

国際化推進事業
「中高温機能性」微生物に関するアジア国際共同研究
研究成果報告書

<研究実施内容>

2009年10月2日に国際戦略室より採択され、以降、半年間の短期に実施された「中高温機能性」微生物に関するアジア国際共同研究における実施内容とその研究成果を、以下に示す海外招聘研究者4名と本学派遣研究者5名の報告を併せ、各共同研究グループ毎に、まとめて、研究内容および研究成果の項に示す。

招聘事業

- 1) Prapaipid Chairattanamanokorn (タイ・カセサート大・講師) を今井 剛 (工学部・教授) が受け入れ、「Municipal Solid Waste and Wastewater Management in Thailand」についての共同研究を推進するとともに、工学部において、同タイトルのセミナーおよび実験指導を行った。期間：2010年1月16日～3月13日
- 2) Bella C. Cruzana (フィリピン・University of the Philippine Los Banos・准教授) を本道栄一 (農学部・准教授) が受け入れ、「コウモリ由来新興感染症の伝播経路」についての共同研究を推進するとともに、農学部において、同タイトルのセミナーおよび実習を行った。期間：2009年12月5日～12月18日
- 3) Ali Azam Talukder (バングラディシュ・Jahangirnagar University・准教授) を山田 守 (農学部・教授) が受け入れ、「Analysis of nucleoid structure of Escherichia coli at high temperature, and characterization of thermotolerant microorganisms from Bangladesh」についての共同研究を推進した。期間：2010年2月25日～3月24日
- 4) Jang hoy-mi (韓国・慶尚大学獣医学部・講師) を度会雅久 (農学部・准教授) が受け入れ、「Prevention and epidemiological study on canine brucellosis」についての共同研究を推進した。期間：2010年2月1日～12日

派遣事業

- 1) 今井 剛 (工学部・教授) は Prapaipid Chairattanamanokorn (タイ・カセサート大学) を訪問し、「中高温機能性微生物を用いたバイオ水素発酵の高機能化」についての共同研究を推進するとともに、「高温耐性酸生成菌によるバイオ水素発酵プロセスの開発」と題する講義をカセサート大学理学部で行った。期間：2009年12月19日～2010年1月4日

- 2) 山田 守(農学部・教授)は Ali Azam Talukder(バングラディシュ・Jahangirnagar University)を訪問し、「耐熱性微生物による高温発酵と耐熱性分子機構」についての共同研究に関連した研究討論およびセミナーを行った。
- 3) 本道栄一(農学部・准教授)および前田 健(農学部・教授)は Bella C. Cruzana 准教授(フィリピン・College of Veterinary Medicine, UPLB)を訪問し、「コウモリ由来新興感染症の伝播経路」についての共同研究に関する研究討論を行うとともに、関連の省庁を尋ね、動物調査および捕獲についての協力および許可申請等を行った。
- 4) 橘 理人(院生・農学部・度会雅久研究室)は Kim Suk(韓国慶尚大学)研究室を訪問し、「ブルセラ、リステリアの感染機構に関する研究」についての共同研究を推進した。

<研究内容および研究成果>

(1) 高温環境浄化および新規エネルギー生産系の確立についての共同研究(今井-Prapaipid)

<派遣>

1. 研究課題名：中高温機能性微生物を用いたバイオ水素発酵の高機能化
(Development of advanced bio-hydrogen production by methophilic and thermotolerant bacteria)

2. メンバー名：

派遣研究者：山口大学工学部 今井 剛

受入研究者：Prapaipid Chairattanamanokorn (Kasetsart University)

3. 事業日程および内容

交流者氏名 今井 剛 平成 21 年 12 月 19 日～平成 22 年 1 月 4 日

交流の具体的内容 タイの未利用農業残渣である豚糞を原料とした水素発酵に関する基礎実験(中温と高温の双方)を実施した。この際に用いる微生物はタイの養豚場の排水を処理するために設けられた嫌気性ラグーンの底泥を植種源としたものを用いた。また、「高温耐性酸生成菌によるバイオ水素発酵プロセスの開発」と題して、カセサート大学理学部環境科学科の学部生、院生、教職員を対象に講義を行った。

4. 研究概要

タイの未利用農業残渣である豚糞を原料とした水素発酵に関する基礎実験を実施した。この際に用いた微生物はタイの養豚場の排水を処理するために設けられた嫌気性ラグー

ンの底泥を植種源としたものである。実験は投入する豚糞の量と微生物の量の比を2段階に変化させ、それぞれについてさらに4段階にpHを変化させたもの(pH=4.5,5.5,6.7,7.5)を35℃の中温と55℃の高温の2ケースに分けて実施した(合計16ケース、なお、各ケース2回ずつ実施)。実験結果から、pHが低いほど安定的に水素が回収でき、微生物の濃度が基質(本実験の場合は豚糞)に比べて高すぎる場合はpHが低く設定されていても、メタン発酵まで反応が進んでしまい、安定的な水素生産が難しいことがわかった。なお、水素発酵は高温の方がより安定的に進行することも明らかとなった。

5. 成果 (発表論文等)

Comparison of biohydrogen production process with extreme-thermophilic with mesophilic anaerobic bacteria: Tsuyoshi Imai, Rafiani Hasyim and Alissara Reungsang; *International Symposium on Southeast Asian Water Environment*, 7, 419-425 (2009)

Cyanide removal from cassava mill wastewater using *Azotobacter vinelandii*, TISTR 1094 with mixed microorganisms in activated sludge treatment system: P. Kaewkannetra, T. Imai, F.J. Garcia-Garcia and T.Y. Chiu; *Journal of Hazardous Materials*, 172 (1), 224-228 (2009)

Key factors regarding decolorization of synthetic anthraquinone and azo dyes: A. Boonyakamol, T. Imai, P. Chairattananokorn, T. Higuchi and M. Sekine; *Applied Biochemistry and Biotechnology*, 158 (1), 180-191 (2009)

Hydrogen production and anaerobic decolorization of wastewater containing Reactive Blue 4 by a bacterial consortium of *Salmonella subteranea* and *Paenibacillus polymyxa*: Ramida Yuwadee Watanapokasin, Anantabhatra Boonyakamol, Aungkana Krajarng, Thanet Sophonnithiprasert, Sungwan Kanso and Tsuyoshi Imai; *Biodegradation*, 20 (6), 411-418 (2009)

Reactive Blue 4 decolorization under mesophilic and thermophilic anaerobic treatments: A. Boonyakamol, T. Imai, P. Chairattananokorn, T. Higuchi, M. Sekine and M. Ukita; *Applied Biochemistry and Biotechnology*, 152 (3), 405-417 (2009)

Decolorization of pulp mill wastewater using thermotolerant white rot fungi: Sehanat Prasongsuk, Pongtharin Lotrakul, Tsuyoshi Imai, and Hunsu Punnapayak; *Science Asia*, 35 (1), 37-41 (2009)

< 招聘 >

1. Research Topic: Municipal Solid Waste and Wastewater Management in Thailand

2. Research Members

Visiting scientist: Dr. Prapaipid Chairattananokorn (Kasetsart University)

Host scientist: Prof. Dr. Tsuyoshi IMAI

3. Research period: January, 16th 2010 to March, 13rd 2010

4. Academic work: For academic works, seminar presentation, proof of manuscripts and advice on design of experiment and data analysis were conducted. Academic seminar was presented on February 13rd, 2010 on the topic “Municipal Solid Waste and Wastewater Management in Thailand”. Some photographs were taken during the lecture as shown in the below and the document for the seminar was attached in the report.



Moreover, I revised an extended abstract conducted by a Master Student (Ms. Wei Jie) as well as two manuscripts written by two doctoral students (Mr. Xuehang Cheng and Mr. Gatot Eko Susilo) were proven and some comments were introduced for the manuscripts. Additionally, I advised a Doctoral student (Ms. Rafiani Hasyim) on the design of her research and data analysis under response surface method.

5. Research work “Utilization of swine manure as nitrogen source for hydrogen production under thermophilic fermentation”

Production of biohydrogen from agricultural residues was conducted by mixed microorganism under thermophilic fermentation (at 55°C). Swine manure taken from a livestock in Thailand was utilized as a substrate for the hydrogen production. Because of high nitrogen, the swine manure was mixed with food waste kindly given from the canteen in Faculty of Engineering, Yamaguchi University to adjust the ratio of carbon and nitrogen of the substrate.

The objectives of the research was to investigate the optimal ratio of swine manure and food waste as well as the concentration of seed sludge for the biohydrogen production at 55°C.

The mixed organism in the sludge from an anaerobic wastewater treatment in Thailand was boiled in water at 100°C for 60 min to inhibit methanogenic bioactivities. The experiment was carried out in triplicate in 75 ml serum bottles filled with 50 ml substrate solution comprised with 20 g/l the substrate. The serum bottles were covered with rubber stoppers and crimped with aluminum seals and flushed with argon gas to create anaerobic culture condition.

During the experiments, the liquid samples were collected to analyze pH and soluble metabolite composition as well as simultaneously biogas production was monitored with the respect to culture time. The cumulative H₂ evolution was calculated with the equation (Eq. 1).

$$V_{H,i} = V_{H,i-1} + C_{H,i} (V_{G,i} - V_{G,i-1}) + V_{H,0}(C_{H,i} - C_{H,i-1}) \quad (1)$$

Where $V_{H,i}$ and $V_{H,i-1}$ denote cumulative H₂ volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i-1}$ are the total biogas volumes at the current and previous time intervals. $C_{H,i}$ and $C_{H,i-1}$ are the fraction of hydrogen in the headspace of the bottle at the current and previous intervals and $V_{H,0}$ is the total volume of headspace in the bottle.

The volume of biogas was measured by a wet glass syringe and the biogas composition (mainly H₂, N₂ and CO₂) was analyzed by gas chromatography (GC, Shimadzu GC-8A) equipped with a thermal conductivity detector and a 3mx3 mm diameter stainless steel column packed with activated charcoal (60/80 mesh). Injector, detector and column temperature were used at 50, 50 and 60°C, respectively, using argon as carried gas. Volatile fatty acid (VFAs) in soluble metabolite samples was analyzed by GC, Shimadzu GC-8A equipped with a flame ionization detector and a 3mx3.2mm diameter glass column packed with unisole F-200 (30/60 mesh). The temperature of injector, detector and column were 250, 140 and 140°C, respectively. N₂ and H₂ were used as carried gas and combustion gas, respectively.

Pretreatment of swine manure

The effect of the pretreatment of swine manure on the hydrogen production was studied by Taguchi experimental design. The manure was pretreated with various conditions as Table 1. The pH of manure solution was adjusted to pH 4, 6 and 8 with several solid contents (3, 5, and 7%, w/v). Thereafter, the mixture of the manure was pretreated under high temperature (35, 65 and 100°C) within 30, 60 and 90 min. The pretreated manure was utilized as nitrogen and mineral sources by mixing with food waste to produce hydrogen. The result of hydrogen production and production yield from the manure pretreated under designed conditions was illustrated in Table 2. The

conditions of heating temperature, heating time, pH and solid content of the manure for the highest cumulative hydrogen production were 65°C, 60 min, pH 8 and 7%, respectively.

Table 1 Pretreatment condition of the manure

Condition	1	2	3
Temperature	30	65	100
Time (min)	30	60	90
pH	4	6	8
Solid content (%)	3	5	7

Table 2 Cumulative hydrogen production and hydrogen production rate from the fermentation of the food waste and manure pretreated under various conditions

	Cumulative H ₂ production (ml/l)	H ₂ production rate (ml/l.hr)
Temperature (°C)		
30	1891.83	35.35
65	2029.92	58.05
100	1989.38	47.83
Time (min)		
30	1968.61	48.71
60	1983.76	40.73
90	1958.77	51.80
pH		
4	1931.79	44.16
6	1978.89	46.15
8	2000.46	50.92
Solid content (%)		
3	1915.46	43.86
5	1966.89	42.60
7	2028.79	54.78

Fermentation condition for hydrogen production

Additionally, the effect of the ratio of the manure and food waste was investigated included the concentration of seed sludge, phosphate buffer and L-cystein as reducing agent. The experiment design was indicated in the Table 3. After the fermentation of the substrate (food waste and pretreated manure) under designed fermentation, the cumulative hydrogen production and yield were presented in the Table 4. The optimal condition (in the range studied) for the ratio of the manure and food waste, the concentration of seed sludge and L-cystein concentration was 1:9, 7.5 and 0.25 mM, respectively. However, the application of phosphate buffer affected the hydrogen production decreased as the concentration of the buffer.

Table 3 The fermentation condition from the mixture of the pretreated manure and food waste

Condition	1	2	3
Ratio of manure and food	9:1	3:7	1:9
Ratio of TVS substrate and seed sludge	2.5	5.0	7.5
Phosphate buffer (mol)	0	0.2	0.4
L-cystein (mmol)	0	0.25	0.5

Table 4 Cumulative hydrogen production and hydrogen production rate from the mixture of pretreated manure and food waste fermented under various conditions

	Cumulative H ₂ production (ml/l)	H ₂ production rate (ml/l.hr)
Ratio of manure and food		
9:1	569.50	9.70
3:7	1499.61	32.23
1:9	1595.84	39.92
Ratio of TVS substrate and seed sludge		
2.5	1082.12	17.20
5.0	1059.74	32.54
7.5	1523.09	32.12
Phosphate buffer (mol)		
0	2504.52	64.12
0.2	944.13	12.40
0.4	216.29	5.24
L-cystein (mmol)		
0	900.70	12.27
0.25	1404.73	34.72
0.5	1359.52	34.86

Optimal condition for hydrogen production from the manure and food waste by response surface method

Since the application of phosphate buffer was negatively affected to the hydrogen production, only the ratio of manure of food waste, ratio of substrate and seed sludge and the concentration of L-cystein were selected for the study designed by response surface method. Because the food waste kept the refrigerator was fermented, the carbon content in the food waste was change. To adjust the ratio of carbon and nitrogen in the substrate, the ratio of the manure and food was changed from the previous studies in the Table 3. The experimental condition, cumulative hydrogen production and production rate conducted following the designed conditions were indicated in the Table 5. Because of the characteristics of food waste was changed, the range of parameters selected in the study could not optimize the fermentation condition.

Table 5 the fermentation condition for optimizing the hydrogen production by response surface method

	Ratio of manure and food waste	Ratio of TVS substrate and seed sludge	L-cystein (mM)	Cumulative production (ml/l)	Production rate (ml/l.hr)
1	26.8	5	0.3	3505.42	10.51
2	13.9	2.5	0.1	3719.33	18.70
3	13.9	2.5	0.5	4076.65	30.35
4	13.9	7.5	0.1	3960.22	37.05
5	13.9	7.5	0.5	3822.99	28.70
6	5.0	1.7	0.3	4461.00	37.75
7	5.0	5	0.04	4201.30	30.38
8	5.0	5	0.3	3804.82	17.68
9	5.0	5	0.3	4472.79	38.32
10	5.0	5	0.3	4754.90	40.71
11	5.0	5	0.3	4629.12	39.79
12	5.0	5	0.3	4101.08	17.78
13	5.0	5	0.3	4393.07	29.57
14	5.0	5	0.56	4465.78	30.57
15	5.0	8.3	0.3	4491.22	30.61
16	2.7	2.5	0.1	4099.98	16.81
17	2.7	2.5	0.5	4565.99	36.17
18	2.7	7.5	0.1	4457.00	33.36
19	2.7	7.5	0.5	4560.30	29.31
20	2.4	5	0.3	4576.47	30.83

Effect of NaHCO₃

The substrate (food waste and swine manure) was organic and easily digested. Therefore, volatile fatty acids were produced more quickly. As the reason, effect of NaHCO₃ was investigated. NaHCO₃ was supplied in the substrate solution at the concentration of 1, 3 and 4 g/l compared with the control condition (without NaHCO₃ added). The optimal concentration of NaHCO₃ was 1 g/l as shown in Table 6.

Table 6 Effect of NaHCO₃ in the substrate on hydrogen production

Concentration NaHCO ₃ (g/l)	Cumulative H ₂ production (ml/l)	H ₂ production rate (ml/l.hr)
0	333.62	5.07
1	1289.86	14.65
3	959.79	19.40
4	1022.14	16.19

Conclusions

Swine manure as agricultural residues and food waste could be more valuable by utilizing for biohydrogen production. Pretreatment of swine manure at 7% w/v by heating at 65°C for 60 min improved the hydrogen production. Application of phosphate buffer negatively affected the hydrogen production. Moreover, NaHCO₃ at 4 g/l provided highest hydrogen production compared with that at 0, 1 and 3 g/l.

Acknowledgements

This project was supported by Yamaguchi University. I am grateful thank for Professor Tsuyoshi Imai as host laboratory and his generosity. Thanks for Ms. Toshimi YAMOTO, Dr. Suksaman Sangyoka, Ms.Jantima Teeka and all lab members for their help during my visit at Yamaguchi University.

(2) 高温発酵系の開発に関する共同研究（山田-Azam）

<派遣>

1. 研究課題名：耐熱性微生物による高温発酵と耐熱性分子機構

(High-temperature fermentation using thermotolerant microbes and molecular mechanism of their thermotolerance)

2. メンバー名：

派遣研究者：山口大学医学系研究科（農学系）山田守

受入研究者：Ali Azam Talkuder (Jahangirnagar University)

3. 事業日程および内容

交流者氏名 山田守 平成 21 年 12 月 15 日～21 日

交流の具体的内容：バングラデシュの Jahangirnagar University、North-South University で高温発酵に関する講演を行うとともに、意見交換を行った。また、Dhaka University でも同様に意見交換を行った。さらに、JSPS - JSPS Fellows Alumni Association of Bangladesh の第一回シンポジウムに招待され、「Fermentation technology with thermotolerant microorganisms: Applicable for tropical countries」と題した講演を行った。

4. 研究概要

耐熱性酵母や耐熱性細菌を用いた高温エタノール発酵のための基礎研究と、大腸菌や耐熱性エタノール生産性細菌の耐熱性分子機構研究を行っている。耐熱性酵母では木質系バ

イオマスのように5単糖を含む複数の糖が混在する系での発酵研究をすすめるとともに、ゲノム解析を NITE と共同で行っている。耐熱性エタノール生産性細菌では、より良い耐熱性株の獲得を行うとともに、本菌の高速発酵性を利用する試みとして乳酸などの有用物質生産系の開発をすすめている。耐熱性分子機構の研究では、大腸菌の解析をほぼ終了し、耐熱性エタノール生産性細菌の解析を継続している。共通の分子機構や耐熱化因子の獲得が今後の課題である。

5. 成果（発表論文等）

A. A. Talukder, S. Alam, Md Ershaduzzaman, S. K. Bashar and M. Yamada: Structure organization of co-regulated genes in *Escherichia coli*. *J. Bacteriology Research*, in press

N. Lertwattanasakul, E. Shigemoto, N. Rodrussamee, S. Limtong, P. Thanonkeo and M. Yamada: Crucial role of alcohol dehydrogenase adh3 in *Kluyveromyces marxianus* mitochondrial metabolism. *Biosci. Biotechnol. Biochem.* 73: 2720-2726 (2009)

藤元 奈保子、山田 守：牛ルーメンからの耐熱性セルロース分解微生物の探索。2010年度日本農芸化学会大会、講演要旨集、p291、東京、3月27-30 2010.

N. Rodrussamee, N. Lertwattanasakul, K. Hirata, S. Limtong and M. Yamada: Glucose repression in thermotolerant *Kluyveromyces marxianus* DMKU. 2010年度日本農芸化学会大会、講演要旨集、p279、東京、3月27-30 2010.

M. Yamada : Fermentation technology with thermotolerant microorganisms: Applicable for tropical countries. The First Symposium for JSPS - JSPS Fellows Alumni Association of Bangladesh 19 Dec 2009, Bangladesh

村田正之、藤本博子、西村香織、山田守：大腸菌における高温生育限界温度必須遺伝子群の同定とパスウェイ解析。第32回日本分子生物学会年会講演要旨、p446、横浜、Dec.9-12, 2009

山田守：次世代発酵技術によるバイオエタノール生産。中高温微生物センター開所記念シンポジウム「地球温暖化に向けた新たな微生物学の展開を」山口大学、Nov. 19, 2009

Kannikar Charoensuk, 入江陽、中島康之、Pornthap Thanonkeo、山田守: *Zymomonas mobilis* のチトクロームCペルオキシダーゼの機能解析。第82回日本生化学会大会講演要旨、p252、神戸、Oct.21-24, 2009

Rashed Noor、村田正之、山田守: 大腸菌定常期初期におけるシグマE依存性プログラム死：PpiDの役割。日本農芸化学会3支部合同大会、要旨集、p124、沖縄、10月30-31日、2009

M. Yamada, R. Noor, H. Nagamitsu, and M. Murata: The higher temperature, the more oxidative stress and lysis in *Escherichia coli*. The 3rd International Conference on Fermentation

Technology for Value Added Agricultural Products, Abstract p63, Khon Kaen, Thailand, Aug 25-26, 2009

村田正之、藤本博子、西村香織、山田守:大腸菌における高温生育限界温度での必須遺伝子とその発現変動. 日本バイオインフォマティクス学会シクテムバイオロジー研究会、8月18日、山口

<招聘>

1. Research subject: Analysis of nucleoid structure of *Escherichia coli* at high temperature, and characterization of thermotolerant microorganisms from Bangladesh

2. Members:

Visiting scientist: Dr. Ali Azam Talukder (Assistant professor; Department of Microbiology, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh)

Host scientist: Dr. Mamoru Yamada (Professor: Yamaguchi University, Japan).

3. Research period: 25 February – 24 March 2010.

4. The results of research:

Bacterial single-circular genome is composed by one or a few bodies known as a nucleoid, which consists of DNA, RNA and DNA-binding proteins. In *E. coli*, growth phase-dependent variations of nucleoid structure have been reported. The compaction and de-compaction states of nucleoid regulate the prokaryotic gene expression in addition to its functional roles for transcription, translation and replication. Here, I tried to isolate membrane-associated nucleoid from different temperature sensitive mutants of *E. coli* by a sucrose density gradient centrifugation. Sedimentation profiles of isolated nucleoids were found to be different among strains examined. Crude nucleoids isolated from temperature-sensitive mutants run faster in the sucrose gradient compared to that from the temperature-resistant wild-type strain. Our preliminary results revealed that unusual/unstable or de-compacted and stable or compacted nucleoids are formed in temperature-sensitive mutants and the wild-type strain, respectively, under the conditions employed in this study.

In order to fulfill our future demands including food safety, energy production as well as to save our environment under the circumstance of recent global warming, we are attempting to isolate useful thermotolerant microorganisms from tropical countries. During this visit for a month,

I also worked with three thermotolerant microorganisms, which were isolated previously from the decomposing jute materials in Bangladesh (self heating piles of jute). Cultural, morphological and some biochemical analyses were carried out under various physiological conditions. Temperature profiles were tested in liquid culture and on solid agar-plates of PDA, YPD and MPD media under shaking and static conditions at 37-48°C. Three strains of microorganisms examined, *Thermomyces lanuginosus*, *Penicillium* sp. and *Streptomyces* sp., could grow well on all temperatures tested. Microscopic observation indicated that the former two and the latter are spore-forming filamentous fungus and actinomyces, respectively. Relatively high levels of reducing sugar were detected in culture medium in all three strains. They also showed amylase and cellulase activities when starch and CMC were added as a sole carbon source, respectively, in liquid culture. Under the conditions employed here, the amount of reducing sugar and activities of cellulase and amylase became maximum at 48°C. All together, our preliminary results indicated that the three strains are potential candidates as useful thermotolerant microorganisms.

(3) 感染症伝播ルートの予測に関する共同研究（本道・前田-Bella）

<派遣>

1. 派遣の目的：

オオコウモリ由来新興感染症がわが国へ進入してくる経路として最も疑われるのは、フィリピンー台湾ー沖縄ルートである。従って、このルート間の研究者が感染症に関する知識を共有する必要があるが、これまで本学とフィリピンの大学間には、同テーマを共有する研究者の交流がなかった。そこで、本事業では、同テーマに関する、本学教員と国立フィリピン大学教員との研究者交流を初めとして、将来的な学生間の交流を視野に入れた国際学術交流を行う。

2. メンバー名：

派遣研究者：山口大学農学部 前田健、本道栄一

受入研究者：University of Philippines at Los Banos, Associate Professor Bella Cruzana

3. 事業日程および内容

交流者氏名 前田健、本道栄一 平成22年3月6日～平成22年3月12日

交流の具体的内容 最初に、フィリピン大学獣医学部 Baldez 学部長を訪ね、Cruzana 博士とともに共同研究（事業）の概要説明を行い、今後の協力に対しての要請を行った。そして、学部内の研究施設の視察を行い、我々が実験を行う際の病原微生物の扱いおよび使用

機器の注意事項の確認を行った。また、本学学生が訪問した際の協力体制についての確認を行った。

4. 研究概要

本事業のベースになる研究は、オオコウモリ由来新興感染症の防圧である。上記のように、オオコウモリ由来新興感染症がわが国へ進入してくる経路として最も疑われるのは、フィリピンー台湾ー沖縄ルートである。従って、来年度に行う研究は、フィリピンバタン諸島において、日本のクビワオオコウモリと同種であるクビワオオコウモリを捕獲し、その行動について Argos 衛星を用いて追跡調査することである。従って、クビワオオコウモリの捕獲に伴う協力体制を築く必要がある。そこで、同大森林博物館を訪れ、Philip 研究員に協力を要請した。そして快諾を頂いたため、クビワオオコウモリの捕獲許可願いのため、Cruzana 博士、Philip 学芸員とともにマニラのフィリピン野生動物管理局を訪れた。同局 Manila 理事補佐および同秘書 2 名に対し、研究の概要を説明し、協力をいただけることになった。ただし、捕獲許可取得のためには、フィリピンバタン諸島の役所の同意が必要であり、本件に関しては、今後 Cruzana 博士、Philip 博士に任せることになった。また、本研究の結果、新たな新興感染症が発見された場合には、本事業による研究者-フィリピン野生動物管理局-バタン諸島の役所職員の 3 者で、その情報の取り扱いを協議して、社会的な影響を最小限に留めるような公開の仕方をしていくことで合意した。

今回の渡比により、実際の研究開始の目処が立った。今後は、他の事業と共同で、本研究の遂行段階に、本学学生およびフィリピン大学学生を参画させる予定である。

< 招聘 >

1. 受入の目的: 国立フィリピン大学との国際交流を目的として、フィリピン大学獣医学部教員を招聘し、当該教員が、本学教員及び学生に対し、セミナー及び実習を行う

2. 担当者

招聘者 : Bella Cruzana

受入担当教員 : 前田健、本道栄一

3. 受入期間: Bella Cruzana; 平成 21 年 12 月 6 日 – 平成 21 年 12 月 19 日

4. 事業報告

上記期間、国立フィリピン大学より Bella Cruzana 博士が来学した。来学中には、コウモ

り由来新興感染症に関するセミナーを行った。特にフィリピンに固有のオオコウモリ類についてその分布と、媒介する感染症の実情について、本学教員および学生に対し教授を行った。詳細は以下の通りである。

The Philippines with over 7,100 islands has one of the world's remarkable terrestrial mammalian fauna. The major island groups in the Philippines (Luzon, Mindanao, Palawan, Negros and Panay) with different topography, geologic age and size contain a unique set of mammalian fauna which include bats. The Philippines is home to the smallest and the largest bats among the 1,000 known species in the world. There were a total of 73 species of bats recognized in the Philippines (25 are fruit bats or microchiropterans while 48 are insectivorous bats). This lecture will focus on fruit bats or microchiropterans (Old world fruit bats, Family Pteropodidae) because of their ecological importance and association with some emerging zoonotic diseases. Ecologically, they disperse thousands of seeds a night throughout the forest floor. With more than 300 plant species relying on the pollinating and seed dispersal services of bats which include bananas, mangoes, avocados and cashews. They leave guano which is one of the best natural fertilizers available to man and hunted by man for food because they are considered a delicacy in many regions since they taste like "chicken". However, these bats are now being associated with emerging zoonotic agents such as Hendra, Nipah, Menangle, SARS- CoV- like virus, Australian bat lyssavirus and Ebola virus. Rabies is a rampant health problem wherein around 600 people died of rabies every year in the Philippines. A serologic study of Lyssavirus infections among bats in the Philippines (Arguin et al, 2002) showed that although no bats were found to have active infection with a *Lyssavirus*, 22 had evidence of neutralizing antibody against the *Australian bat lyssavirus* (ABLV). Seropositivity was statistically associated with one species of bat, *Miniopterus schreibersi*. Results from the virus neutralization assays are consistent with the presence in the Philippines of a naturally occurring *Lyssavirus* related to ABLV. Last year, there was an outbreak of Ebola reston in commercial pig farms. This was the first time that a food animal was infected with this virus. The strain was first found in laboratory monkeys exported from the Philippines to the US in 1989. According to the World Health Organization, the strain infecting the pigs is not dangerous to humans, unlike the four deadly Ebola subtypes found in Africa. A group of foreign health experts have arrived in the Philippines to see if fruit bats may have infected pigs with a non-lethal form of the Ebola virus.

さらに、本学学生に対し、免疫組織化学による神経内分泌細胞および神経細胞の同定技術の教授を行った。

(4) 熱帯感染症診断システムの開発に関する共同研究（度会-Kim）

<派遣>

1. 研究課題名：ブルセラ、リステリアの感染機構に関する研究

(Study on infection mechanism of *Brucella* and *Listeria*)

2. メンバー名：

派遣研究者：山口大学連合獣医学研究科 大学院3年生 橋 理人

受入研究者：韓国慶尚大学 Kim Suk（キム スク）

3. 事業日程および内容

交流者氏名 橋 理人 平成21年12月4日～7日

交流の具体的内容 ブルセラの病原性を解析する目的で、共同研究先の韓国慶尚大学金スク博士の研究室を訪れ、ブルセラ症に罹患した牛から菌株の分離を行った。病原性の解析を行うとともに、リステリアの病原性との共通点に関して討論を行った。

4. 研究概要

現在韓国において牛のブルセラ症が流行している。韓国において、ブルセラ症に罹患した牛からブルセラ菌を分離し、病原性の検討を行った。その結果、研究室内で保存されている菌株に比べ、マウスへの病原性が高くなっていることが示唆された。マクロファージ内における菌の増殖能の増加も認められた。韓国における流行と何らかの関係があるものと考えられるため、今後も引き続き解析を行う予定である。

5. 結果（発表論文等）

Watanabe, K., Tachibana, M., Kim, S. and Watarai, M. (2009) Participation of ezrin in bacterial uptake by trophoblast giant cells. *Reprod. Biol. Endocrinol.* 7: 95.

Watanabe, K., Tachibana, M., Kim, S. and Watarai, M. (2009) EEVD motif of heat shock cognate protein 70 contributes to bacterial uptake by trophoblast giant cells. *J. Biomed. Sci.* 16: 113.

<招聘>

1. Research subject: Prevention and epidemiological study on canine brucellosis

2. Members

Visiting scientist: Jang Hyo-mi

(Graduate student; Gyeongsang National University, College of Veterinary Medicine)

Host scientist: Masahisa Watarai (Faculty of Agriculture, Yamaguchi University)

3. Research period: Jang Hyo-mi; 1 February - 12 February 2010

4. The results of the research

Diagnosis of brucellosis is based on bacteriological examination and serological tests. Serological diagnosis is usually performed by the tube agglutination test, rapid slide agglutination test, and gel immunodiffusion test. However, agglutination tests sometimes give false-positive reactions due to cross-reactions with other pathogens, and a general strategy for eliminating such cross-reactions is to use purified antigen with unique epitopes. We have found that Cu-Zn superoxide dismutase (SOD) of *Brucella* showed the strongest antigenic reaction. We tried a screening method for canine brucellosis using an enzyme-linked immunosorbent assay (ELISA) with recombinant SOD as antigen.

To identify sera infected with *B. canis*, we performed the tube agglutination test on canine serum samples ($n = 224$) randomly selected from dogs consecutively admitted to animal hospitals in Korea by hospital staff. In the test, antibodies to *B. canis* were detected in 30 of the 224 serum samples (Table 1). We then determined whether ELISA using recombinant SOD can be applied to screening for canine brucellosis using the dog serum samples from Korea. All serum samples having absorbance values of over 0.410 or under 0.400 (OD_{405}) were either positive or negative in the tube agglutination test. Immunoreactive bands were detected for sera having an absorbance value of over 0.41 (OD_{405}) in Western blotting (Table 1). Thus, the absorbance measurements in ELISA with recombinant SOD and the titers in the tube agglutination test showed a similar tendency, suggesting that ELISA with recombinant SOD is useful in screening for canine brucellosis.

Table 1. Serological analysis of canine sera

	TAT ^a + 30 (13.4%)		TAT- 194 (86.6%)	
	ELISA ^b +	ELISA-	ELISA+	ELISA-
Sera (n=224)	30 (100%)	0 (0%)	2 (1.0%)	192 (99.0%)
WB ^c + in ELISA+	26 (86.7%)	-	0 (0%)	-

a) Tube agglutination test.

b) Samples with absorbance values of over 0.400 (OD_{405}).

c) Western blot analysis.