

平成 28 年度 医療研究開発推進事業費補助金  
成果報告書

**I. 基本情報**

事業名： (日本語) ナショナルバイオリソースプロジェクト  
(英語) National Bioresource Project

補助事業課題名： (日本語) 病原微生物の収集・保存・提供体制の整備  
(英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms

補助事業担当者 (日本語) 国立大学法人千葉大学 准教授 矢口 貴志  
所属 役職 氏名： (英語) Takashi Yaguchi, Associate Professor, Medical Mycology Research Center, Chiba University

実施期間： 平成 28 年 4 月 1 日 ～ 平成 29 年 3 月 31 日

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性細菌の収集・保存・提供および関連情報の収集・提供)

分担課題名： (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic bacteria)

補助事業分担者 (日本語) 国立大学法人大阪大学 微生物病研究所 教授 飯田 哲也  
所属 役職 氏名： (英語) Tetsuya Iida, Professor, Research Institute for Microbial Diseases, Osaka University

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性細菌の収集・保存・提供および関連情報の収集・提供)

分担課題名： (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic bacteria)

補助事業分担者 (日本語) 国立大学法人岐阜大学 名誉教授・フェロー 江崎 孝行  
所属 役職 氏名： (英語) Takayuki Ezaki, Professor emeritus, Fellow, Gifu University, Center for Conservation of Microbial Genetic Resource, Organization for Research and Community Development

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性原虫の収集・保存と提供)

分担課題名: (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic protozoa)

補助事業分担者 (日本語) 国立大学法人長崎大学 熱帯医学研究所 森田 公一

所属 役職 氏名: (英語) Kouichi Morita, Dean, Institute of Tropical Medicine, Nagasaki University

## II. 成果の概要 (総括成果報告)

NBRP「病原微生物」全体としては、(1) 基準株、(2) 高度病原菌、(3) 新鮮な臨床分離株を収集することを目的とし、今後いかなる感染症が起っても、それに対応できる病原微生物株コレクションを目指した。その中で、千葉大学真菌医学研究センターでは病原真菌、病原放線菌、大阪大学微生物病研究所では腸内感染症を引き起こす病原細菌、岐阜大学・医学部・病原微生物遺伝資源センターでは呼吸気道感染症関連の病原細菌、長崎大学熱帯病研究所では病原原虫株の収集とその保存と提供およびその性状分析を実施した。

毎年の収集、保存、提供における数値目標 (千葉大: 500 株、22,640 株、500 株、大阪大: 150 株、12,100 株、150 株、岐阜大: 200 株、16,330 株、250 株、長崎大: 30 株、820 株、15 株) に対しては、全体としてほぼ目標を達成していると考えられる。

千葉大においては、これまで病原真菌・放線菌として報告された菌種を中心に標準株としての利用価値の高い品揃えを目標とし、収集、提供を実施した。さらに、近年、医真菌分野で着目されている菌種、例えば薬剤耐性を有する *Aspergillus* を重点的に収集した。さらに、新鮮な臨床分離株は、新薬 (抗爪白癬薬 (現在米国で P2 試験)、アゾール系抗真菌薬 (前臨床)) 開発に使用され、医療の発展に貢献している。

大阪大では、腸管病原性細菌を中心に菌株の収集を継続して実施し、これらの生化学的な性状を再確認し、研究上有用な情報を付加して菌株の高品質化を図り、保存、提供した。

岐阜大では、二種、三種病原体 (ボツリヌス菌、炭疽菌、類鼻疽菌、結核菌、野兔菌など) などの気道感染する高度病原体の DNA での提供を行うと同時に、需要が高い血清型、生物型、遺伝子型、耐性株の収集を行い、遺伝子解析、品質管理情報の収集を図った。

長崎大では、国内唯一の病原性原虫リソース・センターとして、原虫株の収集・保存・提供と、国内の研究機関や教育機関等が保有する原虫株所在情報を一挙に収集・公開して、原虫リソースを材料とする研究者や教育者への便宜を図っている。感染症のグローバル化に伴い、その対応策の一環として新薬やワクチン開発用として、特に国外流行地で収集した原虫による風土病の新鮮分離株を数多く揃えた。

いずれの機関においても保存株の性状解析および臨床情報を追加し、菌株の高品質化を図り、データベースとして整備、公開した。病原微生物株の特性を示す情報は細菌、真菌、原虫でそれぞれ異なり、細菌においては国立遺伝学研究所の支援を受け、日本細菌学会員を中心としたアドバイザー委員会の意見を取り入れた新たなフォーマットを作成し、データを移行した。

また、講習会の開催、菌株の同定サービスなどを通じ、医療機関、研究機関との連携を強め、新たなユーザー獲得に繋げている。

The NBRP “Pathogenic Microorganisms” are collections of (1) type strains, (2) high level pathogens, and (3) fresh strains from clinical specimens. Even if any infection develops, this project aims at the pathogenic microorganism collection to deal with it.

The Medical Mycology Research Center, Chiba University handles pathogenic fungi and actinomycetes, the Research Institute for Microbial Diseases, Osaka University and the Gifu University, Center for Conservation of Microbial Genetic Resource, Organization for Research and Community Development do pathogenic bacteria and the Institute of Tropical Medicine, Nagasaki University dose pathogenic protozoa. Their number aims are the following; Chiba University: 500 strains、 22,640 strains、 500 Chiba University, Osaka University: 150 strains、 12,100 strains、 150 strains, Gifu University: 200 strains、 16,330 strains、 250 strains, Nagasaki University: 30 strains、 820 strains、 15 strains. Each institute does collection, preservation, and analysis of characteristics on pathogenic microorganisms.

Chiba University collected, preserved and provided type strains of pathogenic fungi and actinomycetes, high in reusable value. It also collected antifungal-resistant strains of *Aspergillus fumigatus*, notable in clinical field. The fresh clinical strains were used for development of drug medicines and contributed to medical development.

Osaka University collected pathogenic bacteria in the intestine and, analyzed biochemical characteristics to add the information for which the study top is useful.

Gifu University collected air passage infecting high class pathogenic bacteria, including Class II or III Pathogens, and provide their DNA. It also analyzed biochemical and phylogenetic characteristics

Nagasaki University was the only instate collecting, preserving and providing pathogenic protozoa in Japan. These strains were used for development of drug medicines and vaccines.

We also prepared the new format data-base of pathogenic bacteria, providing specific biochemical information of pathogenic bacteria, supported by the National Institute of Genetics and the Japanese Society for Bacteriology.

By holding lectures of pathogenic microorganisms and identification of causative microorganism for infections for medical organizations, we seeked to enhance the partnerships with them and increase the number of users.

### III. 成果の外部への発表

(1) 学会誌・雑誌等における論文一覧（国内誌 35 件、国際誌 384 件）

（千葉大）

1. Ishikawa T, Itoh F, Yoshida S, Saijo S, Matsuzawa T, Gono T, Saito T, Okawa Y, Shibata N, Miyamoto T, Yamasaki S: Identification of distinct ligands for the C-type lectin receptors mincle and dectin-2 in the pathogenic fungus *Malassezia*. Cell Host Microbe. 2013, 13, 477-488.
2. Visagie CM, Houbraken J, Frisvad JC, Hong S-B, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA. Identification and nomenclature of the genus *Penicillium*. Stud Mycology 2014, 78, 343-371.
3. Novakova A, Hubka V, Dudova Z, Matsuzawa T, Kubatova A, Yaguchi T, Kolarik M: New species in *Aspergillus* section *Fumigati* from reclamation sites in Wyoming (U.S.A.) and revision of *A. viridinutans* complex. Fungal Diversity. 2014, 64, 253-274.
4. Herbst S, Shah A, Mazon Moya M, Marzola V, Jensen B, Reed A, Birrell MA, Saijo S, Mostowy S, Shaunak S, Armstrong-James D. Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to *Aspergillus fumigatus*. EMBO

Mol Med. 2015, 7, 240-58.

5. Fraç M, Jezierska-Tys S, Yaguchi T. Occurrence, detection and molecular and metabolic characterization of heat-resistant fungi in soils and plants and their risk to human health. *Advances Agronomy*. 2015, 132, 161-204.
6. Takahata S, Kubota N, Takei-Masuda N, Yamada T, Maeda M, Alshahni MM, Abe S, Tabata Y, Maebashi K. Mechanism of action of ME1111, a novel antifungal agent for topical treatment of onychomycosis. *Antimicrob Agents Chemother*. 2016, 60, 873-880.
7. Hagiwara D, Watanabe A, Kamei K. Sensitisation of an azole-resistant *Aspergillus fumigatus* strain containing the cyp51A-related mutation by deleting the srbA Gene. *Scientific Reports*. 2016, 6, 38833.

(大阪大)

8. Salomon D, Guo Y, Kinch LN, Grishin NV, Gardner KH, Orth K. Effectors of animal and plant pathogens use a common domain to bind host phosphoinositides. *Nat Commun*. 2013;4:2973.
9. Sreelatha A, Bennett TL, Zheng H, Jiang QX, Orth K, Starai VJ. Vibrio effector protein, VopQ, forms a lysosomal gated channel that disrupts host ion homeostasis and autophagic flux. *Proc Natl Acad Sci U S A*. 2013 110(28):11559-64. doi: 10.1073
10. Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, Mirzaei H, Orth K. Marker for type VI secretion system effectors. *Proc Natl Acad Sci U S A*. 2014 111(25):9271-6.
11. Xu H, Yang J, Gao W, Li L, Li P, Zhang L, Gong YN, Peng X, Xi JJ, Chen S, Wang F, Shao F. Innate immune sensing of bacterial modifications of Rho GTPases by the Pysin inflammasome. *Nature*. 2014 513(7517):237-41.
12. Sreelatha A, Bennett TL, Carpinone EM, O'Brien KM, Jordan KD, Burdette DL, Orth K, Starai VJ. Vibrio effector protein VopQ inhibits fusion of V-ATPase-containing membranes. *Proc Natl Acad Sci U S A*. 2015 112(1):100-5.
13. Hubbard TP, Chao MC, Abel S, Blondel CJ, Abel Zur Wiesch P, Zhou X, Davis BM, Waldor MK. Genetic analysis of *Vibrio parahaemolyticus* intestinal colonization. *Proc Natl Acad Sci U S A*. 2016, 113(22):6283-8.
14. Blondel CJ, Park JS, Hubbard TP, Pacheco AR, Kuehl CJ, Walsh MJ, Davis BM, Gewurz BE, Doench JG, Waldor MK. CRISPR/Cas9 screens reveal requirements for host cell sulfation and fucosylation in bacterial type III secretion system-mediated cytotoxicity. *Cell Host Microbe*. 2016, 20(2):226-37.

(岐阜大)

15. Hayashi M, Kubota-Hayashi S, Natori T, Mizuno T, Miyata M, Yoshida S, Zhang J, Kawamoto K, Ohkusu K, Makino S, Ezaki T. Use of blood-free enrichment broth in the development of a rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *Int. J. Food Microbiol*. 2013. 163, 41-46.
16. Hayashi M, Natori T, Kubota-Hayashi S, Miyata M, Ohkusu K, Kawamoto K, Kurazono H, Makino S, Ezaki T. A new protocol to detect multiple foodborne pathogens with PCR dipstick DNA chromatography after six-hour enrichment culture in a broad-range food pathogen enrichment broth. *BioMed Research Inter*. 2013, Article ID295050,

10 pages.

17. Kikuchi M, Ito S, Yasuda M, Tsuchiya T, Hatazaki K, Takanashi M, Ezaki T, Deguchi T, Remarkable increase in fluoroquinolone-resistant *Mycoplasma genitalium* in Japan J Antimicrob Chemother. 2014,10.1093/jac/dku164.
18. Ogawa H, Ohnuma M, Squarre D, Mweene AS, Ezaki T, Fujikura D, Ohnishi N, Thomas Y, Hang'ombe BM, Higashi H, *Bacillus cereus* from the environment is genetically related to the highly pathogenic *B. cereus* in Zambia. J. Vet. Med. Sci. 2015, 77(8): 993-5
19. Ogawa H, Fujikura D, Ohnuma M, Ohnishi N, Hang'ombe BM, Mimuro H, Ezaki T, Mweene AS, Higashi H. A novel Multiplex PCR discriminates *Bacillus anthracis* and its genetically related strains from other *Bacillus cereus* group species. PLoS One. 2015, 16;10(3).
20. Ohshiro T, Miyagi C, Tamaki Y, Mizuno T, Ezaki T, Development of a rapid diagnostic method of *Staphylococcus aureus* and the antimicrobial resistance from a positive blood culture bottle using a PCR-DNA-Strip method. J. Infect. Chemoth.2016, 22(6): 372-6.
21. Hata H, Natori T, Mizuno T, Kanazawa I, Eldesouky I, Hayashi M, Miyata M, Fukunaga H, Ohji S, Hosoyama A, Aono E, Yamazoe A, Tsuchikane K, Fujita N, Ezaki T, Phylogenetics of family Enterobacteriaceae and proposal to reclassify *Escherichia hermannii* and *Salmonella subterranea* as *Atlantibacter hermannii* and *Atlantibacter subterranea* gen. nov., comb. nov. Microbiol. Immunol., 2016, 60(5):303-11.  
(長崎大)
22. Bao LQ, Huy NT, Kikuchi M, Yanagi T, Senba M, Shuaibu MN, Honma K, Yui K, Hirayama K. CD19(+) B cells confer protection against experimental cerebral malaria in semi-immune rodent model. PLoS One. 2013, 8, 5, e64836.
23. Cherif MS, Shuaibu MN, Kodama Y, Kurosaki T, Helegbe GK, Kikuchi M, Ichinose A, Yanagi T, Sasaki H, Yui K, Tien NH, Karbwang J, Hirayama K. Nanoparticle formulation enhanced protective immunity provoked by PYGPI8p-transamidase related protein (PyTAM) DNA vaccine in *Plasmodium yoelii* malaria model. Vaccine. 2014, 32, 17, 1998-2006.
24. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, Kimotho J, Mwangi AW, Kiche I, Matsumoto S, Niki M, Osada-Oka M, Ichinose Y, Inoue M, Itoh M, Tachibana H, Ishii K, Tsuboi T, Yoshida LM, Mondal D, Haque R, Hamano S, Changoma M, Hoshi T, Kamo K, Karama M, Miura M, Hirayama K. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. PLoS Negl Trop Dis. 2014, 8, 7, e3040.
25. Abkallo HM, Tangena JA, Tang J, Kobayashi N, Inoue M, Zoungrana A, Colegrave N, Culleton R. Within-host competition does not select for virulence in malaria parasites: studies with *Plasmodium yoelii*. Plos Pathogens. 2015, 11, 2, E1004628.
26. Cox-Singh J & Culleton R. *Plasmodium knowlesi*: from severe zoonosis to animal model. Trends in Parasitology. 2015, 31, 6, 232-238.
27. Kato K, Yahata K, Gopal Dhoubhadel B, Fujii Y, Tachibana H. Novel hemagglutinating, hemolytic and cytotoxic activities of the intermediate subunit of *Entamoeba histolytica* lectin. Sci Rep5. 2015, 13901.

28. Bao LQ, Nhi DM, Huy NT, Hamano S, Hirayama K. Tacrolimus prevents murine cerebral malaria. *Immunology*. 2017, 150(2), 155-61.

(2) 学会・シンポジウム等における口頭・ポスター発表

1. Molecular approaches to the diagnosis of ocular infectious diseases,ポスター, Ohkusu K, Ezaki T, 24th ECCMID, 2014, 国外.
2. Antifungal susceptibility and frequency of *Aspergillus fumigatus*-related species of clinical strains collected as “*A. fumigatus*” from various area in Japan. ポスター, Watanabe A, Kikuchi K, Oku Y, Taguchi H, Yaguchi T, Kamei K. The 6th Advance Against Aspergillosis. 2014/2/27. Madrid, Spain. 国外.
3. New Approach to detect pathogenic bacteria with a rapid portable PCR and DNA chromatography, 口頭 Ezaki T. Ulan Bator, Mongol. 2014/3, 国外.
4. Multilocus phylogeny, antifungal susceptibilities and mating behavior of opportunistic pathogen *Aspergillus virididutans*. ポスター, Dudova Z, Hubka V, Svobodova L, Haml P, Matsuzawa T, Yaguchi T, Kolarik M. The 10th International Mycological Congress 2014. 2014/8/3. Bangkok, Thailand. 国外.
5. Isolation of *Aspergillus fumigatus* related species from the environment in Japan and their antifungal susceptibilities. ポスター, Yaguchi T, Tanaka R, Ito J, Horose D, Watanabe A, Kamei K. The 19th Congress of International Society for Human and Animal Mycology. 2015/5/4. Melbourne, Austraria. 国外.
6. Plasmodium knowlesi Skeletal Binding Protein 1 (PkSBP1) localises to Sinton and Mulligan stipplings in infected monkey and human erythrocytes, ポスター, Lucky AB, Sakaguchi M, Katakai Y, Kawai S, Yahata K, Templeton TJ, Kaneko O, London-Nagasaki Networking Meeting, London School of Hygiene and Tropical Medicine Week, 2016/9/19, 国外.
7. *Plasmodium knowlesi* skeleton-binding protein 1 Localizes to the ‘Sinton and Mulligan’ stipplings in the cytoplasm of monkey and human erythrocytes, ポスター, Lucky AB, Sakaguchi M, Katakai Y, Kawai S, Yahata K, Templeton TJ, Kaneko O, TICAD VI Pre-event: The 3rd International Symposium for the Promotion of Science and Technology Innovation Cooperation between Africa and Japan.-Life Innovation and Green Innovation-, 2016/7/13, 国内.

(3) 「国民との科学・技術対話社会」に対する取り組み

1. アフリカ “健康イノベーション～「長崎発グローバルヘルス」今、世界をめざす若者が長崎に集う。” ～, 平山謙二, 長崎大学グローバルヘルス基金設立帰任セミナー, 2015/9/4, 国内.
2. 熱帯医学研究所における研究・教育・社会貢献, 平山謙二, 新潟大学脳研究所 創立記念講演会, 2016/6/13, 国内
3. Kongo Bololo(*Morinda morindoides*)の抗マラリア薬として持つ可能性の検討, 水上修作, 和漢研セミナー(第 371 回), 2016/6/21, 国内

(4) 特許出願

日本：特願 2012-156859 号

特願 2012-156860 号

特願 2012-156861 号

特願 2012-156862 号

特願 2013-151337 号

特願 2014-076417 号

特願 2014-076418 号

平成 28 年度 医療研究開発推進事業費補助金  
成果報告書

**I. 基本情報**

事業名： (日本語) ナショナルバイオリソースプロジェクト  
(英語) National Bioresource Project

補助事業課題名： (日本語) 病原微生物の収集・保存・提供体制の整備  
(英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms

補助事業担当者 (日本語) 国立大学法人千葉大学 准教授 矢口 貴志  
所属 役職 氏名： (英語) Takashi Yaguchi, Associate Professor, Medical Mycology Research Center, Chiba University

実施期間： 平成 28 年 4 月 1 日 ～ 平成 29 年 3 月 31 日

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性細菌の収集・保存・提供および関連情報の収集・提供)

分担課題名： (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic bacteria)

補助事業分担者 (日本語) 国立大学法人大阪大学 微生物病研究所 教授 飯田 哲也  
所属 役職 氏名： (英語) Tetsuya Iida, Professor, Research Institute for Microbial Diseases, Osaka University

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性細菌の収集・保存・提供および関連情報の収集・提供)

分担課題名： (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic bacteria)

補助事業分担者 (日本語) 国立大学法人岐阜大学 名誉教授・フェロー 江崎 孝行  
所属 役職 氏名： (英語) Takayuki Ezaki, Professor emeritus, Fellow, Gifu University, Center for Conservation of Microbial Genetic Resource, Organization for Research and Community Development



分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性原虫の収集・保存と提供)

分担課題名: (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic protozoa)

補助事業分担者 (日本語) 国立大学法人長崎大学 熱帯医学研究所 森田 公一  
所属 役職 氏名: (英語) Kouichi Morita, Dean, Institute of Tropical Medicine, Nagasaki University

## II. 成果の概要 (総括成果報告)

補助事業代表者: 国立大学法人千葉大学・真菌医学研究センター・矢口 貴志 の総括成果報告を参照。

## III. 成果の外部への発表

(1) 学会誌・雑誌等における論文一覧 (国内誌 35 件、国際誌 384 件)  
(千葉大)

1. Ishikawa T, Itoh F, Yoshida S, Saijo S, Matsuzawa T, Gono T, Saito T, Okawa Y, Shibata N, Miyamoto T, Yamasaki S: Identification of distinct ligands for the C-type lectin receptors mincle and dectin-2 in the pathogenic fungus *Malassezia*. *Cell Host Microbe*. 2013, 13, 477-488.
2. Visagie CM, Houbraken J, Frisvad JC, Hong S-B, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA. Identification and nomenclature of the genus *Penicillium*. *Stud Mycology* 2014, 78, 343-371.
3. Novakova A, Hubka V, Dudova Z, Matsuzawa T, Kubatova A, Yaguchi T, Kolarik M: New species in *Aspergillus* section *Fumigati* from reclamation sites in Wyoming (U.S.A.) and revision of *A. viridinutans* complex. *Fungal Diversity*. 2014, 64, 253-274.
4. Herbst S, Shah A, Mazon Moya M, Marzola V, Jensen B, Reed A, Birrell MA, Saijo S, Mostowy S, Shaunak S, Armstrong-James D. Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to *Aspergillus fumigatus*. *EMBO Mol Med*. 2015, 7, 240-58.
5. Fraç M, Jezierska-Tys S, Yaguchi T. Occurrence, detection and molecular and metabolic characterization of heat-resistant fungi in soils and plants and their risk to human health. *Advances Agronomy*. 2015, 132, 161-204.
6. Takahata S, Kubota N, Takei-Masuda N, Yamada T, Maeda M, Alshahni MM, Abe S, Tabata Y, Maebashi K. Mechanism of action of ME1111, a novel antifungal agent for topical treatment of onychomycosis. *Antimicrob Agents Chemother*. 2016, 60, 873-880.
7. Hagiwara D, Watanabe A, Kamei K. Sensitisation of an azole-resistant *Aspergillus fumigatus* strain containing the cyp51A-related mutation by deleting the srbA Gene. *Scientific Reports*. 2016, 6, 38833.

(大阪大)

8. Salomon D, Guo Y, Kinch LN, Grishin NV, Gardner KH, Orth K. Effectors of animal and plant pathogens use a common domain to bind host phosphoinositides. *Nat Commun.* 2013;4:2973.
  9. Sreelatha A, Bennett TL, Zheng H, Jiang QX, Orth K, Starai VJ. *Vibrio* effector protein, VopQ, forms a lysosomal gated channel that disrupts host ion homeostasis and autophagic flux. *Proc Natl Acad Sci U S A.* 2013 110(28):11559-64. doi: 10.1073
  10. Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, Mirzaei H, Orth K. Marker for type VI secretion system effectors. *Proc Natl Acad Sci U S A.* 2014 111(25):9271-6.
  11. Xu H, Yang J, Gao W, Li L, Li P, Zhang L, Gong YN, Peng X, Xi JJ, Chen S, Wang F, Shao F. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature.* 2014 513(7517):237-41.
  12. Sreelatha A, Bennett TL, Carpinone EM, O'Brien KM, Jordan KD, Burdette DL, Orth K, Starai VJ. *Vibrio* effector protein VopQ inhibits fusion of V-ATPase-containing membranes. *Proc Natl Acad Sci U S A.* 2015 112(1):100-5.
  13. Hubbard TP, Chao MC, Abel S, Blondel CJ, Abel Zur Wiesch P, Zhou X, Davis BM, Waldor MK. Genetic analysis of *Vibrio parahaemolyticus* intestinal colonization. *Proc Natl Acad Sci U S A.* 2016, 113(22):6283-8.
  14. Blondel CJ, Park JS, Hubbard TP, Pacheco AR, Kuehl CJ, Walsh MJ, Davis BM, Gewurz BE, Doench JG, Waldor MK. CRISPR/Cas9 screens reveal requirements for host cell sulfation and fucosylation in bacterial type III secretion system-mediated cytotoxicity. *Cell Host Microbe.* 2016, 20(2):226-37.
- (岐阜大)
15. Hayashi M, Kubota-Hayashi S, Natori T, Mizuno T, Miyata M, Yoshida S, Zhang J, Kawamoto K, Ohkusu K, Makino S, Ezaki T. Use of blood-free enrichment broth in the development of a rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *Int. J. Food Microbiol.* 2013. 163, 41-46.
  16. Hayashi M, Natori T, Kubota-Hayashi S, Miyata M, Ohkusu K, Kawamoto K, Kurazono H, Makino S, Ezaki T. A new protocol to detect multiple foodborne pathogens with PCR dipstick DNA chromatography after six-hour enrichment culture in a broad-range food pathogen enrichment broth. *BioMed Research Inter.* 2013, Article ID295050, 10 pages.
  17. Kikuchi M, Ito S, Yasuda M, Tsuchiya T, Hatazaki K, Takanashi M, Ezaki T, Deguchi T, Remarkable increase in fluoroquinolone-resistant *Mycoplasma genitalium* in Japan *J Antimicrob Chemother.* 2014,10.1093/jac/dku164.
  18. Ogawa H, Ohnuma M, Squarre D, Mweene AS, Ezaki T, Fujikura D, Ohnishi N, Thomas Y, Hang'ombe BM, Higashi H, *Bacillus cereus* from the environment is genetically related to the highly pathogenic *B. cereus* in Zambia. *J. Vet. Med. Sci.* 2015, 77(8): 993-5
  19. Ogawa H, Fujikura D, Ohnuma M, Ohnishi N, Hang'ombe BM, Mimuro H, Ezaki T, Mweene AS, Higashi H. A novel Multiplex PCR discriminates *Bacillus anthracis* and its genetically related strains from other *Bacillus cereus* group species. *PLoS One.* 2015,

16;10(3).

20. Ohshiro T, Miyagi C, Tamaki Y, Mizuno T, Ezaki T. Development of a rapid diagnostic method of *Staphylococcus aureus* and the antimicrobial resistance from a positive blood culture bottle using a PCR-DNA-Strip method. *J. Infect. Chemoth.* 2016, 22(6): 372-6.
21. Hata H, Natori T, Mizuno T, Kanazawa I, Eldesouky I, Hayashi M, Miyata M, Fukunaga H, Ohji S, Hosoyama A, Aono E, Yamazoe A, Tsuchikane K, Fujita N, Ezaki T. Phylogenetics of family Enterobacteriaceae and proposal to reclassify *Escherichia hermannii* and *Salmonella subterranea* as *Atlantibacter hermannii* and *Atlantibacter subterranea* gen. nov., comb. nov. *Microbiol. Immunol.*, 2016, 60(5):303-11.  
(長崎大)
22. Bao LQ, Huy NT, Kikuchi M, Yanagi T, Senba M, Shuaibu MN, Honma K, Yui K, Hirayama K. CD19(+) B cells confer protection against experimental cerebral malaria in semi-immune rodent model. *PLoS One*. 2013, 8, 5, e64836.
23. Cherif MS, Shuaibu MN, Kodama Y, Kurosaki T, Helegbe GK, Kikuchi M, Ichinose A, Yanagi T, Sasaki H, Yui K, Tien NH, Karbwang J, Hirayama K. Nanoparticle formulation enhanced protective immunity provoked by PYGPI8p-transamidase related protein (PyTAM) DNA vaccine in *Plasmodium yoelii* malaria model. *Vaccine*. 2014, 32, 17, 1998-2006.
24. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, Kimotho J, Mwangi AW, Kiche I, Matsumoto S, Niki M, Osada-Oka M, Ichinose Y, Inoue M, Itoh M, Tachibana H, Ishii K, Tsuboi T, Yoshida LM, Mondal D, Haque R, Hamano S, Changoma M, Hoshi T, Kamo K, Karama M, Miura M, Hirayama K. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. *PLoS Negl Trop Dis*. 2014, 8, 7, e3040.
25. Abkallo HM, Tangena JA, Tang J, Kobayashi N, Inoue M, Zoungrana A, Colegrave N, Culleton R. Within-host competition does not select for virulence in malaria parasites: studies with *Plasmodium yoelii*. *Plos Pathogens*. 2015, 11, 2, E1004628.
26. Cox-Singh J & Culleton R. *Plasmodium knowlesi*: from severe zoonosis to animal model. *Trends in Parasitology*. 2015, 31, 6, 232-238.
27. Kato K, Yahata K, Gopal Dhoubhadel B, Fujii Y, Tachibana H. Novel hemagglutinating, hemolytic and cytotoxic activities of the intermediate subunit of *Entamoeba histolytica* lectin. *Sci Rep*5. 2015, 13901.
28. Bao LQ, Nhi DM, Huy NT, Hamano S, Hirayama K. Tacrolimus prevents murine cerebral malaria. *Immunology*. 2017, 150(2), 155-61.

(2) 学会・シンポジウム等における口頭・ポスター発表

1. Molecular approaches to the diagnosis of ocular infectious diseases,ポスター, Ohkusu K, Ezaki T, 24th ECCMID, 2014, 国外.
2. Antifungal susceptibility and frequency of *Aspergillus fumigatus*-related species of clinical strains collected as “*A. fumigatus*” from various area in Japan. ポスター, Watanabe A, Kikuchi K, Oku Y, Taguchi H, Yaguchi T, Kamei K. The 6th Advance Against Aspergillosis. 2014/2/27. Madrid, Spain. 国外.

3. New Approach to detect pathogenic bacteria with a rapid portable PCR and DNA chromatography, 口頭 Ezaki T. Ulan Bator, Mongol. 2014/3, 国外.
4. Multilocus phylogeny, antifungal susceptibilities and mating behavior of opportunistic pathogen *Aspergillus virididutans*. ポスター, Dudova Z, Hubka V, Svobodova L, Haml P, Matsuzawa T, Yaguchi T, Kolarik M. The 10th International Mycological Congress 2014. 2014/8/3. Bangkok, Thailand. 国外.
5. Isolation of *Aspergillus fumigatus* related species from the environment in Japan and their antifungal susceptibilities. ポスター, Yaguchi T, Tanaka R, Ito J, Horose D, Watanabe A, Kamei K. The 19th Congress of International Society for Human and Animal Mycology. 2015/5/4. Melbourne, Austraria. 国外.
6. Plasmodium knowlesi Skeletal Binding Protein 1 (PkSBP1) localises to Sinton and Mulligan stipplings in infected monkey and human erythrocytes, ポスター, Lucky AB, Sakaguchi M, Katakai Y, Kawai S, Yahata K, Templeton TJ, Kaneko O, London-Nagasaki Networking Meeting, London School of Hygiene and Tropical Medicine Week, 2016/9/19, 国外.
7. *Plasmodium knowlesi* skeleton-binding protein 1 Localizes to the 'Sinton and Mulligan' stipplings in the cytoplasm of monkey and human erythrocytes, ポスター, Lucky AB, Sakaguchi M, Katakai Y, Kawai S, Yahata K, Templeton TJ, Kaneko O, TICAD VI Pre-event: The 3rd International Symposium for the Promotion of Science and Technology Innovation Cooperation between Africa and Japan.-Life Innovation and Green Innovation-, 2016/7/13, 国内.

(3) 「国民との科学・技術対話社会」に対する取り組み

1. アフリカ “健康イノベーション～「長崎発グローバルヘルス」今、世界をめざす若者が長崎に集う。” ～, 平山謙二, 長崎大学グローバルヘルス基金設立帰任セミナー, 2015/9/4, 国内.
2. 熱帯医学研究所における研究・教育・社会貢献, 平山謙二, 新潟大学脳研究所 創立記念講演会, 2016/6/13, 国内
3. Kongo Bololo(*Morinda morindoides*)の抗マラリア薬として持つ可能性の検討, 水上修作, 和漢研セミナー(第 371 回), 2016/6/21, 国内

(4) 特許出願

日本：特願 2012-156859 号

特願 2012-156860 号

特願 2012-156861 号

特願 2012-156862 号

特願 2013-151337 号

特願 2014-076417 号

特願 2014-076418 号

平成 28 年度 医療研究開発推進事業費補助金  
成果報告書

**I. 基本情報**

事業名： (日本語) ナショナルバイオリソースプロジェクト  
(英語) National Bioresource Project

補助事業課題名： (日本語) 病原微生物の収集・保存・提供体制の整備  
(英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms

補助事業担当者 (日本語) 国立大学法人千葉大学 准教授 矢口 貴志  
所属 役職 氏名： (英語) Takashi Yaguchi, Associate Professor, Medical Mycology Research Center, Chiba University

実施期間： 平成 28 年 4 月 1 日 ～ 平成 29 年 3 月 31 日

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性細菌の収集・保存・提供および関連情報の収集・提供)

分担課題名： (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic bacteria)

補助事業分担者 (日本語) 国立大学法人大阪大学 微生物病研究所 教授 飯田 哲也  
所属 役職 氏名： (英語) Tetsuya Iida, Professor, Research Institute for Microbial Diseases, Osaka University

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性細菌の収集・保存・提供および関連情報の収集・提供)

分担課題名： (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic bacteria)

補助事業分担者 (日本語) 国立大学法人岐阜大学 名誉教授・フェロー 江崎 孝行  
所属 役職 氏名： (英語) Takayuki Ezaki, Professor emeritus, Fellow, Gifu University, Center for Conservation of Microbial Genetic Resource, Organization for Research and Community Development

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性原虫の収集・保存と提供)

分担課題名: (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic protozoa)

補助事業分担者 (日本語) 国立大学法人長崎大学 熱帯医学研究所 森田 公一  
所属 役職 氏名: (英語) Kouichi Morita, Dean, Institute of Tropical Medicine, Nagasaki University

## II. 成果の概要 (総括成果報告)

補助事業代表者: 国立大学法人千葉大学・真菌医学研究センター・矢口 貴志 の総括成果報告を参照。

## III. 成果の外部への発表

(1) 学会誌・雑誌等における論文一覧 (国内誌 35 件、国際誌 384 件)  
(千葉大)

1. Ishikawa T, Itoh F, Yoshida S, Saijo S, Matsuzawa T, Gono T, Saito T, Okawa Y, Shibata N, Miyamoto T, Yamasaki S: Identification of distinct ligands for the C-type lectin receptors mincle and dectin-2 in the pathogenic fungus *Malassezia*. *Cell Host Microbe*. 2013, 13, 477-488.
2. Visagie CM, Houbraken J, Frisvad JC, Hong S-B, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA. Identification and nomenclature of the genus *Penicillium*. *Stud Mycology* 2014, 78, 343-371.
3. Novakova A, Hubka V, Dudova Z, Matsuzawa T, Kubatova A, Yaguchi T, Kolarik M: New species in *Aspergillus* section *Fumigati* from reclamation sites in Wyoming (U.S.A.) and revision of *A. viridinutans* complex. *Fungal Diversity*. 2014, 64, 253-274.
4. Herbst S, Shah A, Mazon Moya M, Marzola V, Jensen B, Reed A, Birrell MA, Saijo S, Mostowy S, Shaunak S, Armstrong-James D. Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to *Aspergillus fumigatus*. *EMBO Mol Med*. 2015, 7, 240-58.
5. Fraç M, Jezierska-Tys S, Yaguchi T. Occurrence, detection and molecular and metabolic characterization of heat-resistant fungi in soils and plants and their risk to human health. *Advances Agronomy*. 2015, 132, 161-204.
6. Takahata S, Kubota N, Takei-Masuda N, Yamada T, Maeda M, Alshahni MM, Abe S, Tabata Y, Maebashi K. Mechanism of action of ME1111, a novel antifungal agent for topical treatment of onychomycosis. *Antimicrob Agents Chemother*. 2016, 60, 873-880.
7. Hagiwara D, Watanabe A, Kamei K. Sensitisation of an azole-resistant *Aspergillus fumigatus* strain containing the cyp51A-related mutation by deleting the srbA Gene. *Scientific Reports*. 2016, 6, 38833.

(大阪大)

8. Salomon D, Guo Y, Kinch LN, Grishin NV, Gardner KH, Orth K. Effectors of animal and plant pathogens use a common domain to bind host phosphoinositides. *Nat Commun.* 2013;4:2973.
  9. Sreelatha A, Bennett TL, Zheng H, Jiang QX, Orth K, Starai VJ. *Vibrio* effector protein, VopQ, forms a lysosomal gated channel that disrupts host ion homeostasis and autophagic flux. *Proc Natl Acad Sci U S A.* 2013 110(28):11559-64. doi: 10.1073
  10. Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, Mirzaei H, Orth K. Marker for type VI secretion system effectors. *Proc Natl Acad Sci U S A.* 2014 111(25):9271-6.
  11. Xu H, Yang J, Gao W, Li L, Li P, Zhang L, Gong YN, Peng X, Xi JJ, Chen S, Wang F, Shao F. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature.* 2014 513(7517):237-41.
  12. Sreelatha A, Bennett TL, Carpinone EM, O'Brien KM, Jordan KD, Burdette DL, Orth K, Starai VJ. *Vibrio* effector protein VopQ inhibits fusion of V-ATPase-containing membranes. *Proc Natl Acad Sci U S A.* 2015 112(1):100-5.
  13. Hubbard TP, Chao MC, Abel S, Blondel CJ, Abel Zur Wiesch P, Zhou X, Davis BM, Waldor MK. Genetic analysis of *Vibrio parahaemolyticus* intestinal colonization. *Proc Natl Acad Sci U S A.* 2016, 113(22):6283-8.
  14. Blondel CJ, Park JS, Hubbard TP, Pacheco AR, Kuehl CJ, Walsh MJ, Davis BM, Gewurz BE, Doench JG, Waldor MK. CRISPR/Cas9 screens reveal requirements for host cell sulfation and fucosylation in bacterial type III secretion system-mediated cytotoxicity. *Cell Host Microbe.* 2016, 20(2):226-37.
- (岐阜大)
15. Hayashi M, Kubota-Hayashi S, Natori T, Mizuno T, Miyata M, Yoshida S, Zhang J, Kawamoto K, Ohkusu K, Makino S, Ezaki T. Use of blood-free enrichment broth in the development of a rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *Int. J. Food Microbiol.* 2013. 163, 41-46.
  16. Hayashi M, Natori T, Kubota-Hayashi S, Miyata M, Ohkusu K, Kawamoto K, Kurazono H, Makino S, Ezaki T. A new protocol to detect multiple foodborne pathogens with PCR dipstick DNA chromatography after six-hour enrichment culture in a broad-range food pathogen enrichment broth. *BioMed Research Inter.* 2013, Article ID295050, 10 pages.
  17. Kikuchi M, Ito S, Yasuda M, Tsuchiya T, Hatazaki K, Takanashi M, Ezaki T, Deguchi T, Remarkable increase in fluoroquinolone-resistant *Mycoplasma genitalium* in Japan *J Antimicrob Chemother.* 2014,10.1093/jac/dku164.
  18. Ogawa H, Ohnuma M, Squarre D, Mweene AS, Ezaki T, Fujikura D, Ohnishi N, Thomas Y, Hang'ombe BM, Higashi H, *Bacillus cereus* from the environment is genetically related to the highly pathogenic *B. cereus* in Zambia. *J. Vet. Med. Sci.* 2015, 77(8): 993-5
  19. Ogawa H, Fujikura D, Ohnuma M, Ohnishi N, Hang'ombe BM, Mimuro H, Ezaki T, Mweene AS, Higashi H. A novel Multiplex PCR discriminates *Bacillus anthracis* and its genetically related strains from other *Bacillus cereus* group species. *PLoS One.* 2015,

16;10(3).

20. Ohshiro T, Miyagi C, Tamaki Y, Mizuno T, Ezaki T. Development of a rapid diagnostic method of *Staphylococcus aureus* and the antimicrobial resistance from a positive blood culture bottle using a PCR-DNA-Strip method. *J. Infect. Chemoth.* 2016, 22(6): 372-6.
21. Hata H, Natori T, Mizuno T, Kanazawa I, Eldesouky I, Hayashi M, Miyata M, Fukunaga H, Ohji S, Hosoyama A, Aono E, Yamazoe A, Tsuchikane K, Fujita N, Ezaki T. Phylogenetics of family Enterobacteriaceae and proposal to reclassify *Escherichia hermannii* and *Salmonella subterranea* as *Atlantibacter hermannii* and *Atlantibacter subterranea* gen. nov., comb. nov. *Microbiol. Immunol.*, 2016, 60(5):303-11.  
(長崎大)
22. Bao LQ, Huy NT, Kikuchi M, Yanagi T, Senba M, Shuaibu MN, Honma K, Yui K, Hirayama K. CD19(+) B cells confer protection against experimental cerebral malaria in semi-immune rodent model. *PLoS One*. 2013, 8, 5, e64836.
23. Cherif MS, Shuaibu MN, Kodama Y, Kurosaki T, Helegbe GK, Kikuchi M, Ichinose A, Yanagi T, Sasaki H, Yui K, Tien NH, Karbwang J, Hirayama K. Nanoparticle formulation enhanced protective immunity provoked by PYGPI8p-transamidase related protein (PyTAM) DNA vaccine in *Plasmodium yoelii* malaria model. *Vaccine*. 2014, 32, 17, 1998-2006.
24. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, Kimotho J, Mwangi AW, Kiche I, Matsumoto S, Niki M, Osada-Oka M, Ichinose Y, Inoue M, Itoh M, Tachibana H, Ishii K, Tsuboi T, Yoshida LM, Mondal D, Haque R, Hamano S, Changoma M, Hoshi T, Kamo K, Karama M, Miura M, Hirayama K. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. *PLoS Negl Trop Dis*. 2014, 8, 7, e3040.
25. Abkallo HM, Tangena JA, Tang J, Kobayashi N, Inoue M, Zoungrana A, Colegrave N, Culleton R. Within-host competition does not select for virulence in malaria parasites: studies with *Plasmodium yoelii*. *Plos Pathogens*. 2015, 11, 2, E1004628.
26. Cox-Singh J & Culleton R. *Plasmodium knowlesi*: from severe zoonosis to animal model. *Trends in Parasitology*. 2015, 31, 6, 232-238.
27. Kato K, Yahata K, Gopal Dhoubhadel B, Fujii Y, Tachibana H. Novel hemagglutinating, hemolytic and cytotoxic activities of the intermediate subunit of *Entamoeba histolytica* lectin. *Sci Rep*5. 2015, 13901.
28. Bao LQ, Nhi DM, Huy NT, Hamano S, Hirayama K. Tacrolimus prevents murine cerebral malaria. *Immunology*. 2017, 150(2), 155-61.

(2) 学会・シンポジウム等における口頭・ポスター発表

1. Molecular approaches to the diagnosis of ocular infectious diseases,ポスター, Ohkusu K, Ezaki T, 24th ECCMID, 2014, 国外.
2. Antifungal susceptibility and frequency of *Aspergillus fumigatus*-related species of clinical strains collected as “*A. fumigatus*” from various area in Japan. ポスター, Watanabe A, Kikuchi K, Oku Y, Taguchi H, Yaguchi T, Kamei K. The 6th Advance Against Aspergillosis. 2014/2/27. Madrid, Spain. 国外.
3. New Approach to detect pathogenic bacteria with a rapid portable PCR and DNA



chromatography, 口頭 Ezaki T. Ulan Bator, Mongol. 2014/3, 国外.

4. Multilocus phylogeny, antifungal susceptibilities and mating behavior of opportunistic pathogen *Aspergillus virididutans*. ポスター, Dudova Z, Hubka V, Svobodova L, Haml P, Matsuzawa T, Yaguchi T, Kolarik M. The 10th International Mycological Congress 2014. 2014/8/3. Bangkok, Thailand. 国外.
5. Isolation of *Aspergillus fumigatus* related species from the environment in Japan and their antifungal susceptibilities. ポスター, Yaguchi T, Tanaka R, Ito J, Horose D, Watanabe A, Kamei K. The 19th Congress of International Society for Human and Animal Mycology. 2015/5/4. Melbourne, Austraria. 国外.
6. Plasmodium knowlesi Skeletal Binding Protein 1 (PkSBP1) localises to Sinton and Mulligan stipplings in infected monkey and human erythrocytes, ポスター, Lucky AB, Sakaguchi M, Katakai Y, Kawai S, Yahata K, Templeton TJ, Kaneko O, London-Nagasaki Networking Meeting, London School of Hygiene and Tropical Medicine Week, 2016/9/19, 国外.
7. *Plasmodium knowlesi* skeleton-binding protein 1 Localizes to the 'Sinton and Mulligan' stipplings in the cytoplasm of monkey and human erythrocytes, ポスター, Lucky AB, Sakaguchi M, Katakai Y, Kawai S, Yahata K, Templeton TJ, Kaneko O, TICAD VI Pre-event; The 3rd International Symposium for the Promotion of Science and Technology Innovation Cooperation between Africa and Japan.-Life Innovation and Green Innovation-, 2016/7/13, 国内.

(3) 「国民との科学・技術対話社会」に対する取り組み

1. アフリカ “健康イノベーション～「長崎発グローバルヘルス」今、世界をめざす若者が長崎に集う。” ～, 平山謙二, 長崎大学グローバルヘルス基金設立帰任セミナー, 2015/9/4, 国内.
2. 熱帯医学研究所における研究・教育・社会貢献, 平山謙二, 新潟大学脳研究所 創立記念講演会, 2016/6/13, 国内
3. Kongo Bololo(*Morinda morindoides*)の抗マラリア薬として持つ可能性の検討, 水上修作, 和漢研セミナー(第 371 回), 2016/6/21, 国内

(4) 特許出願

日本：特願 2012-156859 号

特願 2012-156860 号

特願 2012-156861 号

特願 2012-156862 号

特願 2013-151337 号

特願 2014-076417 号

特願 2014-076418 号

平成 28 年度 医療研究開発推進事業費補助金  
成果報告書

**I. 基本情報**

事業名： (日本語) ナショナルバイオリソースプロジェクト  
(英語) National Bioresource Project

補助事業課題名： (日本語) 病原微生物の収集・保存・提供体制の整備  
(英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms

補助事業担当者 (日本語) 国立大学法人千葉大学 准教授 矢口 貴志  
所属 役職 氏名： (英語) Takashi Yaguchi, Associate Professor, Medical Mycology Research Center, Chiba University

実施期間： 平成 28 年 4 月 1 日 ～ 平成 29 年 3 月 31 日

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性細菌の収集・保存・提供および関連情報の収集・提供)

分担課題名： (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic bacteria)

補助事業分担者 (日本語) 国立大学法人大阪大学 微生物病研究所 教授 飯田 哲也  
所属 役職 氏名： (英語) Tetsuya Iida, Professor, Research Institute for Microbial Diseases, Osaka University

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性細菌の収集・保存・提供および関連情報の収集・提供)

分担課題名： (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic bacteria)

補助事業分担者 (日本語) 国立大学法人岐阜大学 名誉教授・フェロー 江崎 孝行  
所属 役職 氏名： (英語) Takayuki Ezaki, Professor emeritus, Fellow, Gifu University, Center for Conservation of Microbial Genetic Resource, Organization for Research and Community Development

分担研究 (日本語) 病原微生物の収集・保存・提供体制の整備  
(病原性原虫の収集・保存と提供)

分担課題名: (英語) Developing a system for collection, preservation, and provision of pathogenic microorganisms  
(Developing a system for collection, preservation, and provision of pathogenic protozoa)

補助事業分担者 (日本語) 国立大学法人長崎大学 熱帯医学研究所 森田 公一  
所属 役職 氏名: (英語) Kouichi Morita, Dean, Institute of Tropical Medicine, Nagasaki University

## II. 成果の概要 (総括成果報告)

補助事業代表者: 国立大学法人千葉大学・真菌医学研究センター・矢口 貴志 の総括成果報告を参照。

## III. 成果の外部への発表

(1) 学会誌・雑誌等における論文一覧 (国内誌 35 件、国際誌 384 件)  
(千葉大)

1. Ishikawa T, Itoh F, Yoshida S, Saijo S, Matsuzawa T, Gono T, Saito T, Okawa Y, Shibata N, Miyamoto T, Yamasaki S: Identification of distinct ligands for the C-type lectin receptors mincle and dectin-2 in the pathogenic fungus *Malassezia*. *Cell Host Microbe*. 2013, 13, 477-488.
2. Visagie CM, Houbraken J, Frisvad JC, Hong S-B, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA. Identification and nomenclature of the genus *Penicillium*. *Stud Mycology* 2014, 78, 343-371.
3. Novakova A, Hubka V, Dudova Z, Matsuzawa T, Kubatova A, Yaguchi T, Kolarik M: New species in *Aspergillus* section *Fumigati* from reclamation sites in Wyoming (U.S.A.) and revision of *A. viridinutans* complex. *Fungal Diversity*. 2014, 64, 253-274.
4. Herbst S, Shah A, Mazon Moya M, Marzola V, Jensen B, Reed A, Birrell MA, Saijo S, Mostowy S, Shaunak S, Armstrong-James D. Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to *Aspergillus fumigatus*. *EMBO Mol Med*. 2015, 7, 240-58.
5. Fraç M, Jezierska-Tys S, Yaguchi T. Occurrence, detection and molecular and metabolic characterization of heat-resistant fungi in soils and plants and their risk to human health. *Advances Agronomy*. 2015, 132, 161-204.
6. Takahata S, Kubota N, Takei-Masuda N, Yamada T, Maeda M, Alshahni MM, Abe S, Tabata Y, Maebashi K. Mechanism of action of ME1111, a novel antifungal agent for topical treatment of onychomycosis. *Antimicrob Agents Chemother*. 2016, 60, 873-880.
7. Hagiwara D, Watanabe A, Kamei K. Sensitisation of an azole-resistant *Aspergillus fumigatus* strain containing the cyp51A-related mutation by deleting the srbA Gene. *Scientific Reports*. 2016, 6, 38833.

(大阪大)

8. Salomon D, Guo Y, Kinch LN, Grishin NV, Gardner KH, Orth K. Effectors of animal and plant pathogens use a common domain to bind host phosphoinositides. *Nat Commun.* 2013;4:2973.
  9. Sreelatha A, Bennett TL, Zheng H, Jiang QX, Orth K, Starai VJ. *Vibrio* effector protein, VopQ, forms a lysosomal gated channel that disrupts host ion homeostasis and autophagic flux. *Proc Natl Acad Sci U S A.* 2013 110(28):11559-64. doi: 10.1073
  10. Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, Mirzaei H, Orth K. Marker for type VI secretion system effectors. *Proc Natl Acad Sci U S A.* 2014 111(25):9271-6.
  11. Xu H, Yang J, Gao W, Li L, Li P, Zhang L, Gong YN, Peng X, Xi JJ, Chen S, Wang F, Shao F. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature.* 2014 513(7517):237-41.
  12. Sreelatha A, Bennett TL, Carpinone EM, O'Brien KM, Jordan KD, Burdette DL, Orth K, Starai VJ. *Vibrio* effector protein VopQ inhibits fusion of V-ATPase-containing membranes. *Proc Natl Acad Sci U S A.* 2015 112(1):100-5.
  13. Hubbard TP, Chao MC, Abel S, Blondel CJ, Abel Zur Wiesch P, Zhou X, Davis BM, Waldor MK. Genetic analysis of *Vibrio parahaemolyticus* intestinal colonization. *Proc Natl Acad Sci U S A.* 2016, 113(22):6283-8.
  14. Blondel CJ, Park JS, Hubbard TP, Pacheco AR, Kuehl CJ, Walsh MJ, Davis BM, Gewurz BE, Doench JG, Waldor MK. CRISPR/Cas9 screens reveal requirements for host cell sulfation and fucosylation in bacterial type III secretion system-mediated cytotoxicity. *Cell Host Microbe.* 2016, 20(2):226-37.
- (岐阜大)
15. Hayashi M, Kubota-Hayashi S, Natori T, Mizuno T, Miyata M, Yoshida S, Zhang J, Kawamoto K, Ohkusu K, Makino S, Ezaki T. Use of blood-free enrichment broth in the development of a rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *Int. J. Food Microbiol.* 2013. 163, 41-46.
  16. Hayashi M, Natori T, Kubota-Hayashi S, Miyata M, Ohkusu K, Kawamoto K, Kurazono H, Makino S, Ezaki T. A new protocol to detect multiple foodborne pathogens with PCR dipstick DNA chromatography after six-hour enrichment culture in a broad-range food pathogen enrichment broth. *BioMed Research Inter.* 2013, Article ID295050, 10 pages.
  17. Kikuchi M, Ito S, Yasuda M, Tsuchiya T, Hatazaki K, Takanashi M, Ezaki T, Deguchi T, Remarkable increase in fluoroquinolone-resistant *Mycoplasma genitalium* in Japan *J Antimicrob Chemother.* 2014,10.1093/jac/dku164.
  18. Ogawa H, Ohnuma M, Squarre D, Mweene AS, Ezaki T, Fujikura D, Ohnishi N, Thomas Y, Hang'ombe BM, Higashi H, *Bacillus cereus* from the environment is genetically related to the highly pathogenic *B. cereus* in Zambia. *J. Vet. Med. Sci.* 2015, 77(8): 993-5
  19. Ogawa H, Fujikura D, Ohnuma M, Ohnishi N, Hang'ombe BM, Mimuro H, Ezaki T, Mweene AS, Higashi H. A novel Multiplex PCR discriminates *Bacillus anthracis* and its genetically related strains from other *Bacillus cereus* group species. *PLoS One.* 2015,

16;10(3).

20. Ohshiro T, Miyagi C, Tamaki Y, Mizuno T, Ezaki T. Development of a rapid diagnostic method of *Staphylococcus aureus* and the antimicrobial resistance from a positive blood culture bottle using a PCR-DNA-Strip method. *J. Infect. Chemoth.* 2016, 22(6): 372-6.
21. Hata H, Natori T, Mizuno T, Kanazawa I, Eldesouky I, Hayashi M, Miyata M, Fukunaga H, Ohji S, Hosoyama A, Aono E, Yamazoe A, Tsuchikane K, Fujita N, Ezaki T. Phylogenetics of family Enterobacteriaceae and proposal to reclassify *Escherichia hermannii* and *Salmonella subterranea* as *Atlantibacter hermannii* and *Atlantibacter subterranea* gen. nov., comb. nov. *Microbiol. Immunol.*, 2016, 60(5):303-11.  
(長崎大)
22. Bao LQ, Huy NT, Kikuchi M, Yanagi T, Senba M, Shuaibu MN, Honma K, Yui K, Hirayama K. CD19(+) B cells confer protection against experimental cerebral malaria in semi-immune rodent model. *PLoS One*. 2013, 8, 5, e64836.
23. Cherif MS, Shuaibu MN, Kodama Y, Kurosaki T, Helegbe GK, Kikuchi M, Ichinose A, Yanagi T, Sasaki H, Yui K, Tien NH, Karbwang J, Hirayama K. Nanoparticle formulation enhanced protective immunity provoked by PYGPI8p-transamidase related protein (PyTAM) DNA vaccine in *Plasmodium yoelii* malaria model. *Vaccine*. 2014, 32, 17, 1998-2006.
24. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, Kimotho J, Mwangi AW, Kiche I, Matsumoto S, Niki M, Osada-Oka M, Ichinose Y, Inoue M, Itoh M, Tachibana H, Ishii K, Tsuboi T, Yoshida LM, Mondal D, Haque R, Hamano S, Changoma M, Hoshi T, Kamo K, Karama M, Miura M, Hirayama K. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. *PLoS Negl Trop Dis*. 2014, 8, 7, e3040.
25. Abkallo HM, Tangena JA, Tang J, Kobayashi N, Inoue M, Zoungrana A, Colegrave N, Culleton R. Within-host competition does not select for virulence in malaria parasites: studies with *Plasmodium yoelii*. *Plos Pathogens*. 2015, 11, 2, E1004628.
26. Cox-Singh J & Culleton R. *Plasmodium knowlesi*: from severe zoonosis to animal model. *Trends in Parasitology*. 2015, 31, 6, 232-238.
27. Kato K, Yahata K, Gopal Dhoubhadel B, Fujii Y, Tachibana H. Novel hemagglutinating, hemolytic and cytotoxic activities of the intermediate subunit of *Entamoeba histolytica* lectin. *Sci Rep*5. 2015, 13901.
28. Bao LQ, Nhi DM, Huy NT, Hamano S, Hirayama K. Tacrolimus prevents murine cerebral malaria. *Immunology*. 2017, 150(2), 155-61.

(2) 学会・シンポジウム等における口頭・ポスター発表

1. Molecular approaches to the diagnosis of ocular infectious diseases,ポスター, Ohkusu K, Ezaki T, 24th ECCMID, 2014, 国外.
2. Antifungal susceptibility and frequency of *Aspergillus fumigatus*-related species of clinical strains collected as “*A. fumigatus*” from various area in Japan. ポスター, Watanabe A, Kikuchi K, Oku Y, Taguchi H, Yaguchi T, Kamei K. The 6th Advance Against Aspergillosis. 2014/2/27. Madrid, Spain. 国外.

3. New Approach to detect pathogenic bacteria with a rapid portable PCR and DNA chromatography, 口頭 Ezaki T. Ulan Bator, Mongol. 2014/3, 国外.
4. Multilocus phylogeny, antifungal susceptibilities and mating behavior of opportunistic pathogen *Aspergillus virididutans*. ポスター, Dudova Z, Hubka V, Svobodova L, Haml P, Matsuzawa T, Yaguchi T, Kolarik M. The 10th International Mycological Congress 2014. 2014/8/3. Bangkok, Thailand. 国外.
5. Isolation of *Aspergillus fumigatus* related species from the environment in Japan and their antifungal susceptibilities. ポスター, Yaguchi T, Tanaka R, Ito J, Horose D, Watanabe A, Kamei K. The 19th Congress of International Society for Human and Animal Mycology. 2015/5/4. Melbourne, Austraria. 国外.
6. Plasmodium knowlesi Skeletal Binding Protein 1 (PkSBP1) localises to Sinton and Mulligan stipplings in infected monkey and human erythrocytes, ポスター, Lucky AB, Sakaguchi M, Katakai Y, Kawai S, Yahata K, Templeton TJ, Kaneko O, London-Nagasaki Networking Meeting, London School of Hygiene and Tropical Medicine Week, 2016/9/19, 国外.
7. *Plasmodium knowlesi* skeleton-binding protein 1 Localizes to the 'Sinton and Mulligan' stipplings in the cytoplasm of monkey and human erythrocytes, ポスター, Lucky AB, Sakaguchi M, Katakai Y, Kawai S, Yahata K, Templeton TJ, Kaneko O, TICAD VI Pre-event: The 3rd International Symposium for the Promotion of Science and Technology Innovation Cooperation between Africa and Japan.-Life Innovation and Green Innovation-, 2016/7/13, 国内.

(3) 「国民との科学・技術対話社会」に対する取り組み

1. アフリカ “健康イノベーション～「長崎発グローバルヘルス」今、世界をめざす若者が長崎に集う。” ～, 平山謙二, 長崎大学グローバルヘルス基金設立帰任セミナー, 2015/9/4, 国内.
2. 熱帯医学研究所における研究・教育・社会貢献, 平山謙二, 新潟大学脳研究所 創立記念講演会, 2016/6/13, 国内
3. Kongo Bololo(*Morinda morindoides*)の抗マラリア薬として持つ可能性の検討, 水上修作, 和漢研セミナー(第 371 回), 2016/6/21, 国内

(4) 特許出願

日本：特願 2012-156859 号

特願 2012-156860 号

特願 2012-156861 号

特願 2012-156862 号

特願 2013-151337 号

特願 2014-076417 号

特願 2014-076418 号