李達三葉耀珍
中醫藥研究發展中心
Li Dak Sum Yip Yio Chin R & D Centre for Chinese Medicine

生命科學學院
School of Life Sciences

Virtual Forum on Ready-to-use DNA Authentication Protocols for Herbal Material

Date: 6 March 2021, Saturday
08:50am - 12:00nn Hong Kong time

Invited Speakers

Dr. ZHANG Wenjuan 张文娟 博士
National Institutes for Food and Drug Control, China
中国食品药品检定研究院
"TaqMan实时荧光定量PCR技术在中药掺伪检测中的应用研究"

Dr. LU Zhengfei 陆峥飞 博士
Herbalife Nutrition & United States Pharmacopeia, USA
美国康宝莱国际有限公司 & 美国药典
"Development and validation of species-specific PCR methods for the identification of three ginseng species"

Mr. WONG Ka-Lok 黄家乐 先生
Government Chinese Medicines Testing Institute,
Hong Kong, China
中国香港政府中药检测中心
"Analysis of Cornu Cervi Pantotrichum by DNA Method"

Ms. YANG Zhiye 杨志业 女士
Guangdong Institute for Drug Control, China
中国广东省药品检验所
"中药DNA分子鉴定技术在广东省中药标准体系中的应用研究"

Dr. SU Chang 苏畅 博士
Shenzhen Institute for Drug Control, China
中国深圳市药品检验研究院
"以明党参、皂角刺、鹿茸等为例探讨中药DNA分子鉴定质量标准的技术规范模式"

Dr. BUT Grace Wing-Chiu 毕颖超 博士
The Chinese University of Hong Kong,
Hong Kong, China
中国香港中文大学
"Rapid detection of CITES-listed shark fin species by loop-mediated isothermal amplification assay with potential for field use"

Registration:

ORGANIZING COMMITTEE

CHAIRPERSON

Prof. SHAW Pang-Chui
Li Dak Sum Yip Yio Chin R & D Centre for Chinese Medicine,
School of Life Sciences, Institude of Chinese Medicine,
The Chinese University of Hong Kong

MEMBERS

Dr. WU Hoi-Yan Karen
Li Dak Sum Yip Yio Chin R & D Centre for Chinese Medicine,
The Chinese University of Hong Kong

Ms. YIK Hong-Yu Mavis
Li Dak Sum Yip Yio Chin R & D Centre for Chinese Medicine,
The Chinese University of Hong Kong

PROGRAM RUNDOWN

6 MARCH, 2021 | Saturday

Opening Remarks | 09:00- 09:05

Speaker: **Prof. SHAM Mai-Har 岑美霞 教授**
Vice-President, The Chinese University of Hong Kong, Hong Kong, China

Sharing | 09:05- 09:35

Speaker: **Dr. ZHANG Wenjuan 张文娟 博士**
National Institutes for Food and Drug Control, China
TaqMan实时荧光定量PCR技术在中药掺伪检测中的应用研究

Sharing | 09:35- 10:05

Speaker: **Dr. LU Zhengfei 陆峥飞 博士**
Herbalife Nutrition & United States Pharmacopeia, USA
Development and validation of species-specific PCR methods for the identification of three ginseng species

Sharing | 10:05- 10:35

Speaker: **Mr. WONG Ka-Lok 黄家乐 先生**
Government Chinese Medicines Testing Institute, Hong Kong, China
Analysis of Cornu Cervi Pantotrichum by DNA Method

Session Break | 10:35- 10:45

Sharing | 10:45- 11:15

Speaker: **Ms. YANG Zhiye 杨志业 女士**
Guangdong Institute for Drug Control, China
中药DNA分子鉴定技术在广东省中药标准体系中的应用研究

Sharing | 11:15- 11:45

Speaker: **Dr. SU Chang 苏畅 博士**
Shenzhen Institute for Drug Control, China
以明党参、皂角刺、鹿茸等为例探讨中药DNA分子鉴定质量标准的技术规范模式

Sharing | 11:45- 12:10

Speaker: **Dr. BUT Grace Wing-Chiu 毕颖超 博士**
The Chinese University of Hong Kong, Hong Kong, China
Rapid detection of CITES-listed shark fin species by loop-mediated isothermal amplification assay with potential for field use

Closing Remarks | 12:10- 12:15

Speaker: **Prof. SHAW Pang-Chui 邵鹏柱 教授**
Director, Li Dak Sum Yip Yio Chin R & D Centre for Chinese Medicine,
The Chinese University of Hong Kong, Hong Kong, China

BIOGRAPHY AND ABSTRACT

OF INVITED SPEAKERS

TaqMan实时荧光定量PCR技术在中药掺伪检测中的应用研究

Dr. ZHANG Wenjuan 张文娟 博士
National Institutes for Food and Drug Control, China

Biography

张文娟 博士，副研究员，毕业于北京大学医学部，分子免疫学专业，曾赴哈佛大学联合培养。2012年至今就职于中国食品药品检定研究院中药民族药检定所。

率先开展《中国药典》川贝母PCR-RFLP鉴别法以及蕲蛇、乌梢蛇PCR鉴别法的检验业务，并以组织培训班等形式对全国药检系统技术人员展开上述检验方法的培训，促进了中药DNA分子鉴定方法在全国药检系统的推广和应用。负责组织了《中国药典》川贝母PCR-RFLP鉴别法全国范围内的首次能力验证活动。起草和复核《中国药典》标准及补充检验方法20余项。

2019年荣获中国药学会科学技术奖二等奖。迄今主持过国家自然科学基金、科技部重大专项子课题、国家药品监督管理局委托项目等。研究成果发表在权威期刊Molecular Cell、J Biol Chem、J Pharm Biomed Anal等。在国内外发表学术论文20余篇，编著5部。

研究方向与重点领域：基于生物技术的中药质量控制及评价。

Abstract

目前中药掺伪特别是贵细药掺伪问题越来越突出，而现有手段如普通PCR方法抑或DNA条形码鉴定法由于技术本身的缺陷，无法很好的对掺伪样品进行鉴定。TaqMan实时荧光定量PCR技术，增加了探针与模板序列的结合，使反应特异性显著增强；以荧光信号作为检测目标，使方法灵敏度大为提高。此外，该技术尤其适用于检测小片段，在对DNA降解较严重的中药材与饮片、成方制剂、配方颗粒等的检测中具有良好的应用前景。

Development and validation of species-specific PCR methods for the identification of three ginseng species

Dr. LU Zhengfei 陆峥飞 博士
Herbalife Nutrition & United States Pharmacopeia, USA

Biography

Dr. Zhengfei Lu is a Sr. Analytical Scientist at Herbalife Nutrition. Zhengfei holds a Ph.D. in Experimental Pathology from the University of Southern California and received a Medical degree from Peking University. With over 15 years of experience in the field of molecular application, he joined the dietary supplement and food industry to work on DNA-based authentication methods since 2016. His work involves characterization of DNA in botanical materials, development and validation of DNA methods for the identification of both raw herbal materials and processed botanical extracts, implementation and ISO 17025 accreditation of analytical methods for routine botanical qualification. His works has been published in *Food Chemistry*, *Fitoterapia*, and *Journal of AOAC International*. In addition to his role at Herbalife, he also advances public quality standards by volunteering his time and expertise to co-chair the United States Pharmacopeia (USP) Project Team on Botanical Library for Identification using DNA-based Methods (2015-2020 cycle) and as a member of USP Botanical Dietary Supplement and Herbal Medicines Expert Committee (2020-2025 cycle).

Abstract

Establishing the identity of herbal material is a key step to support the reported benefits and safety of the botanical dietary supplements and herbal medicines. Based on fitness for the purpose of establishing identity, morphological, chemical, and genomic evaluations are the most widely used tools to provide herbal material identity at species level. Species-specific PCR can be used as a cost-effective quality control tool for herbal material identification and for discrimination of closely related species. The USP Dietary Supplements Stakeholder Forum formed a Project Team with the aim to provide input for prioritization and materials for establishment of a botanical plant material sample library. This work presents the collaborative laboratory studies on the development and validation of species-specific PCR methods for the identification of three ginseng species samples provided by the project team. The set of species-specific methods was first developed based on variable regions observed in sequences collected from public databases, tested in in silico and in an experimental study to ensure that there is no cross-reactivity with foreign materials and plant species outside the scope. Then, a validation using orthogonally identified ginseng materials collected through the global supply chain was performed to evaluate the specificity of the methods. Finally, the applicability of this method in processed materials, such as red ginseng and ginseng extracts, was also explored. This work demonstrates the value of species-specific PCR for herbal material species identification.

Analysis of Cornu Cervi Pantotrichum by DNA Method

Mr. WONG Ka-Lok 黃家乐 先生
Government Chinese Medicines Testing Institute, Hong Kong, China

Biography

Mr. K. L. WONG is currently DNA Analyst in the Government Chinese Medicines Testing Institute, Department of Health, Hong Kong SAR, PR China. He obtained his M.Phil degree with Professor P.C. SHAW at Department of Biochemistry, The Chinese University of Hong Kong (CUHK). Under supervision of Prof SHAW, he then started R&D work of Chinese medicines (CM) at CUHK focusing on: (1) molecular authentication of Chinese medica materia (CMM); (2) establishing CMM DNA barcode database; (3) determining the properties and functions of proteins in CM.

In 2016, he joined the Department of Health. Following this, he was assigned to establish DNA Laboratory of Government Chinese Medicines Testing Institute. The current role is to implement research project on developing DNA testing method for CMM.

Abstract

This research project aims at developing DNA-based testing methods for the identification of animal-derived CMM - Cornu Cervi Pantotrichum. This project is divided into two parts. In the first part, a screening method for differentiation of two genuine deer species of Cervi Cornu Pantotrichum, i.e. *Cervus nippon* Temminck and *Cervus elaphus* Linnaeus, from each other was developed and validated. By adopting specific-polymerase chain reaction (specific-PCR) technique, the procedure is relatively simple and suitable for rapid screening of decoction pieces of Cervi Cornu Pantotrichum.

In the second part, DNA barcoding testing method for identification of animal-derived CMM and quality control system were developed and validated in accordance with the requirements of the Hong Kong Laboratory Accreditation Program. The generation of reference DNA barcode sequences of Cornu Cervi Pantotrichum, i.e. *C. nippon* and *C. elaphus* was completed in order to provide more comprehensive reference data for identification.

We hope that the developed DNA-based testing methods and quality control system can complement the current testing methods for identification of Cornu Cervi Pantotrichum, which could help CM sectors to distinguish unscrupulous suppliers and avoid buying and selling of problematic products, protect their brand images and interests, and effectively solve the difficulties encountered by CM sectors in discriminating the authenticity of the products.

中药DNA 分子鉴定技术在广东省中药标准体系中的应用研究

Ms. YANG Zhiye 杨志业 女士
Guangdong Institute for Drug Control, China

Biography

杨志业，副主任中药师，广东省药品检验所所中药室副主任。从事中药鉴定、中药质量标准研究等工作。发表研究论文25篇，其中SCI论文3篇；主持及参与中国药典标准提高、国家评价性抽验、广东省科技厅项目等多项。

Abstract

广东省中药标准体系包括了《广东省中药材标准》、《广东省中药饮片炮制规范》、《广东省配方颗粒标准》、《广东省医疗机构制剂规范》等。中药DNA分子鉴定技术在中国药典2010年版得到国家中药法定标准的首次应用，在《广东省中药材标准》（第三册）得到了地方中药法定标准的首次应用。报告围绕DNA鉴定技术在广东省中药标准体系中的应在现状、存在问题及应用前景展开介绍与讨论， 探讨DNA鉴定技术在我国中药法定标准亟需完善的地方，分析DNA鉴定技术在广东省中药地方标准体系中推广应用的可行性、必要性及创新性。

以明党参、皂角刺、鹿茸等为例探讨中药DNA分子鉴定质量标准的技术规范模式

Dr. SU Chang 苏畅 博士
Shenzhen Institute for Drug Control, China

Biography

苏畅，中山大学博士，深圳市药品检验研究院中药检验室副主任。从事中药DNA分子鉴定和质量控制新方法研究，参与完成科技计划项目4项，以及港标、欧洲标准、德国标准等各类中药质量标准制定29项。发表论文10余篇、参编专著5部、获授权发明专利3项、实用新型专利6项。科研项目获广东省科学技术一等奖、中国药学会科学技术三等奖。

Abstract

2015年，以蕲蛇、乌梢蛇、川贝母为示范品种的DNA分子鉴定方法进入《中国药典》，有效填补了中药质量标准分析方法空白。但目前该方法在标准层面的应用进展缓慢，除药检和企业技术人员缺乏相关专业背景、接受程度较慢等原因，另一个重要因素是市售中药材和饮片通常存在贮藏不规范产生发霉、虫蛀等情况，以及炮制高温过程导致DNA降解，增加PCR扩增的难度。同时在标准执行过程中，按照现有标准方法操作，不具备分子生物学背景的技术人员可能产生假阴性和假阳性结果，导致中药检验界对DNA分子鉴定方法持质疑态度。基于以上原因，本文从完善现有中药DNA分子鉴定质量标准的技术操作角度，以三个品种举例探讨更适合于药品检验习惯的标准设置模式，以扩大DNA分子鉴定方法在质量标准层面的接受度和影响力。

Rapid detection of CITES-listed shark fin species by loop-mediated isothermal amplification assay with potential for field use

Dr. BUT Grace Wing-Chiu 毕颖超 博士
The Chinese University of Hong Kong, Hong Kong, China

Biography

Grace But is a research associate at the School of Life Sciences at The Chinese University of Hong Kong. Her research work focuses on developing rapid authentication assays and molecular identification protocols for traditional Chinese food product, seafood product and timber tree samples.

Obtained her Bachelor of Science in Food Science from Cornell University in 2015, Grace received her a PhD in Food and Nutritional Sciences from The Chinese University of Hong Kong in 2019. In 2017, she received a Hop Wai Short-term Research Grant for visiting the National Center for Natural Products Research (USA) and obtained a third prize in Entrepreneurship Proposal of the Competition of Challenge Cup in Hong Kong with development of rapid screening platform for detection of transgenic papaya using LAMP and LOAD techniques. Currently, she has four publications on topics of rapid authentication of CITES-listed shark species and transgenic papaya, identification of sushi products in Hong Kong, and database of medicinal materials.

Abstract

Shark fin is a delicacy in many Asian countries. Overexploitation of sharks for shark fin trading has led to a drastic reduction in shark population. To monitor international trade of shark fin products and protect the endangered species from further population decline, we present rapid, user-friendly and sensitive diagnostic loop-mediated isothermal amplification (LAMP), multiplex-polymerase chain reaction (PCR) and conventional PCR assays for all twelve CITES-listed shark species. Species-specific LAMP and conventional PCR primers were designed based on cytochrome oxidase I (COI) and NADH2 regions. Our LAMP and conventional PCR assays have been tested on 291 samples from 93 shark and related species. Target shark species could be differentiated from non-target species within three hours from DNA extraction to LAMP assay. The LAMP assay reported here is a simple and robust solution for on-site detection of CITES-listed shark species with shark fin products.