS also inhibited the growth of various yeast-like and filamentous fungi (e.g. *Aspergillus flavus*, *Pemphigus betae*, and *Cladosporium herbarum*) responsible for damaging agricultural crops at concentrations 500-1,000 μg/mL.

Taken together, our results demonstrated that EBP-technology is promising for effective and environmentally friendly production of water-soluble COS for agricultural applications.

3. 幾丁(聚)寡糖抗腫瘤研究

幾丁寡醣及幾丁聚寡醣在促進免疫功能及抗腫瘤上具顯著的效果，小鼠動物試驗顯示幾丁寡醣及幾丁聚寡醣明顯抑制腫瘤生成、生長及促使腫瘤細胞凋亡，並具有增強免疫之效果，犬隻動物試驗亦顯示幾丁寡醣及幾丁聚寡醣同樣具有抗腫瘤效果。

PL-16

Anti-tumor effects of orally administered chitin and chitosan oligosaccharides

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The current study evaluated the anti-tumor activities of N-acetyl-d-glucosamine oligomer (NACOS) and glucosamine oligomer (COS) after their oral administration in a tumor (colon 26)-bearing mouse model. Compared to the control group, NACOS and COS groups showed significantly suppressed tumor growth, and apparent, marked apoptosis in tumor tissues. Furthermore, serum interleukin-12 and interferon-γ levels significantly increased in the NACOS and COS groups compared to the corresponding levels in the control group. Collectively, the results indicate the oral administration of NACOS and COS could enhance innate immunity. Results of experiments in Myd-88 knockout mice revealed that the apparent effects were related to both Myd-88-dependent and Myd-88-independent pathways. The data indicated that oral administration of NACOS and COS produced anti-tumor effects through the induction of apoptosis and stimulation of the immune system.

The dogs with tumor were administered orally NACOS or COS every day at dose of 1-4g/head, according to the weight (less than 7kg: 1g/day, 7-20 kg: 2g/day, 21-30 kg: 3g/day, 31-50 kg: 4g/day). Median time of oral administration was 5.2 courses (1 course = one month). In 8 out of 17 cases, NACOS or COS was administered alone, and in the remaining 9 cases, local therapy including surgery, hyperthermia, and cryuosurgery was combined. In the one case (liposarcoma) with administration of COS alone, the tumor was disappeared completely. In 4 out of 8 cases, the tumors were shrunk. In the remaining 4 cases, stable and progressing cases were each 2 cases. In the 4 cases with a combination of complete surgery and administration of NACOS or COS, there was no recurrence in 2 out of 4 cases, the remaining 2 cases recurred. In the 5 cases with combination of partial surgery, hyperthermia and administration of NACOS or COS, 4 cases were stable. There were no side effects including diarrhea. These data suggest that NACOS and COS have anti-tumor effects in canine spontaneous tumors. These results suggest that NACOS and COS are candidate anti-tumor functional foods.

另一個研究則實際以癌症病人進行人體試驗研究，結果發現幾丁聚醣對癌症具有治療效果，尤以幾丁六醣的比例愈高的話效果愈佳。

PB-24

Therapeutic effect of chitin oligosaccharide mixtures by *per os* administration on human cancer

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In response to the request of a Ministry in Japan to utilize crab shell in 1985, 13-universities in Japan entered into research in the immune effect of chitin and chitosan component of crab shell for various diseases. As a result, the usefulness was recognized by every university. Moreover, in 1986, Suzuki recognized more useful therapeutic effect of chitin oligosaccharide mixture (NACOS) and its effective component is NACOS-6, based on animal experiment on cancer. In 1990, NACOS has become available commercially as foods supplement in Japan. Since then, a number of personal experiences have been reported by the people who took the foods and recognized its effectiveness for various diseases including cancer.

Experiencing successful immune effect for my own cancer by NACOS, in 2006, the author entered into a series of studies on therapeutic effect of NACOS by *per os* administration on patients in Kei Clinic. This report is for cancer patients. Observing significant regression of the cancer in most of them, the data for 41 patients were reported in this Society in 2013.

Now the data have reached to 103 patients as shown below. These also show regression of the cancer in particular those with early stage cancer.

Regression achievement (CR, PR, PFS, RFS)

Treatment	Stage Ⅰ + Ⅱ	Stage Ⅲ	Stage Ⅳ	Total
Only by NACOS	95%(20/21)	80% (4/5)	80% (4/5)	90%(28/31)
With the others	90%(26/29)	70% (7/10)	45% (15/33)	67%(48/72)

For 72 patients treated with chemotherapy and/or surgical operation, 67% of the patients achieved the regression. Since significant decrease of NLR is seen in most of them, it is considered the contribution of the NACOS is high. These anticancer effects were observed regardless of organ treated. Therefore the author recognizes high therapeutic effect of NACOS by *per os* administration on human cancers. It is noted that the anticancer effect is remarkable when the peripheral lymphocytes average before treatment is above 1550/μL, NLR after treatment is below 2.8, and that the effect depends on the quantity of NACOS-6 that requires of the order of 1gr/day. These real data will be useful for the anticancer-therapy treatment of the human. Further studies by investigators are expected so that the effectiveness of NACOS would widely be experienced and applied preventing human cancer.

4. 幾丁質酶研究

Matsumiya 等人及 Yaguchi 等人分別從金烏賊(*Sepia esculenta*)及槍魷魚(*Heterololigo bleekeri*)肝臟分離出幾丁質酶並完成純化、分析，各得到兩個幾丁質酶 cDNAs。蛋白質結構分析顯示金烏賊及槍魷魚其中一個幾丁質酶含一個幾丁質結合域，而另一個幾丁質酶含 2 個幾丁質結合域，幾丁質結合域為幾丁質酶分解活性的重要因素，可作為生技中心研究幾丁質酶研究參考。

PL-12

Characteristics of chitinase isozymes from the liver of golden cuttlefish *Sepia esculenta*

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The distribution of chitinolytic enzymes in eight organs of the golden cuttlefish *Sepia esculenta* was determined. High chitinase activity was observed in the liver, posterior salivary gland, and stomach. Two chitin-binding proteins (CBP-A, CBP-B) were detected from the liver using a chitin affinity column. A chitinase was purified from the liver of *S. esculenta* by ammonium sulfate fractionation and column chromatography on Chitin EX, DEAE-Toyopearl 650S, and hydroxyapatite columns. The purified enzyme (SeChi, CBP-B) was identified as a single protein band on SDS-PAGE with an apparent molecular mass of 62 kDa. When pNp-$(GlcNAc)_2$ was used as a substrate, the optimum pH and optimum temperature for SeChi activity were 3.5 and 50 °C, respectively. The activity of SeChi was increased to 164% in the presence of 0.5 M NaCl. SeChi showed the highest activity towards *pNp*-$(GlcNAc)_2$, followed by *pNp*-$(GlcNAc)_3$ and *pNp*-$(GlcNAc)_4$. When crystalline chitins were used as substrates, SeChi degraded α-chitins derived from crab shells and shrimp shells and β-chitin derived from squid pen; however, it did not degrade α-chitin derived from silkworm cuticle. These results suggested that SeChi possesses a characteristic specificity towards crystalline chitins derived from organisms the golden cuttlefish usually ingest as feed in seawater.

Two full-length cDNAs (*SeChi*-1: 1484 bp; *SeChi*-2: 1748 bp) encoding chitinases were obtained from the liver of *S. esculenta*. *SeChi*-1 contained a 1377-bp open reading frame (ORF) encoding 459 amino acids, and *SeChi*-2 contained a 1656-bp ORF encoding 552 amino acids. Domain structures predicted from the deduced amino acid sequences of *SeChi*-1 and *SeChi*-2 (Se-Chi-1, SeChi-2) contained signal peptides, a GH Family 18 catalytic domain, one chitin binding domain (CBD) in SeChi-1, and two CBDs in SeChi-2. Proteome analysis revealed that 125 peptide residues of CBP-A were present in SeChi-1, and 116 peptide residues of CBP-B were present in SeChi-2. Organ expression analysis revealed that *SeChi*-1 and *SeChi*-2 were expressed only in the liver of *S. esculenta*. Phylogenetic analysis of SeChi-1, SeChi-2, and GH family 18 chitinases revealed that SeChi-2 belongs to a group of previously reported squid chitinases, while SeChi-1 does not belong to any previously reported group of mollusk chitinases.

PE-05

Molecular cloning of chitinase isozyme genes from the liver of spear squid *Heterololigo bleekeri*

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Chitin is the second most abundant biopolymer in nature next to cellulose. It is a main constituent of fungal cell walls, insect exoskeletons, and mollusk shells. Chitinases are capable hydrolases of cleaving the β-1,4-glycosidic bonds in chitin polymer. Chitinases are found in many species including mammals, bacteria, fungi, viruses, nematodes, arthropods, and plants. Chitinases serve important biological roles such as nutrient intake, morphological change, defense, and attack. Squid is one of mollusks, which quickly grows to become adult in one year. Along with this, squid is known to have a very high ability to digest diet. In this study, we tried cDNA cloning of liver-derived chitinase, a secretory organ of digestive enzyme, using spear squid *Heterololigo bleekeri*.

Total RNA was extracted from the liver of *H. bleekeri.* cDNA was synthesized using Prime Script Reverse Transcriptas and oligo (dT) primer and used as a template. Degenerate primers were designed from conserved amino acid sequences of several species of squid chitinases already reported, and gene fragment amplification was carried out by PCR. From the cDNA cloning, two chitinase genes, *HbChi-1* (full length: 1,622 bp. ORF: 1,377 bp) and *HbChi-2* (partial sequence: 1,319 bp), were obtained. The deduced amino acid sequences of *HbChi-1* and *HbChi-2* contained one chitin binding domain (CBD) and two CBDs, respectively. This result was in agreement with the report at golden cuttlefish *Sepia esculenta.* Currently we are trying to amplify the full-length gene of *HbChi-2*.

由植物來源之幾丁質酶將其轉變為醣苷合成酶(glycosynthases)，由二醣製備生產幾丁四醣，幾丁四醣可再由醣苷合成酶作用生成幾丁七醣，另一家族幾丁質酶可以經由轉醣苷反應(transglycosylation)來產生具生物活性之寡醣。

YG-06

Plant chitinases: structure, function, and exploitation in chitin oligosaccharide synthesis

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Family GH19 chitinase is an endo-acting enzyme that hydrolyses the glycosidic bonds of chitin through an inverting mechanism. We determined crystal structures of "loopful" and "loopless" GH19 chitinases from plants and successfully converted these enzymes into glycosynthases. These enzymes were found to consist of a conserved, α-helical core-region and different numbers of loop structures, located at both ends of the substrate-binding groove and which extend over the glycon- and aglycon-binding sites. Glycosynthase derived from the loopless GH19 chitinase produced $(GlcNAc)_4$ from α-$(GlcNAc)_2$ fluoride and $(GlcNAc)_2$.[1] $(GlcNAc)_4$ could be further elongated to $(GlcNAc)_7$ by glycosynthetic reaction catalyzed by glycosynthase derived from the loopful GH19 chitinase.[2] These are the first cases of glycosynthases that synthesize chitin oligosaccharides.

Family GH18 enzymes hydrolyze the glycosidic bonds of chitin through a retaining mechanism. A number of retaining glycoside hydrolases are capable of catalyzing the transglycosylation reaction, which has been employed to synthesize bioactive oligosaccharides. The transglycosylation activity of retaining GH18 chitinases has also been applied to the synthesis of chitin oligosaccharides. So far, we isolated family GH18 (plant class V) chitinases from three plant species and determined their crystal structures. The class V chitinases were found to adopt an $(\beta/\alpha)_8$ fold with a small insertion domain composed of an α-helix and a five-stranded β-sheet.[3] We found that their transglycosylation reaction could be significantly enhanced by introducing a tryptophan side chain into subsite +1 of the enzymes.[4] The enhancement seemed to be due to protection of the oxazolinium ion intermediate from nucleophilic attack by a water molecule and also due to higher affinity for the acceptor-binding site of the enzyme.

1. Ohnuma et al. *Biochem J.* 444, 437-43, 2012
2. Ohnuma et al. *J Biochem.* 160, 93-100, 2016
3. Ohnuma et al. *Planta* 234, 123-37, 2011
4. Umemoto et al. *Glycobiology* 23, 81-90, 2013

5. NAG 皮膚保養功效研究

細胞試驗結果顯示 NAG 促進人類表皮角質細胞及真皮纖維細胞之玻尿酸形成，老鼠口服試驗結果亦顯示 NAG 可促進玻尿酸形成，人體臨床試驗結果 NAG 具有皮膚保濕抗皺功效。以人類表皮角質細胞及真皮纖維細胞建構人類皮膚 3D 模式進行試驗結果顯示NAG可增加玻尿酸形成，實際進行人體試驗以皮膚水份測定儀測量皮膚水分含量，1% NAG 乳液可有效增加角質層水分含量。

PB-11

Skin moisturizing effect of *N*-acetylglucosamine assessed by a three-dimensional human skin model and its application in cosmetics

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N-acetylglucosamine (GlcNAc) is a sugar that constitutes hyaluronan, which has an important biological role of retaining skin moisture.

Previous studies revealed that GlcNAc dose-dependently increases hyaluronan synthesis in human epidermal keratinocytes and dermal fibroblasts, but excess GlcNAc (over 32 mM) has reduced effect on dermal fibroblasts. Furthermore, oral administration of GlcNAc promoted hyaluronan synthesis in rats, and moisturized dry skin and relieved wrinkle formation in a clinical study.

Although GlcNAc is also used for cosmetics, most of the previous studies are limited to monolayer culture and oral intake. In this study, we investigated the topical route, thereby more closely mimicking the application of cosmetics on the human skin.

We aimed to confirm hyaluronan production on a three-dimensional human skin cell model. Human epidermal keratinocytes and dermal fibroblasts (TIG-108) were cultured to differentiation in EpiLife/DMEM medium for 14 days and embedded into collagen gel at the air-liquid interface. After 2 days of incubation the medium was supplemented with GlcNAc or saline. GlcNAc upregulated hyaluronan production in comparison to saline.

To confirm the application effect of GlcNAc-containing cosmetic products on the skin, we applied skin milk containing 1, 2, or 4% GlcNAc or placebo on the inside of the arm of 7 subjects with dry skin and measured skin moisture by a corneometer. The results indicated that transdermal administration of 1% GlcNAc significantly improves stratum corneum hydration.

This result suggests that cosmetics containing GlcNAc have skin-moisturizing effects. Because GlcNAc is reported to improve stratum corneum exfoliation and reduce facial hyperpigmentation, GlcNAc is a suitable cosmetic ingredient for skin care products.

(三)心得與建議

幾丁質廣泛分布在自然界許多生物中，在自然界中產量次高的一種生物長鏈高分子聚合醣，僅次於纖維素。幾丁質及幾丁聚醣，具有優秀的親水性、抗菌性、化學反應性、生物相容性和生物可降解性，可用於製備功能凝膠、高效吸附材料、生物支架、藥物載體、酶和細胞固定化模板、生物傳感器和創傷敷料等，在生醫產業上、食品加工、保健食品、農業、化妝品產業、環保產業等都有相當廣泛的應用。

此次會議主要的心得為瞭解世界上各國專家學者於 N-乙醯葡萄糖胺、幾丁寡醣、幾丁質酶研究的進展。利用幾丁質酶分解幾丁質來生產幾丁寡醣為目前研究重點，幾丁寡醣具有多樣的生物活性，中油生技利用自行開發菌種 *Chitinibacter tainanensis*，篩選出 12 個幾丁質分解酶，也經過結構分析幾丁質分解酶的功能。在篩選出的幾丁質分解酶中，有多個酵素具有幾丁質內切酶(endo-chitinase)的活性，透過基因工程技術製備幾丁質酶，鑑定出較具水解寡醣活性的幾丁質分解酶。而幾丁質分解酶的活性不單只與酵素催化活性有關，還需要其他幾丁質結合域來增強對幾丁質的分解作用。目前進一步透過基因工程技術，進行酵素胺基酸定點突變，建構改良型幾丁質分解酵素，期望提高幾丁質分解活性及寡醣生產效率。而除了增強酵素分解活性外，結合物理性的方法，例如以電漿、微波處理先破壞幾丁質結構，可以增加酵素分解生產寡醣的效率，亦是重要參考之方向之一。