WORLD FEDERATION FOR CULTURE COLLECTIONS Newsletter (No.49)–DECEMBER 2010

FAREWELL TO THE PAST PRESIDENT

Dr David Smith

at the launch of the IMCAS BRC, China

Culture Collection Community Activities

I have really enjoyed and have been very proud to represent the WFCC as President for the last six years and I welcome Philippe Desmeth into the role and wish him well in his duties. I was fortunate to visit 26 collections and participate in many conferences and symposium discussions and, as a result, I hope more people understand what the WFCC does and can do for the life sciences. I have participated in a number of projects and learned of many initiatives that impact on culture collection activities. Consolidating the many initiatives is crucial to establishing a systematic and networked approach. This will bring advantages to both the users and the collections themselves importantly, providing a research infrastructure to underpin research and development enabling the harnessing of microbial and cell diversity that will contribute to providing solutions to the world's big challenges.

Harnessing the power of networking is bearing fruit. The World Federation for Culture Collections has been promoting the activities of culture collections for over 4 decades (http://www.wfcc.info) and is joined in the battle by regional organisations such as the European Culture Collection's Organisation (ECCO – http://www.eccosite.org) and over 20 national federations. However, a lot of work still needs to be done both by collections and Governments if the goal to harness the power of microbial diversity is to be realised. Already microorganisms have been the source of wonder drugs and chemical products. We need to harness the properties and products of microorganisms more efficiently if we are to tackle the big global challenges of today in poverty alleviation, food security, healthcare, climate change and the environment. There have been many initiatives over the years that have moved collections forward and delivered useful output, but just imagine if we could all work together to deliver quicker, provide better output that is targeted and of higher quality.

Over decades collections have been supported in Europe by ECCO, an incubator for several European initiatives that has helped collections develop to better meet user needs. For example, recent European Community Framework Programme projects including Common Access to Biological Resources and Information (http://www.cabri.org), EBRCN (European Biological Resource Centres Network – www.ebrcn.net) and the European Consortium of Microbial Resource Centres (http://www.embarc.eu). However, project funding alone is not the answer. Networking is needed to improve coverage and services and help build the new generation culture collection, the microbial Biological Resource Centre (mBRC) for delivery of the pipeline of resources and services for research, development and the bioeconomy.

The European projects above have resulted in technical guidelines and focussed information documents covering requirements with which modern day microbial collections are challenged[1]. Importantly they provided some of the basic information needed for the OECD Biological Resource Centre (BRC) initiative[2] which started in 1999. This initiative demanded a paradigm shift from traditional culture collections to high quality biological resource centres (BRCs). The

[1] *EBRCN Information Resource (Online)*, www.wfcc.info

[2] *Biological Resource Centres – Underpinning the Future of Life Sciences and Biotechnology (Online)*, http://oecdpublications.gfi-nb.com/cgi-bin/oecdbookshop.storefront).

recommendations of the OECD BRC Task Force towards governments, policy makers and other stakeholders embraced the importance of safe and legitimate access to high quality biological material for research and development. They requested: *BRCs must meet the standards of quality and expertise demanded by the international community of scientists and industry for the delivery of biological information and materials that will enable research. Adequate funding is required to achieve these standards and assure sustainability.* Guidance Documents for the Operation of such BRCs including quality management and biosecurity as well as strategies for setting up a Global Biological Resources Centres Network (GBRCN) were a major outcome of this work[3]. As a consequence, the demonstration project for a GBRCN (http://www.gbrcn.org) commenced at the end of 2008. In its present composition it has partners in North- and South America, Africa, Asia and a strong base in Europe. On a global level, the project aims to build a structured long-lasting network which will pave the way for collections to meet user needs. It addresses technical, legal and administrative challenges presented in this globalised, fast developing world. This project will be my focus for some years to come.

One of the key efforts in this development is the EMbaRC project which lays down the foundations of the European node of the GBRCN (EMbaRC is the European Consortium of Microbial Resource Centres, a project funded under the EU 7th Framework Programme). Key issues addressed are biosafety, development of added-value techniques and the improvement, coordination and validation of microbial resource centre protocols. It aims to optimise conservation and identification of bacteria and fungi, and the generation of high quality DNA. It is developing a strategy to increase deposits of strains described in the scientific literature into collections.

The networking elements will give better access to authentic microorganisms and validated associated data and provide a set of business models to increase sustainability of BRCs. Additional capacity is needed to address the avalanche of novel material as a result of modern techniques. The accession of this material into microbiological resource centres (mBRCs) will require new expertise and greater capacity at a time when the very existence of some collections is under threat. Additional funding on a broad scale is needed although not simply money for a good cause but as an investment to deliver into scientific research, bioindustry and biotechnology. To do this efficiently and effectively there are many obstacles to overcome but 'a problem shared is a problem solved'. Collections need to increase the availability of biological material for the verification of experimental data and the authenticity of reference material used in research. Deplorably, the scientific literature is full of data which cannot be verified because the material is either no longer available and/or the material once used to generate the data has changed or deteriorated.

More microbial collections are needed around the world, equipped with techniques and expertise to cope with the depth and breadth of emerging biodiversity; providing access to high quality biological material and scientific services while at the same time observing donor countries' rights, intellectual property rights, biosafety and biosecurity requirements. The challenge is to keep abreast of developments in taxonomy and systematics, against a background of diminishing expertise as well as the development of new methods for the authentication and identification, cultivation and maintenance of cultures. As this will be especially difficult on an individual collection basis, cooperation and harnessing the power of networking on a national, regional and global level will be the only way forward to achieve sustainable development. It was for these very reasons that the GBRCN, ECCO and EMbaRC submitted a proposal via the French delegation to the European Strategy Forum for Research Infrastructures (ESFRI). To this end the Microbial Resources Research Infrastructure (MIRRI) has been accepted on to the ESFRI Road Map. MIRRI brings together European microbial resource collections with stakeholders (their users, policy makers, potential funders and the plethora of microbial research efforts) aiming at improving access to enhanced quality microbial resources in an appropriate legal framework, thus underpinning and driving life sciences research.

The WFCC will continue to provide input to these initiatives. Together the modern day culture collections, the mBRCs, will work more closely with policy makers, funders, researchers to deliver resources and services needed for innovation and thus together they will become sustainable. I am grateful to the project funders: **E**uropean Consortium of **M**icro**b**i**a**l **R**esources **C**entres (EMbaRC) project (EU Seventh Framework Programme Research Infrastructures (INFRA-2008-1.1.2.9: Biological Resources Centres (BRCs) for microorganisms (Grant agreement number: FP7-228310) and for the GBRCN, the Bundesministerium für Bildung und Forschung (BMBF), the German Federal

[3] *OECD Best Practice Guidelines for Biological Resource Centres (Online),* http://www.oecd.org/dataoecd/6/27/38778261.pdf. Accessed July 28, 2010.

Ministry of Research and Education. It would impossible for the President of WFCC to be able to do their job if it was not for the support of work colleagues and their employer. CABI has contributed enormously to enabling my participation in WFCC activities for which I am most grateful. Most importantly I thank the old WFCC board for their support and guidance and I wish the new President, Dr Philippe Desmeth, and the new board well. Together the WFCC will continue to make a difference.

David Smith

President of the WFCC 2004-2010

WFCC Past President Lindsay Sly receives the WFCC Medal for outstanding contribution to the WFCC

The WFCC Medal is presented to members of the WFCC that have made major contributions to the delivery of the WFCC objectives, principles and programme of work. The first medal was awarded by the WFCC Executive Board to Professor Hideaki Sugawara on his retirement for his sterling efforts with the WDCM.

Professor Lindsay Sly received the medal at ICCC12, Florianopolis, Brazil for his many years of active work and contribution to the WFCC.

He recently retired from the University of Queensland as Head of the *Australian Collection of Microorganisms* (ACM). His endeavours were extended nationally through the Australian Microbial Resources Research Network (AMRRN), the establishment of the *Council of Heads of Australian Collections of Microorganisms* (CHACM) and the involvement of microbial resources in the *Atlas of Living Australia*.

He is a past President of the WFCC and has worked with the WFCC from its origins indeed working with Professor Skerman who first established the World Data Centre for Microorganisms. Professor Sly has made a substantial contribution to the WFCC not least organising ICCC-9 in Brisbane which was so successful that it has contributed to subsequent ICCC´s (including ICCC12) by funding the participation of scientists from countries of developing economies.

In addition to the WFCC medal Prof. Lindsay Sly has been awarded honorary membership to the WFCC.

Past and Present WFCC Presidents; David Smith, Lindsay Sly, Phillippe Desmeth (current), Vanderlei Canhos

REPORT FROM THE 12th INTERNATIONAL CONFERENCE FOR CULTURE COLLECTIONS

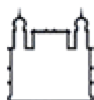
Florianópolis, Santa Catarina, Brazil September 26-October 1, 2010

Organized by:

The Centro de Referência em Informação Ambiental

Sponsored by:

Financiadora de Estudos e Projetos

 FIOCRUZ FUNDAÇÃO OSWALDO CRUZ FAPESP *Fundação de Amparo à Pesquisa do Estado de São Paulo*

The World Federation for Culture Collections (WFCC) International Conference for Culture Collections held in Florianópolis, Brazil, was organised by the Centro de Referência em Informação Ambiental and the Sociedade Brasiliera de Microbiologia in close cooperation with the WFCC Executive Board. It offered the opportunity for participants to meet with collaborators, scientists, culture collection staff and collection stakeholders from all over the world. The scientific programme included 160 posters and 120 oral presentations (120); the report from the conference and the minutes of WFCC meetings will be made available on the WFCC web site. The presentations are available for all to enjoy on the ICCC12 website as attachments to the programme www.iccc12.info.

There were several pre-and after-symposium courses and satellite meetings including the courses:

- Preservation of Laboratory Held Biological Material and Related Quality Management
- A Practical Approach to Microbial Identification by MALDI-TOF ICMS and a workshops
- Self assessments (internal audits) according to the OECD Best Practice Guidelines
- IDAs-Depositary Centres for Biological Material for Patent Purposes

Session 1 - *Biotechnological innovation in the knowledge based bio-economies* included key note talks on *Developing the Bioeconomy* by Iain Gillespie, Head of the Organisation for Economic Cooperation and Development's (OECD) Science and Technology Policy Division and the *Knowledge-based bio-economy: challenges and opportunities for Latin America* by George Tzotzos representing UNIDO. These presentations emphasized the importance of access to high quality resources and the role of Biological Resource Centre, the modern day culture collection. Gillespie stressing how important the GBRCN was to the process and again stressed that growth of the network must be inclusive and output must deliver societal benefit. International cooperation and networking was essential and in this regard the OECD were driving the development of Governance structures for multilateral partnerships (Key countries in this were China, South Africa and now Brazil). There was a need for facilitating policy but to date this was not happening. Biofuels were important and there was a move to unlock investment for industrial development. To drive improvement there was a need to pool ideas and the patent pool idea to enable group benefits was strengthening. BRCs needed to secure IP, needed interoperable data systems and had to address public concerns such as the "big challenges" to engage Governments. George Tzotzos from UNIDO described a plant genetics network recently established to provide plant resources into Biotech; addressing under researched, underexplored areas that the big companies were not investigating niche applications.

Session 2 - *Innovation and intellectual property in the bio-economy age* included the talks *Biotechnologies revolutionizing healthcare* by Carlos Morel, FIOCRUZ, Brazil and *Managing intellectual property in the bioeconomy age* by Jorge Ávila, INPI, Brazil. The full programme covered Bioprospecting, healthcare, biofuels, biotechnological applications, networking, taxonomy and ecology, data management, resource management, policy and legislation impact and quality issues and provided something for everyone.

Session 11 *From culture collections to biological resource centres* and Session 12 *GBRCN implementation and coordination with national and regional efforts* covered topics key to global networking including implementation of best practices, legal and safety issues, intellectual property and innovation in biotechnology and data management, networking and information systems. A Global Biological Resource Centre Network (GBRCN) closed meeting was held to discuss current outputs and to discuss the next steps with partners and interested stakeholders. The conference provided the opportunity to interact with current and future partners and enabled the detailed development of action plans for the GBRCN and Microbial Resources Research Infrastructure which is now on the European Strategy Forum for Research Infrastructures road map 2010.

The EMbaRC session presented the outputs from the 18 month reports in some of the work packages. Initial discussions were held on establishing the GBRCN cluster (focus group) on information systems and data interoperability and standards. Vanderlei Canhos

outlined the system requirements in his presentation in Session 9 *The Global BRC Network Architecture* (see presentations available at www.iccc12.info).

Session 13B on legal issues offered the opportunity to interact with the WHO, USA, UK and Brazil initiatives in areas of legislative compliance particularly biosafety and biosecurity. Marie Isabelle Chevrier, University of Texas at Dallas gave an overview of this complex arena. Peter Kämpfer, Head of Committee for Biological Agents, Germany described the German legislation resource. The WHO position was described by Nicoletta Previsani, they endorsed the CEN standard on Biorisk and offered training courses to help implement best practices.

The Skerman Award was presented to Kostas Konstantinidis, Georgia Institute of Technology, USA who presented the lecture - *Microbial Taxonomy and Phylogeny: Extending from rRNAs to Genomes*. ICCC12 was also the stage for the award of the second WFCC medal for outstanding contribution to the WFCC to Lindsay Sly.

NEWS FROM MEMBERS

The Philippine Network of Microbial Culture Collection (PNMCC)

Rosario G. Monsalud

On last November 13, 2010, the Philippine Network of Microbial Culture Collection (PNMCC) conducted its 10th Annual Symposium and General Assembly with the theme "Linking Microbial Systematics with Biotechnology". Four plenary lectures, research poster papers, product exhibits of microbial ID kits and diagnostics, and the PNMCC Inc. business meeting were the highlights of the event. This was attended by 137 participants from the academia, research institutions and private companies. Fifteen new members were sworn-in bringing the total individual membership to 229.

Since 2001, the PNMCC organized annual symposia and workshops (Figure 1a,b,c,d) about conservation, diversity, taxonomy, potential applications of microbial resources, culture collection management and impact of microbial resources on public health. Laws and policies on biosafety and bioprospecting have also been tackled. The PNMCC since then realized the need to disseminate significant information on microbial resources and to develop and upgrade the technical capability of educators and researchers who are current and potential users of microbial resources.

The Philippines is a country of 7,100 islands covering 297,179 km^2 in Southeast Asia. Being part of the coral triangle, its coral-reefs support among the highest levels of marine biodiversity in the world. It also has one of the most rugged rainforests in the world. It is said that the archipelago was formed from a series of isolated fragments some dating back 30-50 million years. These islands are part of the "Ring of Fire" of the Pacific Basin having at least 17 volcanoes. The archipelago stretches over 1,810 kilometres from north to south. The patchwork of isolated islands, the tropical location of the country, and the once extensive areas of rainforests have resulted in high species diversity in some groups of organisms and a very high level of endemism. The Philippines has among the highest rates of discovery in the world with sixteen new species of mammals discovered in the last ten years This megadiversity country, however, is now one of the world's most threatened biodiversity hotspots. Destructive fishing and sedimentation due to poor land management practices have dramatically affected its coral reefs (www.biodiversityhotspots.org/xp/hotspots/philippines/pages/biodiversity.aspx). Only less than 6% of its original forest cover remained intact and only roughly 5% of its ocean ecosystems are still pristine (www.conservation.org/explore/asia-pacific/philippines).

Along with habitat destruction, the country's microbial diversity is likewise threatened, and they have barely been studied and untapped. Relatively, only a handful of micro-bioprospecting projects have been carried out by a few researchers and students in some universities.

The significant projects in the country were embarked by the National Institute of Molecular Biology & Biotechnology (BIOTECH) at the University of the Philippines Los Banos in the 80's searching for microorganisms with high nitrogen-fixing ability for agriculture, and yeast strains for biofuel production in answer to a national crisis. Then, from 2003-2006, the Philippine National Collection of Microorganisms (PNCM) took on isolation and preservation of microorganisms from different mangrove areas and screening them for various enzymes and antimicrobials adding ~1500 strains in the collection. A more recent project is that of the Marine Science Institute at the University of the Philippines Diliman campus where some 2,000 marine microbes associated with sponges were isolated under its PharmaSeas Project with the

University of Utah for the discovery of anti-cancer and anti-malarial marine natural products.

Fig 1: PNMCC organized annual symposia and workshops

(a)

(b)

(c)

(d)

The reality described above is what prompted the PNMCC to conduct symposia and workshops to spread the importance of conserving our bioresources so that more people will embark on this important and immediate task. Microbiology is a recent field of study in the Philippines. Microbiology as a course was offered at UPLB only in the mid 70's and even much later in three other universities. Hence the need to disseminate significant information on microbial resources and develop/upgrade the technical capability of educators and researchers.

The PNMCC was established in 1996 as a project of the PNCM. It aims to:

- Provide a permanent secretariat for all Philippine Culture Collections and a central contact point for Philippine scientists and any institutions seeking advice and information on microbiological materials and on culture collection-related matters;
- Establish an effective liaison between persons and organizations concerned with culture collections and among the users of the cultures;
- Collect information on the strains and services offered by the various culture collections;
- Publicize the resources within the culture collections in terms of materials and scientific expertise by preparing printed and visual materials for distribution as well as producing informative literature for scientifically and industrially oriented publications;
- Encourage the study of procedures for the isolation, culture, characterization, conservation, and distribution of microorganisms and to make known the most recommendable methods which will take the form of training;
- Promote the training of personnel for the operation of culture collections, and to promote the establishment of a national data service concerned with the location of and information

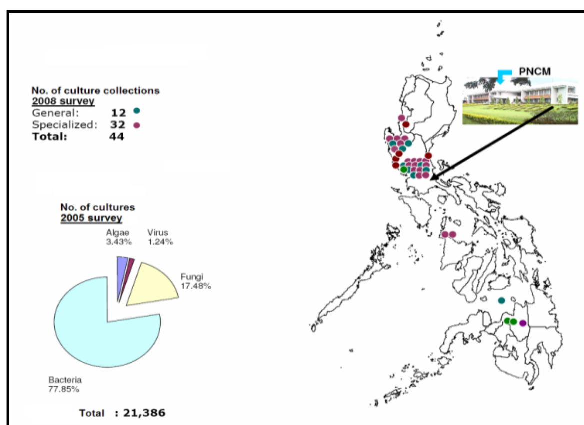
about microorganisms maintained in culture collections; and

- Publish a Philippine Directory of Culture Collections.

Membership to the PNMCC is open to any individual with a declared interest in culture collections (regular member), culture collections regardless of size and geographical location (affiliate member) represented by the curator, and to individuals or organizations who espouse the cause of the Network (sustaining member). No restriction is placed on the number of members from one region or institution. There are now 8 affiliate culture collections of the PNMCC. Although there were 44 culture collections in the country in the survey conducted in 2005 (Figure 2), most of them are project based thus sustainability is a big question.

Some of the culture holdings of five affiliate culture collections of the PNMCC can now be accessed at the Asian Biological Resource Center Network website (www.abrcn.net).

Figure 2: Culture Collections in the Philippines



PNMCC Affiliate Member Culture Collections

1. Philippine National Collection of Microorganisms (*PNMCC Headquarters)*

Acronym of the collection: BIOTECH
Host Institute: National Institute of Molecular Biology and Biotechnology (BIOTECH)
Address: University of the Philippines Los Baños, College, Laguna 4031, Philippines
Head Curator: Rosario G. Monsalud, Ph.D.
Email: pncm@uplb.edu.ph

2. Microbial Culture Collection-Museum of Natural History

Acronym of the collection: MCC-MNH
Host institute: Museum of Natural History (MNH)
Address: Upper Forestry Campus, University of the Philippines Los Baños,
College, Laguna 4031, Philippines
Head Curator: Dr. Marian A. Pulido-de Leon, Ph.D.
Email: mpdeleon@uplb.edu.ph

3. UP Natural Sciences Research Institute Culture Collection

Acronym of the collection: UPCC
Host Institute: Natural Sciences Research Institute, University of the Philippines Diliman
Address: Quirino cor. Velasquez Sts., Diliman, Quezon City 1101, Philippines
Head Curator: Ma. Auxilia T. Siringan, Ph.D.
Email: mats@nsri.upd.edu.ph

4. Industrial Technology Development Institute Microbial Culture Collection

Acronym of the collection: ITDI - MCC
Host institute: Industrial Technology Development Institute
Address: Industrial Technology Development Institute Environment and Biotechnology Division, Department of Science and Technology (DOST)
DOST Compound, Gen. Santos Avenue Bicutan, Taguig City 1632,
Metro Manila, Philippines
Head Curator: Elena L. Brillante, MS
Email: ellenbril@yahoo.com

5. Ecosystem Research and Development Bureau Endomycorrhizal Germplasm Bank and Culture Collection

Acronym of the Collection: ERDB
Host Institute: Ecosystems Research and Development Bureau
Address: College of Forestry campus, U.P. Los Banos, College, Laguna 4031, Philippines
Head Curator: Evangeline T. Castillo, Ph.D.
Email: ecotrend_etc@yahoo.com

6. Research Center for the Natural Sciences-Collection of Microbial Strains

Acronym of the Collection: UST-CMS
Host Institute: Research Center for the Natural Sciences, University of Santo Tomas
Address: España, Manila 1015, Philippines
Head Curator: Thomas Edison Dela Cruz, Ph.D.
Email: tedelacruz@mnl.ust.edu.ph

7. United Laboratories Clinical Culture Collection

Acronym of the Collection: UL
Host Institute: United Laboratories, Inc.
Address: 66 United St., Mandaluyong City 1501, Philippines
Head Curator: Leila M. Florento, Ph.D.
Email: lmflorento@unilab.com.ph

8. Dela Salle University Culture Collection

Acronym of the Collection: DLSU

Host Institute: Dela Salle University
Address: Taft Avenue, Manila 1015, Philippines
Head Curator: Esperanza C. Cabrera, Ph.D.
Email: esperanza.cabrera@dlsu.edu.ph

LATIN AMERICA FEDERATION FOR CULTURE COLLECTIONS

F.E.L.A.C.C.

Federación Latinoamericana de Colecciones de Cultivos

Mirtha Floccari [1,2], ***Vanderlei Pêrez Canhos*** [1,3], ***Faustino Siñeriz*** [1,4], ***Nélida Leardini*** [1,2], ***Nidia Lucero*** [1,15], ***Vidal Rodríguez Lemoine*** [1,5], ***Zulia Weng Alemán*** [1,6], ***Elsie Iglesias*** [1,7], ***Sueli Corrêa Marques de Mello*** [1,8], ***Silvia Giono Cerezo*** [1,9], ***Elizabeth Sfreddo*** [1,10], ***Alejandro Perticari*** [1,11], ***Gladys Martos*** [1,12], ***Silvana Levis*** [1,13], ***Lilian Loperena*** [1,14], ***Graciela Davel*** [1,15].

[1] Latin American Federation for Culture Collections, FELACC. [2] SCCM, Argentinean Association for Microbiology. [3] CRIA, Campinas, San Pablo, Brazil. [4] PROIMI, Tucumán, Argentina. [5] CVCM, Venezuela. [6] INHEM, Cuba. [7] Finlay Institute, Cuba. [8] Embrapa, Brasilia, Brazil. [9] CC – ENCB Collection, Mexico. [10] CCMFCA, UNCuyo. Mendoza, Argentina. [11] INTA-IMYZA, Buenos Aires, Argentina. [12] CERELA, Tucuman, Argentina. [13] INEVH, Pergamino, Argentina. [14] Engineering School, Montevideo, Uruguay. [15] ANLIS, Malbrán Institute, Buenos Aires, Argentina.

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The idea of creating an organization able to represent collections in this region has been suggested several times. The first time was in 1974 during the VI Latin American Congress of Microbiology held in Caracas, Venezuela, sponsored by the Latin American Association for Microbiology (ALAM). Here it was proposed to create a regional organization called "Federación Latinoamericana de Colecciones de Cultivos Microbianos, (FLCC)". The topic was examined again at several International Symposiums and Microbiological Congresses after this. At a meeting granted by UNESCO, with the World Federation for Culture Collections(WFCC) also in attendance, held in Buenos Aires (1977); at XIII Latin American Congress of Micology, ALAM, Caracas, Venezuela (1996) (3,4); XIV ALAM – Paraguay (1998) and XV ALAM – Mexico (2000); IV Symposium for Genetics Resources for Latin American and The Caribbean (SIRGEALC), Mar del Plata, Argentina (2003) and other national scientific meetings (2).

Finally during the XVIIº ALAM, held in Buenos Aires in October 2004, a workshop on *Microbial Culture Collections* took place during which several countries agreed and they were summoned to the Federation Constitutive Assembly. On 19th October representatives from Mexico, Venezuela, Cuba, Brazil and Argentina gathered in the Central Office of the Argentinean Association of Microbiology, AAM, and Buenos Aires. Here they chose the initial Executive Board and approved the Statute for the Latin American Federation of Culture Collections, with FELACC as the acronym (1).

Since then, FELACC as an Association, or its members, have been invited to participate in several scientific regional events, for example, the V SIRGEALC, Montevideo, Uruguay (2005); XVIII ALAM, Pucón, Chile (2006); XIX ALAM, Quito, Ecuador (2008); VI Latin American Congress for Mycology, Mar del Plata (2008); II Brazilian Symposium for Genetics Resources, Brasilia, Brazil (2008); VII SIRGEALC, Pucón, Chile (2009); XX ALAM, Montevideo, Uruguay (2010) and the 12th International Conference on Culture Collections, ICCC-12, Florianopolis, Brazil (2010).

In 1991 an important precedent was set when a bi-national Brazilian-Argentinean Project, granted by CABBIO (Brazilian-Argentinean Centre for Biotechnology), recognised the importance of Culture Collections in the region. Dr Faustino Siñeriz, representing Argentina and Dr Vandelei Pêrez Canhos, representing Brazil, were the respective Directors of CABBIO. A course on "Culture Collections and their Services" was run by CABBIO, under the direction of Dr Vandelei Pêrez Canhos, in Campinas, Brazil, attended by students from both countries. This course emphasized the relevance of Biological Resource Centers (BRCs) in Latin America. Under this Project equipment used in Collections was supplied to those BRCs which were already well established, in order to help them become more efficient.

FELACC operates like other non-profit organizations, relying on the voluntary association of its members. There are no fees or other commitments except expecting members to combine their efforts in order to contribute to the rational use of microbial diversity for the benefit of the community as a whole.

From 2004, FELACC began the construction of a database that gathers information about cultures preserved *ex situ*, in the BRCs of the Region. This database is constantly upgraded and has nine entries, these are: Full Name / Acronym ; Institution ; Postal Address, Tel/Fax, Email address, Homepage ; Type of Collection ; Name of the Director ; Name of the Curator ; Microorganisms Preserved ; Number of Strains and Services Carried Out. It may be consulted on the AAM home page, http://www.aam.org.ar. Recently the database has also become available on the WFCC-Network page, http://www.wfcc.info/network.html.

FELACC is made up of 48 Institutional (affiliated collections), 23 Ordinary (individual affiliations) and 2 Cooperative (sustaining affiliations) members from Argentina, Brazil, Colombia, Cuba, Ecuador, Mexico, Paraguay, Uruguay and Venezuela (Table 1, Figure 1).

On the whole, the regional system maintains nearly 53.000 strains of bacteria, archeae, filamentous fungi and yeasts, which include isolates from a wide variety of ecological niches in the region.

FELACC activities are directed to promote the development of regional collections under rules and regulations internationally applied to this kind of organization. For the development of these purposes, the federation is organized into subcommittees allowing the development of a cooperative action. The topics of these subcommittees include: Quality Management, Preservation Methods, Bio-protection and Transport of Biological Materials, Organization of Courses and Scientific Meetings and Publications. Every four months an electronic Newsletter is published with general information and articles contributed by members.

Figure 1: FELACC Affiliate Countries

Affiliate collections
Ordinary members
Cooperative members

Until now the number of Culture Collections affiliated to FELACC is considered small in relation to the total number existing in the region, but this organization represents a significant effort in the maintenance of a network between regional institutions, in order to make the conservation *ex situ* microbial diversity more efficient.

Table 1: FELACC Associate Collections

Further information in WFCC-Network page: http://www.wfcc.info/network.html and http://www.aam.org.ar

COLLECTION / ACRONYM	COUNTRY
	ARGENTINA
LPSC	**Spegazzini Institute FCNyM. La Plata National University. BUENOS AIRES.** Angélica Arambarri anmabarr@fcnym.unlp.edu.ar Ana María Bucsinszky *anabucsi@hotmail.com*
CCMFCA.	**Agricultural Cs. School. National University from Cuyo. MENDOZA.** Elizabeth Sfreddo *esfreddo@fca.uncu.edu.ar*
CEPAVE.	**CEPAVE- CONICET. La Plata National University. BUENOS AIRES.** Claudia C. López Lastra claudiacll58@yahoo.com.ar
EEA-INTA Balcarce	**INTA. Balcarce. BUENOS AIRES.** Horacio R. Terzolo *terzolo@lcnet.com.ar, hterzolo@balcarce.inta.gov.ar,*
BAFC	**FCEN - Buenos Aires University. UBA.** María Victoria Novas *vicnovas@bg.fcen.uba.ar*
BRU	**INEI. ANLIS. Dr. Carlos G. Malbrán. BUENOS AIRES.** Nidia Lucero *nidia@elsitio.net, nlucero@anlis.gov.ar*
LIHLCOM–SBIL	**SURMICEL-IMEXTRADE Laboratory.** Silvia N. Blumenfeld *Tel: 54 299 4980-001/002/003.Fax: 54 299 4980-000*
AGRAL	**Agronomic School. Buenos Aires University, UBA.** Silvia Miyasaki *miyazaki@mail.agro.uba.ar*
DMic	**INEI - ANLIS. Dr. Carlos G. Malbrán. BUENOS AIRES.** Graciela Davel *gdavel@anlis.gov.ar, godavel25@hotmail.com*
CCC	**Mycological Reference Centre. CEREMIC. Biochemical and Pharmacological Sciences School. Rosario National University. SANTA FE.** Alicia Luque *agluquear@yahoo.com.ar*
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Molecular Techniques in the Diagnosis of Fungal Infections and Preservation and Collection of Fungal Genomic DNA

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The frequency of invasive fungal infections has risen dramatically in recent years, the use of immunosuppressive therapies to treat cancer and enable solid organ or bone marrow transplants, together with advances in the development of broad spectrum antibiotics, have created an increasing population of immuno-compromised patients (55,58). In addition, the HIV pandemic has created a large increase in the number of immuno-suppressed individuals. These patients are at significant risk from systemic fungal infections. The incidence of invasive fungal infection in bone marrow transplant patients has been reported to be as high as 50%, and the subsequent mortality rates are generally around 80% (53). The availability of accurate and timely diagnoses could reduce the use of empirical antifungal therapy, thereby reducing antifungal selection pressure and the emergence of antifungal resistance. Unfortunately, a major obstacle to the successful treatment of invasive fungal infections is the lack of sensitive and specific methods for the early diagnosis of invasive fungal infections. If appropriate therapy is to improve the prognosis of the immuno-compromised patient with systemic fungal infection; early diagnosis is required (6).

Over the past century microbiologists have searched for more rapid and efficient means of microbial identification. The identification and differentiation of microorganisms have principally relied on microbial morphology and growth variables. Advances in molecular biology over the past 10 years have opened new avenues for microbial identification and characterization (14,38,39,41,45). The traditional methods of microbial identification rely solely on the phenotypic characteristics of the organism. Standard approaches to the laboratory diagnosis of invasive fungal infections include (a) direct microscopic visualization for the presence of organisms in freshly obtained body fluids, (b) histopathologic demonstration of fungi within tissue sections, and (c) cultivation of the causative fungus and its subsequent identification. However, these approaches often are not sufficiently sensitive and/or specific to diagnose invasive fungal infections, and they sometimes require invasive procedures to obtain the necessary specimens. Most phenotypic variables commonly observed in the microbiology laboratory are not sensitive enough for strain differentiation. When methods for microbial genome analysis became available, a new frontier in microbial identification and characterization was opened. Inability or delay in diagnosing fungal infection defers the administration of appropriate therapy. This has grave implications for the prognosis of the patient: reliable and rapid diagnostic tests for systemic mycoses are imperative to improve rates of patient survival. Different types of molecular identification approaches have been evaluated to date for fungi (4,20,21,34,46).

Restriction Enzyme Pattern

Restriction endonucleases recognize specific nucleotide sequences in DNA and produce double-stranded cleavages that break the DNA into small fragments. The number and sizes of the restriction fragments, called restriction fragment length polymorphisms (RFLPs), generated by digesting fungal DNA are influenced by both the recognition sequence of the enzyme and the composition of the DNA. In conventional restriction endonuclease analysis, chromosomal DNA is extracted from fungi specimens and then digested with endonucleases into small fragments. These fragments are then separated by size with use of agarose gel electrophoresis. The nucleic acid electrophoretic pattern can then be visualized by ethidium bromide staining and examination under UV light (47). Restriction endonuclease analysis has the advantage of being highly reproducible, very accurate in determining the relatedness of fungi strains, and well within the technical capabilities of experienced laboratory technologists. However, the major limitation of this technique, especially for chromosomal DNA, is the difficulty of comparing the complex profiles generated, which consist of hundreds of fragments. To this end, pulse-field gel electrophoresis (PFGE) has been developed to enable the separation of large DNA fragments. PFGE provides a chromosomal restriction profile typically composed of 5 to 20 distinct, well-resolved fragments ranging from; 10–800 kilobases (kb) (54). The relative simplicity of the RFLP profiles generated by PFGE facilitates the application of the procedure in identification and epidemiological survey of fungal pathogens (13, 24, 44). In postamplification RFLP analysis, the amplified DNA fragments are cut by a restriction endonuclease, separated by gel electrophoresis, and then transferred to a nitrocellulose or nylon membrane. The fragments containing specific sequences may then be detected by using a labelled homologous oligonucleotide as a probe. Variations in the number and sizes of the fragments detected are referred to as RFLPs and reflect variations in both the number of loci that are homologous to the

probe and the location of restriction sites within or flanking those loci (24, 50).

Random amplified polymorphic (RAPD) typing, originally developed by Welsh and McClelland in 1990, involves the use of a short (usually 10 to 15 mers), arbitrarily chosen primer to amplify nearly homologous sequences of the genomic DNA under low-stringency conditions (56). RAPD has been used to differentiate strains of various species, various serotypes within species, and various subtypes within a serotype. It is, therefore, useful for determining whether two isolates of same species are epidemiologically related. RAPD may be a better choice than PFGE because the technique requires fewer open manipulations and the organisms are kept viable for a shorter period. These methods have provided some unique insights into the epidemiology of infections due to *Candida* species. As no laborious cloning, nucleotide sequencing or Southern blot hybridization is required, the method appears to permit the rapid and cost-effective detection of polymorphisms in genetic studies of bacteria, protozoan and pathogenic fungi including *Candida* strain identification (22).

in Situ Hybridization Assays

In situ hybridization assays have been used effectively to localize the DNA and RNA of infectious agents in routinely processed tissues, and no DNA extraction is required (44). This technique has been reported for the identification of *Candida* spp. (27, 28), and *Aspergillus* spp. (37,40). Although the entire procedure is rapid and easy to perform, the sensitivity of the assay is often lower than those of other molecular biological assays, especially those including nucleic acid amplification (9, 23,44).

Polymerase Chain Reaction (PCR)

PCR is the best-developed and most widely used method of nucleic acid amplification. An ingenious procedure, PCR is based on the ability of DNA polymerase to copy a strand of DNA by elongation of complementary strands initiated from a pair of closely spaced chemically synthesized oligonucleotide primers. The basic technique of PCR includes repeated cycles of amplifying selected nucleic acid sequences (38,39). Each cycle consists of three steps: (*a*) a DNA denaturation step, in which the double strands of the target DNA are separated; (*b*) a primer annealing step, performed at a lower temperature, in which primers anneal to their complementary target sequences; and (*c*) an extension reaction step, in which DNA polymerase extends the sequences between the primers. At the end of each cycle (each consisting of the above three steps), the quantities of PCR products are theoretically doubled. The whole procedure is carried out in a programmable thermal cycler. Generally, performance of 30 to 50 thermal cycles results in an exponential increase in the total number of DNA copies synthesized (11,57). The PCR generated product is usually analysed by ethidium bromide-stained gel electrophoresis. Although gel electrophoresis is simple and inexpensive, it is much less sensitive than Southern blotting. Detection of PCR products by ethidium bromide staining and by Southern blotting is time-consuming, and the interpretation of results may be subjective (17,36,43,52).

PCR Based Techniques

A) Polymerase Chain Reaction-Enzyme Immun Assay (PCR-EIA)

Including PCR amplification, hybridization with the complementary labeled probe, and detection of reaction products in an EIA that provides either a colorimetric or fluorescence readout was developed (12,17,26,29). The sensitivity of PCR-EIA to detect candidemia and aspergillosis was higher than that by ethidium bromide staining (18). In addition, the PCR-EIA format provided further amplification without losing the species-specific binding associated with Southern blotting, multiple samples can be assayed in parallel, and semi-quantitation of DNA is possible (7).

B) TaqMan PCR

The TaqMan probe consists of a reporter dye with a fluorescein derivative at the 5’ end, a 3’ quencher dye, and a 3’ blocking phosphate group. The fluorescence emission of the reporter dye is suppressed in the intact probe by Forster-type energy transfer. During PCR, the probe is cleaved by the 5’ nuclease activity of *Taq* polymerase only when it is hybridized to a complementary target. When probe-specific PCR probe has been generated, an increase in reporter dye fluorescence, resulting from the cleavage between the reporter and quencher, occurs. The amount of reporter dye released is proportional to the amount of DNA amplified by PCR.

The Taq-Man fluorescence assay enables samples to be analysed as soon as 5 to 10 min after PCR is complete, and no post-amplification manipulation, which might reduce a significant source of laboratory contamination, is required (5). In addition, it was shown to be 10-fold more sensitive than detection by ethidium bromide-stained agarose gel. This method could detect isolates of *Candida* species and *Aspergillus fumigatus* (44).

C) Real-time PCR

A quantitative PCR assay with the LightCycler (Roche Diagnostics, Mannheim, Germany) amplification and detection system has been described (30). This technology combines rapid thermocycling with glass capillaries with online fluorescence detection of the PCR amplicon; cycling is achieved by alternating heated air and air of ambient temperature. The detection system is based on fluorescence resonance energy transfer with two different specific oligonucleotides: hybridization probe 1 is labelled with fluorescein, while the second hybridization probe is labelled with the fluorophore LightCycler Red 640. Both probes can hybridize in a head-to-tail arrangement, bringing the two fluorescent dyes into close proximity. A transfer of energy between the two probes results in emission of red fluorescent light, which is measured by photohybrids, and the level of fluorescence is proportional to the amount of DNA generated during the PCR process. This technology is promising because (a) specific detection of *C. albicans* and *A. fumigatus* has been achieved, (b) the fungal load in clinical samples can be determined, (c) amplification and post-amplification analyses are performed in closed glass capillaries, thus minimizing the risk of carryover contamination; and (d) the whole amplification and detection process requires only 45 min (15).

D) Nested PCR

Nested PCR, designed mainly to increase sensitivity (detects smaller quantities of target), uses two sets of amplification primers (14). One set of primers is used for the first round of amplification, which consists of 15 to 30 cycles. The amplification products of the first reaction are then subjected to a second round of amplification with another set of primers that are specific for an internal sequence that was amplified by the first primer pair (19).

Nested PCR has extremely high sensitivity because of the dual amplification process. The DNA product from the first round of amplification contains the hybridization sites for the second primer pair. The amplification by the second primer set, therefore, verifies the specificity of the first-round product.

The major disadvantage of the nested-amplification protocol is the high probability of contamination during the transfer of the first round amplification products to a second reaction tube. This can be avoided either by physically separating the two amplification mixtures with a layer of wax or oil, or by designing the primer sets to utilize substantially different annealing temperatures (14,25).

E) Multiplex PCR

Multiplex PCR is an amplification reaction in which two or more sets of primer pairs specific for different targets are introduced in the same tube. Thus, more than one unique target DNA sequence in a specimen can be amplified at the same time. Primers used in multiplex reactions must be carefully designed to have similar annealing temperatures, which often require extensive empirical testing. This co-amplification of multiple targets can be used for various purposes. For diagnostic uses, multiplex PCR can be set up to detect internal controls or to detect multiple pathogens from a single specimen. This quick method has become routine for the amplification of DNA from cultures of yeast species (8,32).

F) *Aspergillus* RNA

Aspergillus RNA in blood samples that does not require temperature cycling has recently been described. This method was more sensitive than PCR for detecting *Aspergillus* 18S ribosomal sequences, but it has not yet been assessed in patients with proven presence or absence of invasive aspergillosis (31).

Preservation, Collection and Storage of Fungal Genomic DNA

There is a growing interest in the preservation of DNA from a variety of fields including pharmaceutical science, forensics, homeland defence, biology, and bio-repository management. Although each of these fields is concerned with maintenance of DNA integrity, the requirements in terms of storage time and fidelity are significantly different. Both storage time and fidelity must be carefully considered and defined when discussing strategies for “preservation”. Current sequencing technology depends on the PCR to make copies of stored DNA that are ultimately used for sequence identification. As a result of this process, accurate sequence information can be obtained even from samples in which the DNA has been reduced to fragments. Although the desire for sequence identification does allow substantially greater levels of degradation to be tolerated, bio-repositories typically strive to preserve samples “permanently” so that future scientists can utilize specimens for studies at some undefined point in time. In reality, some finite degradation rate is occurring in any sample regardless of storage conditions, and the goal is to minimize this rate of degradation. In most instances, DNA samples are stored at -80°C or in liquid nitrogen (-196°C), but there is a significant expense associated with maintaining these conditions in hundreds of millions of samples. In contrast, dehydrated samples could be stored at room

temperature, thereby greatly reducing cost and increasing convenience. Previous studies of DNA in solution have clearly shown that degradation rates can be precisely predicted, and buffer conditions can be adjusted to obtain remarkable stability at room temperature. Three strategies of DNA preservation are common: a) room temperature storage on a “dry” solid matrix, b) frozen DNA (−20°C, −80°C, and −196°C), c) dehydrated DNA but with some notable variations (3,35,49).

A) Solution Stability

To avoid chemical and enzymatic degradation in a laboratory setting, molecular biologists commonly store DNA as a precipitate in ethanol at -80°C. Under these conditions, nucleic acids are stable for prolonged periods, but must be isolated from the ethanol, transferred to aqueous buffers, and are typically quantified prior to use. In addition, it was shown that demetalation of all components (DNA, buffers, water) can significantly reduce degradation during storage. Utilizing this strategy, (16) combined 200µM EDTA (a chelator) with 1% ethanol (a radical scavenger) to minimize strand breakage in plasmid DNA.

This straightforward approach was capable of preserving >90% of the initial supercoil content after 2 years of storage at room temperature. However, the inclusion of components such as ethanol and salts can be incompatible with some applications, for example, maintenance of viable cells, intact tissue storage, and the use of lipids that are electrostatically associated with the DNA in a delivery vehicle (1,16).

B) Frozen DNA

Storage at elevated subfreezing temperatures (−20°C to −80°C) may well provide adequate conditions depending on the quality and quantity of DNA desired and the time frame in which the sample will be stored. Neither of these conditions will, however, provide long-term storage quality equivalent to maintenance at liquid nitrogen temperatures (3). Although studies demonstrate sufficient solution stability on a pharmaceutical time scale, maintenance of DNA integrity over prolonged periods (e.g. decades) will be likely to require storage conditions in which molecular mobility is more restricted, that is, low temperature and/or dehydrated. Despite the fact that DNA is routinely frozen, little is known about the effects of freezing and thawing on DNA integrity. DNA was preserved after freezing to temperatures as low as -196°C. Lyscov and Moshkovsky (33) later described a mechanism of “cryolysis”; DNA degradation that was dependent on the rate of cooling of the frozen sample (10). However, previous studies have investigated plasmid stability during rapid freeze-thawing (immersion in liquid nitrogen), and demonstrated that supercoil content could be effectively preserved if sugars and EDTA were present (2). Although the authors did not specifically address cryolysis as described by Lyscov and Moshkovsky, it seems possible that the presence of sugars reduced cracking by entrapping DNA in a glass (33).

C) Dehydrated DNA

In contrast to freezing, drying offers a more practical alternative by eliminating the need for cold storage. In addition to a reduction in molecular mobility, dehydration also removes water that can participate in hydrolytic reactions (48,59). There are several methods of removing water from liquid preparations to produce dehydrated DNA formulations, for example, spray drying, spray freeze drying, air drying, or lyophilization. These authors demonstrated that dry DNA stored at 0–4°C undergoes significant changes in molecular weight (i.e., strand breakage) within 6 months. Studies incorporating sugars to stabilize purified, dried DNA reported that lactose, glucose, and sucrose were able to preserve biological activity for 3 weeks of storage at 75°C (42). This study also monitored structural changes in the DNA upon rehydration, and reported that sugars partially prevented dehydration-induced structural changes. Although the latter study suggests that the addition of sugars may be sufficient to preserve DNA during prolonged storage, other studies by Shirkey *et al.* have shown that purified plasmid DNA desiccated in the presence of trehalose and stored at room temperature is initially protected against aggregation and light-induced damage, but that degradation is clearly observed after 8 weeks (51).

CONCLUSIONS

In conclusion, during the past 10 years, genetic, molecular, and genomic approaches have been widely used to study a number of medically important fungi. New approaches will be developed to assess the value of these clinical samples in daily routine diagnosis. Laboratory conditions will determine how to store the DNA, and further studies are needed on the optimal DNA storage conditions and preservation solutions.

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ANNOUNCEMENTS AND USEFUL LINKS

Establishment of the Bergey's International Society for Microbial Systematics

http://www.bergeys.org/bismis.html

The purpose of the society is to promote excellent research in microbial systematics as well as enhance global communication among taxonomists who study the *Bacteria* and *Archaea*. The society will also serve

internationally as an advocate for research efforts on microbial systematics and diversity.

CONFERENCES AND WORKSHOPS

- The first meeting of the **Bergey's International Society for Microbial Systematics (BISMiS)** Friendship Hotel Beijing, China, May 19-23 2011
  http://www.bismis.org/dct/page/1

- **European Culture Collections' Organisation Annual meeting**

  The **30th Annual Meeting of ECCO** will be organized in 2011 by CBS, Centraalbureau voor Schimmelcultures, the Netherlands. It will be held on 16-17 June, 2011 at the **CBS** building and the nearby Hotel **Mitland** in Utrecht.

  Further information will be posted on the ECCO website (www.eccosite.com)

- **Microbial Resources Research Infrastructure (MIRRI) preparatory meeting**

  A workshop to discuss the preparatory phase of the MIRRI project will be held back to back with the ECCO meeting on the 18th June 2011.

- **VAAM-Annual Conference of the Association for General and Applied Microbiology 2010, Stadthalle, Karlsruhe, Germany, April 3-6 2011**
  http://www.vaam2011.de

  Main Topics:

  - Cell Biology
  - Environmental Microbiology
  - Food Microbiology
  - Microbial Interactions
  - New Imaging and other innovative Techniques
  - Stress Responses
  - White Biotechnology

- **4th FEMS Congress of European Microbiologists, June 26–30, 2011**
  http://www2.kenes.com/fems2011/Pages/Home.aspx

- **BIO International Convention, June 27 -30, 2011, Washington, DC, USA**
  http://convention.bio.org/

- **36th FEBS Congress, June 25-30, 2011, Torino (Turin), Italy**
  http://www.febs2011.it/

- **14th Annual Conference of the European BioSafety Association, April 14-15, 2011, Estoril, Portugal**
  http://www.ebsaweb.eu/ebsa_14

- **BioSystematics Berlin February 21 - 27, 2011, Berlin, Germany**, hosting the 7th International Congress of Systematic and Evolutionary Biology (ICSEB VII) of IOSEB (International Organization for Systematic and Evolutionary Biology), 12th Annual Meeting of the Society of Biological Systematics (GfBS), and 20th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)
  http://www.biosyst-berlin-2011.de/

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