

Yeasts biodiversity in Ecuador: Amazonia, Andes, Pacific Coast and Galápagos Archipelago and the CLQCA role as the repository of the Ecuadorian yeasts biodiversity.

Personal introduction

Professor Javier Carvajal Barriga is a researcher at the Pontifical Catholic University of Ecuador in Quito. He is the director at the Neotropical Centre for the Research of Biomass, which embraces the Catholic University Yeast Collection in Quito.

He is the Ecuadorian Representative to the International Commission on Yeast (ICY). He also belongs to several scientific networks such as the Iberoamerican Network for the Energies, and the Iberoamerican Society for the Development of Biorefineries. Additionally he has been awarded by the Ecuadorian Government as well as other international institutions such as the Fulbright Commission, ISTIC, TWAS, and, more recently by the Chinese Academy of Sciences.

He has been invited to give presentations in more than 20 international scientific venues; he coauthored a number of research papers, chapters and a book. Currently he is a full professor who has in charge the biochemistry cathedra.

Name of the culture collection

Catholic University Yeasts Collection in Quito (CLQCA)

Colección de Levaduras Quito-Católica (CLQCA)

ABSTRACT

The Catholic University Yeast Collection in Quito (CLQCA) is the very first collection dedicated to yeasts in Ecuador. It has been founded in 2006 as a biodiversity study in the Country. The CLQCA is part of the Neotropical Centre for the Research of the Biomass (CNIB) which is supported by the Pontifical Catholic University of Ecuador (PUCE).

Both, the PUCE and the Ecuadorian Science and Technology Secretariat (SENESCYT) had been supporting the research by funding the infrastructure as well as the human talent training. The CLQCA maintain relationships with a number of other similar collections abroad. A big push to the networking of this collection was given by the International Commission of Yeasts (ICY) by designating in 2013 the CLQCA's Director as the representative of Ecuador for this international organization of yeasts experts.

The CLQCA is also member to the Latin-American Federation of Culture Collections (FELACC). Recently the Director of the CLQCA was elected to be part of the directive of this Latin American organization.

The contributions of the CLQCA to the yeast's basic and applied science during the last 8 years have been focused on biodiversity studies, Microbial Archaeology and Biorefinery applications. A summary of the contributions, especially in terms of the discovery and description of novel yeast species as well as other achievements are presented in this report.

Key words: (Times New Roman, 14)

Yeast, Biodiversity, Microbial Archaeology, Biorefinery

1. Brief introduction of your Culture Collection.

1.1 Contributions and Study Areas of the CLQCA

1.1.1 Biodiversity

The CLQCA has organized a number of expeditions to a wide variety of ecosystems in Ecuador and the Antarctica aiming to recover yeasts isolates from natural environments. Seven novel yeast species have been described up to date and several others are being characterized for future description. An increasing number of isolates are being preserved in the CLQCA, being up to date, more than 3230 yeast isolates from 146 different species preserved, most of them from the four natural regions of Ecuador. Figure 1 shows the percent composition of the isolates maintained at the CLQCA.

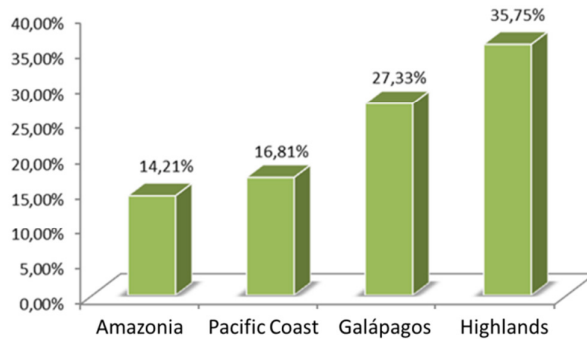


Figure 1. Percent composition of the yeast isolates preserved in the CLQCA. Source: CLQCA data base

Furthermore, CLQCA in its database contains abundant information that has been the fundament of ecological studies carried out on yeasts collected in the Ecuadorian territory. From these studies a mathematical analysis of the yeasts diversity ecology has been developed. This analysis allows determining the specialization degree that exhibit the different yeast species found in the Ecuadorian territory, including the Galápagos Islands.

The analysis is based upon two novel indicators, namely the Specialization index (S_i) and the Abundance index (I_a). It was determined that about $2/3$ of the yeasts species accessed in the CLQCA are highly specialized, regarding the kind of substrates and ecosystems where the yeasts were found. Conversely, a 4% of the yeasts are generalistic species. This data demonstrates the high potential of yeasts group for bioprospection, given the high metabolic versatility exhibited. These data are shown in Figure 2.

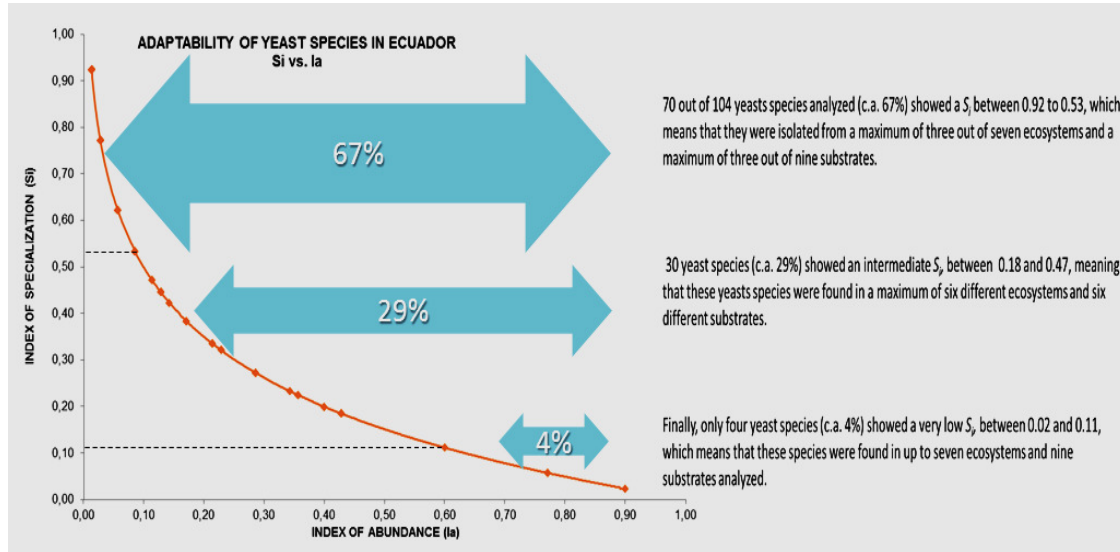


Figure 2. Curve of Adaptability of the yeast species found in Ecuador.

Source: modified from Carvajal et al., 2014

1.1.2 Microbial Archaeology

Regarding the works in the Microbial Archaeology which have been developed along the last years, it is remarkable the invention of a method to recover ancient yeast cells adhered to the walls of the fermentation recipients for chicha (corn beer) and oldest brewery in America. This method combines the use of nanoparticles to restore cell structures; one further hydration step followed by a metabolic activation period.

By the use of this method more than 30 different yeast isolates from fermentation vessels from ancient indigenous peoples from Ecuador, as well as beer wooden fermentation barrels have been recovered.

One of the most important achievements of this yeast collection has been its direct participation in recreating the very first beer brewed in the Americas in 1566 by using a resuscitated yeast strain from the original

fermenters located in the San Francisco Convent in Quito.

1.1.3 Biorefinery

Another achievement which had been generated from the fundamental studies carried out on second generation ethanol fermentation at CLQCA has been the setting up and operation of the very first biorefinery at pilot scale in Ecuador, where yeasts from the CLQCA are being tested for different types of developments in the biofuels area as well as renewable chemicals study by small scale production and experimentation. Nevertheless, the biorefinery project pursues its own goals different to the ones from the yeast collection, but it is to be remarked that it found its origin from the CLQCA studies and now it is another unit integrating the Neotropical Centre for the Research of the Biomass (CNIB) where the yeast collection is the biotechnology platform.

Figure 4 shows a picture of the biorefinery settled at the Campus of the Pontifical Catholic University of Ecuador.

1.1.4 Publications

During the last 8 years, the CLQCA has contributed with seven yeasts novel species which have been published in collaboration with other collections such as the NCYC in Norwich, UK; the microbial collection of the University Federal of Minas Gerais, Brazil; the National Hsinchu University of Education in Taiwan; and the University of West Ontario, in Canada.

The following yeast species have been described and published since 2009.

***Candida carvajalis* sp. nov., an ascomycetous yeast species from the Ecuadorian Amazon jungle.**

James SA, Carvajal Barriga EJ, Bond CJ, Cross K, Núñez NC, Portero PB, Roberts IN.

In the course of a yeast biodiversity survey of different ecological habitats found in Ecuador, two yeast strains (CLQCA 20-011(T) and CLQCA20-014) were isolated from samples of rotten wood and fallen leaf debris collected at separate sites in the central region of the Ecuadorian Amazonia. These strains were found to represent a novel yeast species based on the sequences of their D1/D2 domain of the large-subunit (LSU) rRNA gene and their physiological characteristics. Phylogenetic analysis based on LSU D1/D2 sequences revealed this novel species to be most closely related to *Candida asparagi*, *Candida fructus*, *Candida musae* and two as yet undescribed *Candida* species, with the six yeast taxa collectively forming a distinct species group within the *Clavispora* clade. The species name of *Candida carvajalis* sp. nov. is proposed to accommodate these strains, with CLQCA 20-011(T) (NCYC 3509(T), CBS 11361(T)) designated as the type strain.

***Saturnispora quitensis* sp. nov., a yeast species isolated from the Maquipucuna cloud forest reserve in Ecuador**

Stephen A. James, Geneviève M. Cadet, Enrique Javier Carvajal Barriga, Patricia Portero Barahona, Kathryn Cross, Christopher J. Bond and Ian N. Roberts

A single strain, CLQCA-10-114^T, representing a novel yeast species belonging to the genus *Saturnispora* was isolated from the fruit of an unidentified species of bramble (*Rubus* sp.), collected from the Maquipucuna cloud forest reserve, near Quito, in Ecuador. Sequence analyses of the D1/D2 domains of the large-subunit rRNA gene and ribosomal internal transcribed spacer region indicated that the novel species is most closely related to the recently described species *Saturnispora gosingsensis*, isolated from the fruiting body of a mushroom collected in Taiwan, and *Saturnispora hagleri*, a *Drosophila*-associated yeast found in Brazil. The name *Saturnispora quitensis* sp. nov. is proposed to accommodate this strain; the type strain is CLQCA-10-114^T (=CBS 12184^T=NCYC 3744^T).

The GenBank/EMBL/DDBJ accession numbers for the LSU D1/D2 and ITS sequences of CLQCA-10-114^T are FN908197 and FN985101, respectively.

***Candida ecuadorensis* sp. nov., an ascomycetous yeast species found
in two separate regions of Ecuador**

Stephen A. James, Enrique Javier Carvajal Barriga, Patricia Portero Barahona, Kathryn Cross, Christopher J. Bond and Ian N. Roberts

In the course of an on-going study aimed at cataloguing the natural yeast biodiversity found in Ecuador, two strains (CLQCA 13-025 and CLQCA 20-004^T) were isolated from samples of cow manure and rotten wood collected in two separate provinces of the country (Orellana and Bolívar). These strains were found to represent a novel yeast species based on the sequences of their D1/D2 domain of the large-subunit (LSU) rRNA gene and their physiological characteristics. Phylogenetic analysis based on LSU D1/D2 sequences revealed this novel species to belong to the *Metschnikowia* clade and to be most closely related to *Candida suratensis*, a species recently discovered in a mangrove forest in Thailand. The species name of *Candida ecuadorensis* sp. nov. is proposed to accommodate these strains, with strain CLQCA 20-004^T (=CBS 12653^T=NCYC 3782^T) designated as the type strain.

The GenBank/EMBL/DDBJ accession numbers for the LSU D1/D2 and ITS sequences of CLQCA 20-004^T are FR839617 and FR839619, respectively. The Mycobank deposit number is MB801370.

***Candida theae* sp. nov., a new anamorphic beverage-associated member of the *Lodderomyces* clade**

Chin-Feng Chang, Yu-Ching Lin, Shan-Fu Chen, Enrique Javier Carvaja Barriga, Patricia Portero Barahona, Stephen A. James, Christopher J. Bond, Ian N. Roberts, Ching-Fu Lee

Four strains representing a novel yeast species belonging to the genus *Candida* were independently isolated in Taiwan and Ecuador. Two strains (G17T and G31) were isolated in Taiwan, by pellet precipitation from plastic-bottled tea drinks produced in Indonesia, while two additional strains (CLQCA 10-049 and CLQCA 10-062) were recovered from ancient chicha fermentation vessels found in tombs in Quito, Ecuador. These four strains were morphologically, and phylogenetically identical to each other. No sexual reproduction was observed on common sporulation media. Large-subunit (LSU) rRNA gene sequence analysis revealed the four strains to belong to the *Lodderomyces* clade, closely related to members of the *Candida parapsilosis* species complex. The four strains, which have identical LSU D1/D2 sequences, differ from their closest phylogenetic neighbors, *Candida orthopsilosis* and *Candida parapsilosis*, by 6–9 nt substitutions, respectively.

Physiologically, the four strains are similar to *Candida parapsilosis*, although they can be distinguished from their closest relative by the assimilation of arbutin, nitrite, and creatine. The Indonesian and

Ecuadorian strain sets can also be distinguished from one another based on ITS sequencing, differing by 4 substitutions in ITS1 and 1 single nucleotide indel in ITS2. Collectively, the results indicate that the four strains represent a previously unrecognized species of *Candida*. The name *Candida theae* sp. nov. is proposed to accommodate these strains, with G-17T (BCRC 23242T=CBS 12239T=ATCC MYA-4746T) designated as the type strain.

***Saccharomycopsis fodiens* sp. nov., a rare predacious yeast from three distant localities**

Marc-André Lachance, Carlos A. Rosa, Enrique Javier Carvajal Barriga, Larissa F. D. Freitas and Jane M. Bowles

Three strains representing a novel yeast species were recovered as part of independent collections from flower-associated nitidulid beetles in Australia, Costa Rica and the Galapagos Islands, Ecuador. Analysis of the D1/D2 domains of the large subunit rRNA gene indicated that the species belongs to the genus *Saccharomycopsis*, although the formation of ascospores was not observed. The yeast is capable of necrotrophic parasitism by means of infection pegs when mixed with other yeasts or filamentous fungi. Of particular interest is the fact that despite the large distances separating the isolation sites of the three strains, other strains of the species have not been recovered in other samples of flower-associated nitidulids even though these habitats have been sampled extensively. It is

suggested that the dispersal of the yeast may be linked to human historical factors. The name *Saccharomycopsis fodiens* sp. nov. is proposed for the yeast. The type strain is UWOPS 95-697.4^T (=CBS 8332^T=NRRL Y-48786^T).

The GenBank/EMBL/DDBJ accession number for the D1/D2 LSU rRNA gene sequence of strain UWOPS 95-697.4^T is AF203418. The MycoBank number for *Saccharomycopsis fodiens* sp. nov. UWOPS 95-697.4^T is MB564897.

***Kodamaea transpacific* f.a., sp. nov., a yeast species isolated from ephemeral flowers and insects in the Galápagos Islands and Malaysia: further evidence for ancient human transpacific contacts**

Larissa F. D. Freitas, Enrique Javier Carvajal Barriga, Patricia Portero Barahona, Marc-André Lachance and Carlos A. Rosa

Twenty-four yeast strains were isolated from ephemeral flowers of *Ipomoea* spp. and *Datura* sp. and their associated insects in the Galápagos Archipelago, Ecuador, and from *Ipomoea* spp. and associated insects in the Cameron Highlands, Malaysia. Sequences of the D1/D2 domains of the large subunit rRNA gene indicated that these strains belong to a novel yeast species of the *Kodamaea* clade, although the formation of ascospores was not observed. The closest relative is *Candida restingae*. The human-mediated dispersion of this species by transpacific contacts in ancient times is suggested. The name *Kodamaea*

transpacifica f.a., sp. nov. is proposed to accommodate these isolates. The type strain is CLQCA-24i-070T (5CBS 12823T5 NCYC 3852T); MycoBank number MB 803609.

***Wickerhamomyces arborarius* sp. nov., a novel ascomycetous yeast species found in arboreal habitats on three different continents**

James S. A., Carvajal E. J. B., Barahona P. P., Harrington T. C., Lee C. F., Bond C. J., Roberts I. N.

Five strains representing a novel yeast species belonging to the genus *Wickerhamomyces* were independently isolated from Ecuador, Taiwan and the USA. One strain (CLQCA 10-161^T) was isolated from the white flower of an unidentified plant species collected in the Maquipucuna cloud forest reserve, near Quito, in Ecuador. A second strain (GY7L12) was isolated from the leaf of a Chinese sumac or nutgall tree (*Rhus chinensis* var. *roxburghiana*) collected in the Taoyuan mountain area, Kachsiung, in Taiwan. Three additional strains (A543, A546 and A563) were isolated from two species of wood-boring beetle (*Xyleborus glabratus* and *Xyleborinus saxeseni*) collected near Clio, Georgia, USA. Analysis of the D1/D2 domains of the large-subunit (LSU) ribosomal RNA gene indicated that the novel species belongs to the genus *Wickerhamomyces*, and is most closely related to *Wickerhamomyces sydowiorum*, an insect-associated

species predominantly found in South Africa. The North American and Taiwanese strains have identical ITS sequences and can be distinguished from the Ecuadorian strain based on a single nucleotide substitution in the ITS1 region. The species name of *Wickerhamomyces arborarius* sp. nov. is proposed to accommodate these strains, with CLQCA 10-161^T (NCYC 3743^T) designated as the type strain.

1.2 Final Remarks

The CLQCA is a yeast collection which had a number of activities and contributions to the advance of sciences in Latin America. Furthermore its database (<http://www.cnib.com.ec/index.php/en/clqca-catalogo>) is frequently consulted by visitors all over the world.

Nevertheless, this yeast collection is not a commercial bank, but exchanges strains under specific agreements with scientific institutions interested in the strains for basic and applied studies.

Future challenges for CLQCA are mainly in the framework of the human talent formation in taxonomy, bioinformatics, continuous actualization of informatic platforms and international databases such as WFCC, CCINFO and ABC.

2. Benefit from the training courses.

The training course brought high benefits in the familiarization with the most important databases for information on culture collections as well as related themes such as metagenomics, genomics, biotechnology,

publications, patents and other important subjects involving the culture collections.

Moreover, the opening towards the world's system of information about culture collections was a valuable opportunity to insert the developing countries collections in the world's context and to introduce into a worldwide organization such as WFCC.

Furthermore, the opportunity to establish relationships between colleagues from all over the world and possible future collaborations is a step forward to the unification of the culture collections information to make it readily available for anyone who could be interested.

The visibility of the culture collections, its dedication, its achievements and specialties was also a remarkable ingredient which reinforced and enriched the substance of the course itself.

3. Suggestion on WDCM work.

I propose to work on the evaluation of the microbiota registered in the WFCC regarding its adaptability and the evolutionary state of the species, by using the concepts and calculations developed to obtain the Specialization index (S_i) and the Index of Abundance (I_a). The two indexes are possible to be calculated by formulas given in this report, which will be very valuable for the understanding of evolution, adaptability and ecology of microorganisms. The WFCC database has the potential to be used in such an important task that has never been

regarded for the lack of a methodology properly designed with that end.

Moreover, we, as CLQCA offer training on the use, understanding and interpretation of the results attained regarding the wide comprehension of the current evolutionary/adaptation status of microbes worldwide.

4. Comments or suggestion on the training courses.

I may suggest that in order to get a better understanding of each other's activities the presentation of the culture collections should have been done at second day, in presence of the president of WFCC. The presentation done later was much less interesting without the presence of an authority.

The training course on mycology was poorly presented and questions done to the lecturer were not responded at all. The yeasts were not represented by any microbiologist. It is necessary to find equilibrium in the dedication of the lecturers and trainers, due to the variety of interests among the trainees.

5. Suggestion on further cooperation between WDCM and your collections.

I strongly suggest that future encounters between the already contacted collections are organized with the aim of finding effective and real cooperation. A good way to do that is by proposing to work on specific issues concerning the WFCC. Those collections which are keen an eager to participate in such programs will finally generate tangible products,

namely, papers, chapters, courses, books, manuals, and other scientific production. This will allow sustaining the relationships and establishing a collaboration environment.

Networking is key factor to support the WFCC in order to attain a sustainable organization worldwide.