Fungal Systematics – Current Technologies and Techniques for Fungal Identification

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA and an R&D and Services center in Gaithersburg, MD
- World's premiere biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microbes
  - Genomic & synthetic nucleic acids
  - Media/Reagents
- ATCC collaborates with and supports the scientific community with industry-standard and innovative biological solutions
  - Growing portfolio of products and services
  - Sales and distribution in 150 countries, 12 International distributors
- Talented team of 450+ employees; over one-third with advanced degrees

An innovative global partner for authentic biomaterials, standards, and services

Agenda

Aspergillus fumigatus image courtesy of David Gregory & Debbie Marshall

- Recent updates in Mycology
- The ATCC Mycology Collection and our QC criteria and ID methods
- Recent ATCC research on fungi and yeasts
- Summary

Recent Updates in Mycology

Fungal taxonomy has been significantly affected by:

- Improvements in contemporary molecular techniques
  - Identifying fungi to the species level by DNA sequencing
  - Early clinical diagnostics
  - Compare fungi as "Holomorphs"
- Changes in nomenclatural rules
  - 2012 – Melbourne Code

Aspergillus bread mold

Fungal Nomenclature

International Code of Nomenclature for algae, fungi, and plants

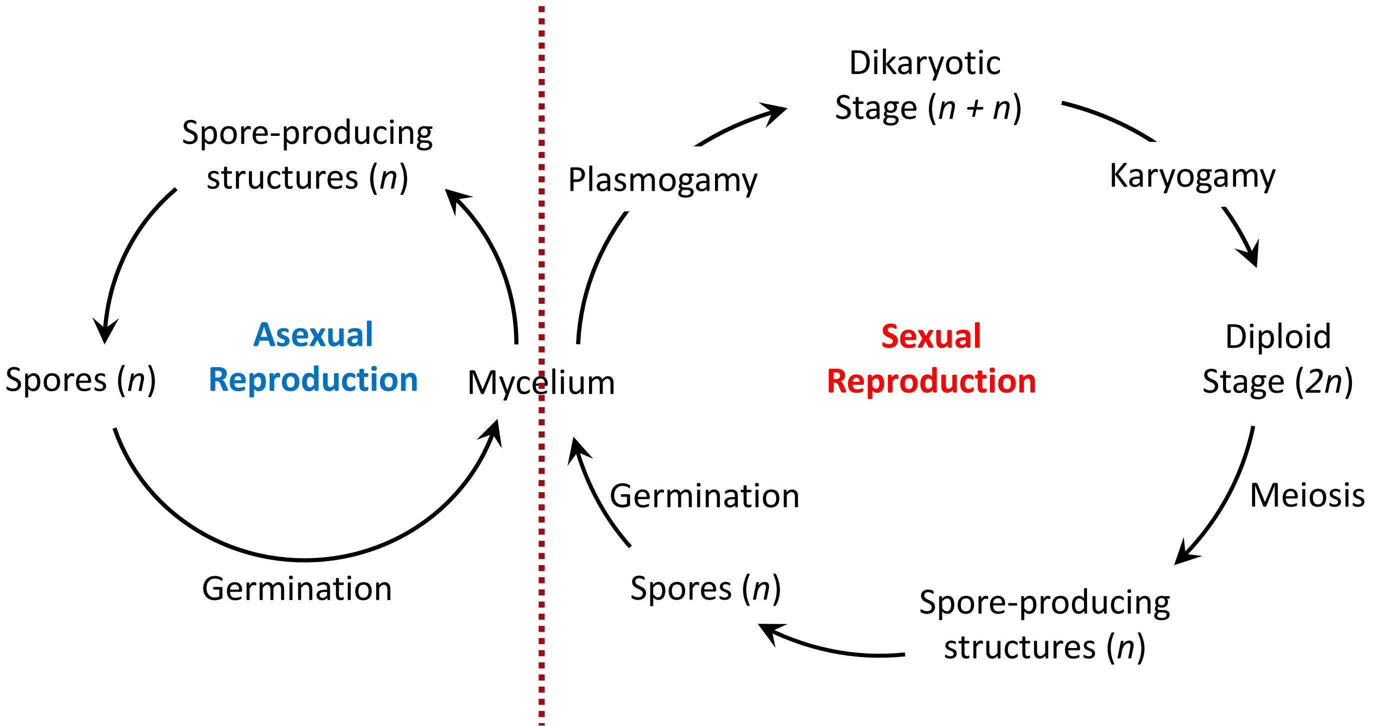
- (www.iapt-taxon.org/nomen/main.php)

Recent updates

- At the International Botanical Congress in Melbourne (July 2011)
  - Major changes in the Melbourne Code – started from 2012
    - Language of diagnosis (English or Latin)
    - Cultures as types (specify how the type specimens are preserved)
    - Governance of the code (set up a subcommittee on fungal nomenclature)
    - Registration of fungal names
    - The end of dual nomenclature

Dual Nomenclature to ‘One fungus, One name’

Fungal life cycle



Spore-producing structures (*n*)

Spores (*n*)

Asexual Reproduction

Germination

Mycelium

Plasmogamy

Dikaryotic Stage (*n* + *n*)

Karyogamy

Sexual Reproduction

Diploid Stage (2*n*)

Meiosis

Spore-producing structures (*n*)

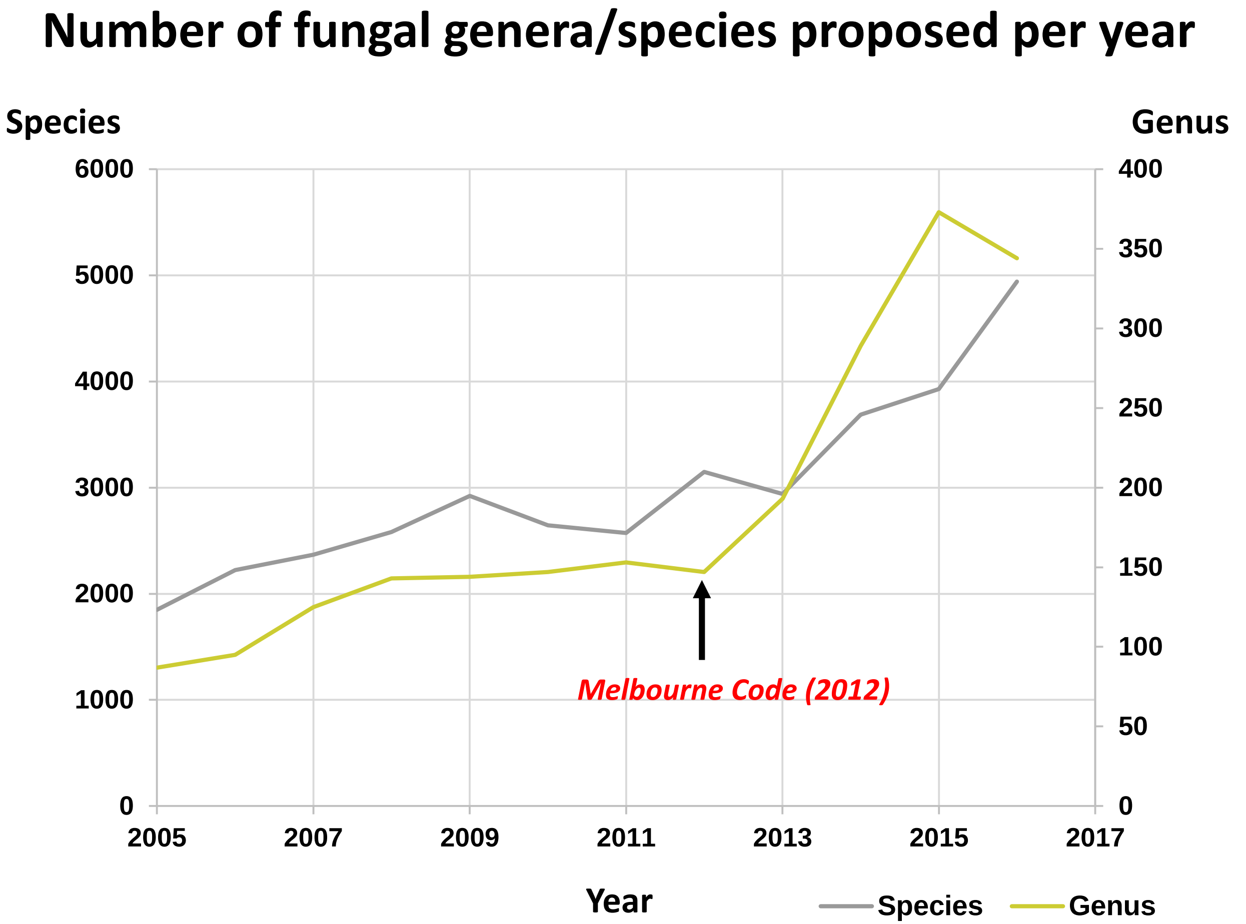
Spores (*n*)

Germination

	ANAMORPH	**TELEOMORPH**
Dual name	***Candida***	***Pichia, Ogataea, Clavispora, Yamadazyma*, and many others**
	Aspergillus	***Eurotium, Neosartorya, Emericella***
	Penicillium	***Talaromyces, Eupenicillium***

HOLOMORPH - Single name (One fungus, One name)

Number of New Fungal Taxa is Increasing



Data is from the MycoBank database (Aug. 2017). Not all the species/genera are still valid nor accepted.

Examples of Recent Name Changes

***Penicillium* – *Talaromyces* (*e.g.*, Samson *et al.* 2011, Yilmaz *et al.* 2014)**

- No longer teleomorph-anamorph
- Represent two individual phylogenetic groups

***Cryptococcus gattii*/*C. neoformans* species complex (Hagen *et al.* 2015)**

- Expanded to 7 *Cryptococcus* spp., including 5 novel species
- No longer use teleomorphic names *Filobasidiella neoformans* or *Filobasidiella bacillispora*

***Trichophyton mentagrophytes* species complex (de Hoog *et al.* 2017)**

- Transferred teleomorphic genera *Arthroderma benhamiae*, *A. simii*, and *A. vanbreuseghemii* to *Trichophyton*

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Aspergillus fumigatus image courtesy of David Gregory & Debbie Marshall

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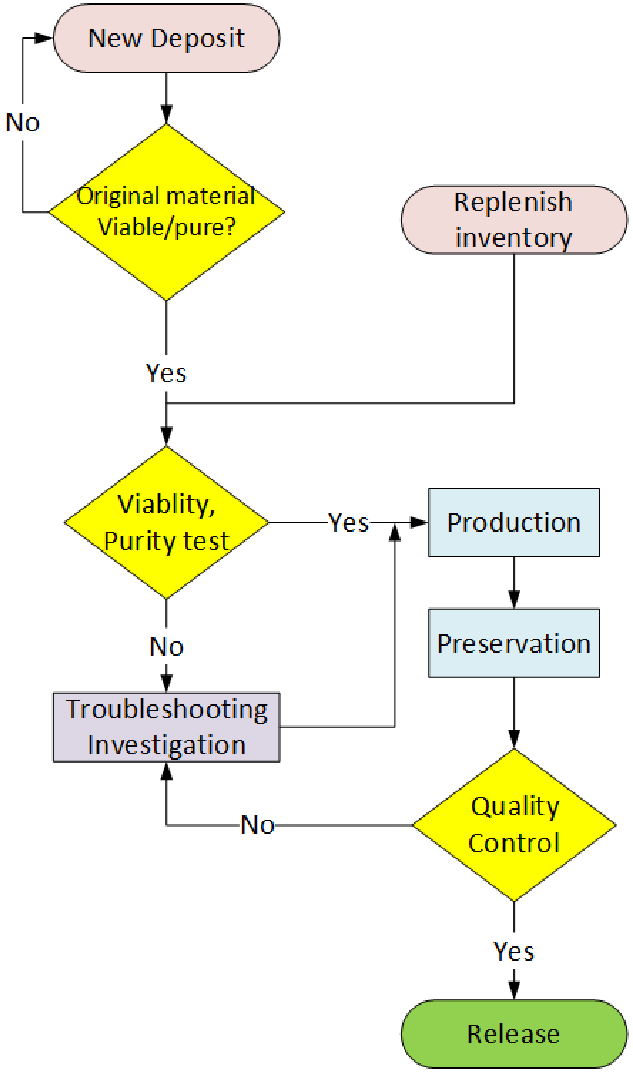
ATCC Mycology Collection

ATCC® is a trusted resource center housing a diverse assortment of filamentous fungi and yeasts, representing over 7,600 species.

- >3,600 type strains
- ≈ 600 genome sequenced strains
- > 32,000 yeast genetic strains
- Many quality control strains for use in media testing, food microbiology, assay development, environmental testing, *etc.*

ATCC Mycology Accession Procedure

- All materials are evaluated for viability and purity
- Newly deposited materials are identified to the species level by ATCC scientists
- Only items confirmed to be viable, pure, and fully-identified are released to customers



QC Criteria for Yeasts and Fungi

Viability

- Recovery condition from the freeze-dried or frozen state

Purity

- On different media at a wide temperature range

Identification

- Morphology – macro & micro
- DNA sequencing
- Optional tests
  - VITEK® 2 YST ID card
  - VITEK® MS (MALDI-TOF)
  - Dropout medium test
  - API® test

Taxonomic References

- MycoBank Database (www.mycobank.org)
- Index Fungorum (www.indexfungorum.org)

DNA Sequencing

Default barcode genes

- ITS (Internal transcribed spacer) ≈ 0.4-0.6 kb
- D1D2 region of LSU rDNA ≈ 0.5-0.7 kb

Optional genes

- SSU rDNA
- IGS (Intergenic Spacer region)
- Protein-coding genes
  - EF-1 α, RPB 1 & 2, calmodulin, β-tubulin, *etc.*

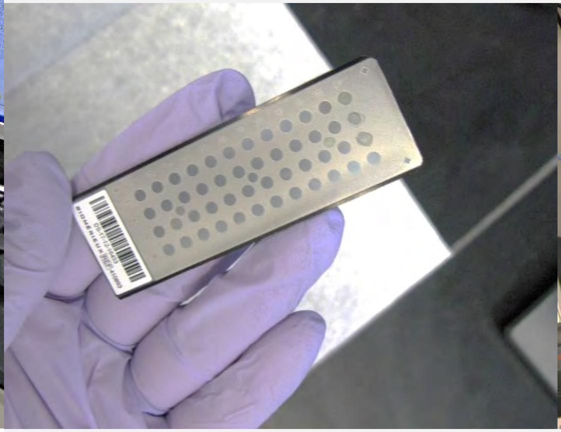
Reference DNA database

- GenBank (www.ncbi.nlm.nih.gov/genbank)
- UNITE (unite.ut.ee/index.php)

MALDI-TOF Mass Spectrometry

VITEK® MS system from bioMérieux

- Quick and easy to prepare test samples
- Immediate and reliable identification results
- Current database available mainly for medically relevant yeasts and fungi – but is expending



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Aspergillus fumigatus image courtesy of David Gregory & Debbie Marshall

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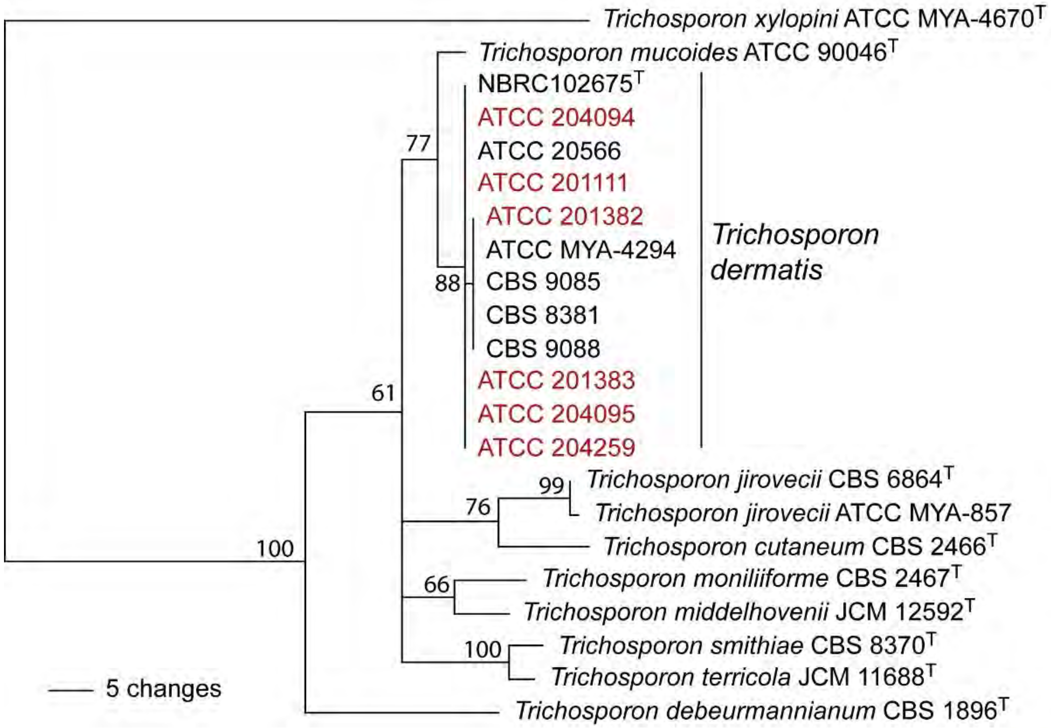
I. *Trichosporon mucoides/T. dermatis* Study

- Clinically important yeast species – causes disseminated infections to humans
- The two species are closely related – difficult to distinguish with conventional methods
- Many *T. dermatis* strains have been mistakenly identified as *T. mucoides* (red)

Onychomycosis caused by *Trichosporon mucoides*

Rizzitelli *et al.* Int J Infect Dis 42:61-63, 2016
DOI: http://dx.doi.org/10.1016/j.ijid.2015.11.011



Data from Suh *et al.* J Med Microbiol 64(10): 1135–1143, 2015
DOI: 10.1099/jmm.0.000134

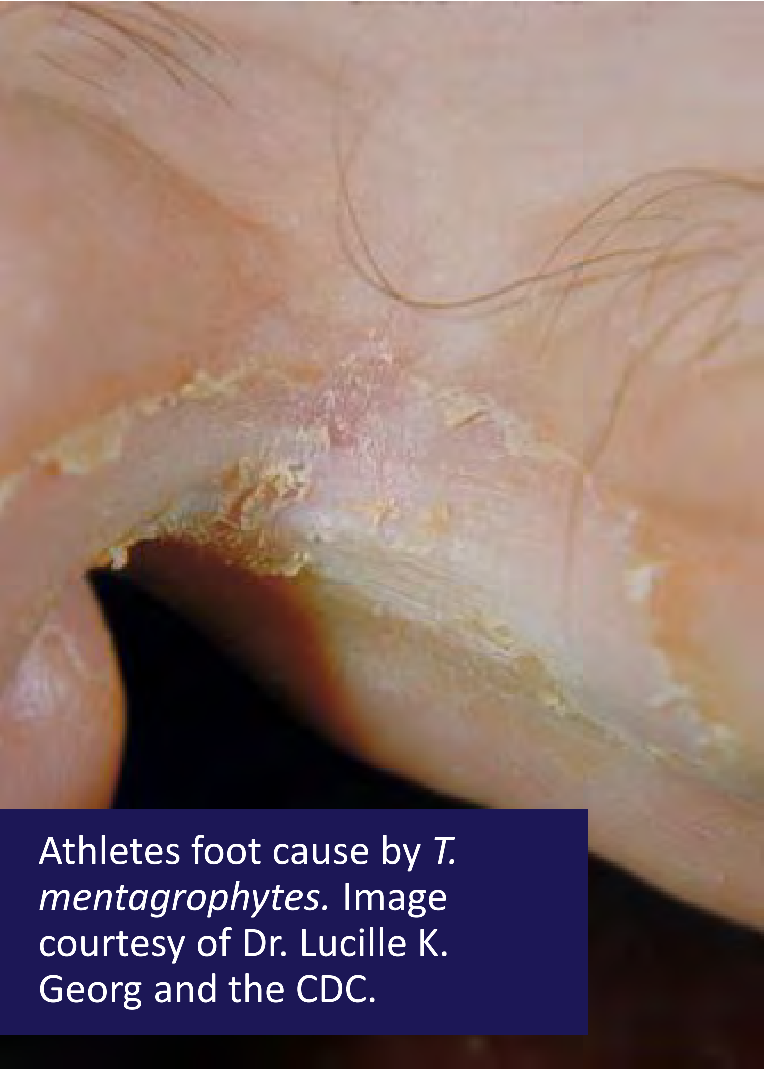
MALDI-TOF MS for *T. mucoides*/*T. dermatis*

- Conventional ID systems (VITEK® 2, VITEK® MS) could not distinguish the two species
- The MALDI-TOF MS successfully identified the two species with 99.9% confidence value when using customized reference spectra for each species

Strain No.	Species by DNA sequences	VITEK® 2 YST card (Probability)	VITEK® MS using the MYLA database (Confidence value)	VITEK® MS using customized superspectra (Confidence value)
ATCC® 90046™T	*T. mucoides*	*T. mucoides* (93%)	*T. mucoides* (99.9%)	*T. mucoides* (99.9%)
NBRC 102675™ T	*T. dermatis*	*T. mucoides* (96%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)
ATCC® 204094™	*T. dermatis*	*T. mucoides* (97%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)
ATCC® 201382™	*T. dermatis*	*T. mucoides* (99%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)
ATCC® MYA-4294™	*T. dermatis*	*T. mucoides* (97%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)
ATCC® 201383™	*T. dermatis*	*T. mucoides* (98%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)
ATCC® 204095™	*T. dermatis*	*T. mucoides* (99%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)
ATCC® 204259™	*T. dermatis*	*T. mucoides* (99%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)
ATCC® 20566™	*T. dermatis*	*T. mucoides* (97%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)
ATCC® 201111™	*T. dermatis*	*T. mucoides* (99%)	*T. mucoides* (99.9%)	*T. dermatis* (99.9%)

Data from Suh *et al.* J Med Microbiol 64: 1135–1143, 2015

II. *Trichophyton mentagrophytes* SC Study



Athletes foot cause by *T. mentagrophytes.* Image courtesy of Dr. Lucille K. Georg and the CDC.

What is the *Trichophyton mentagrophytes* species complex (SC)?

- Dermatophytes causing superficial skin infections by invading the keratinized tissue of human and animal hosts, such as hair, nails, and feathers
- Common dermatophytes worldwide, but their taxonomy has not been well established
- Quick and accurate identification of these fungi is clinically relevant for providing proper treatments to patients

Taxonomic changes in the *T. mentagrophytes* SC

Trichophyton mentagrophytes image courtesy of Dr. Lucille K. Georg and the CDC

- *Arthroderma benhamiae*, *A. simii*, *A. vanbreuseghemii* and related anamorphic *Trichophyton* spp.

  (*e.g.*, Gräser *et al*. 2008)

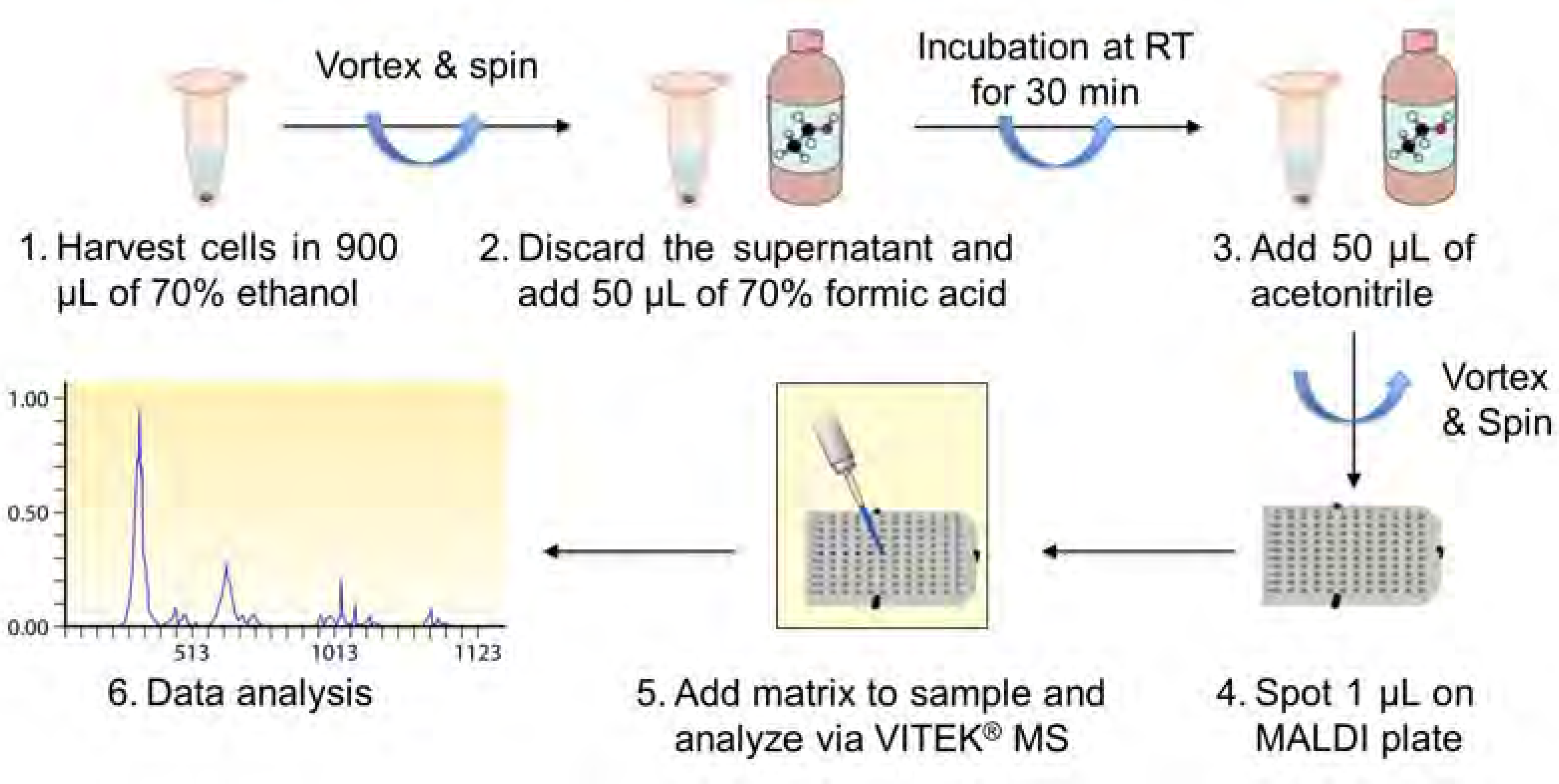
↓

- Transferred *Arthroderma* spp. To *Trichophyton*
- No longer use teleomorphic *Arthroderma* in the SC
- Designated neotypes for accurate identification

  (de Hoog *et al.* 2017)

Approach

- Multi-locus DNA sequence analysis – ITS, D1D2, β-tubulin
- MALDI-TOF MS analysis – rapid identification



1. Harvest cells in 900 µL of 70% ethanol

Vortex & spin

2. Discard the supernatant and add 50 µL of 70% formic acid

Incubation at RT for 30 min

3. Add 50 µL of acetonitrile

Vortex & Spin

4. Spot 1 µL on MALDI plate

5. Add matrix to sample and analyze via VITEK® MS

6. Data analysis

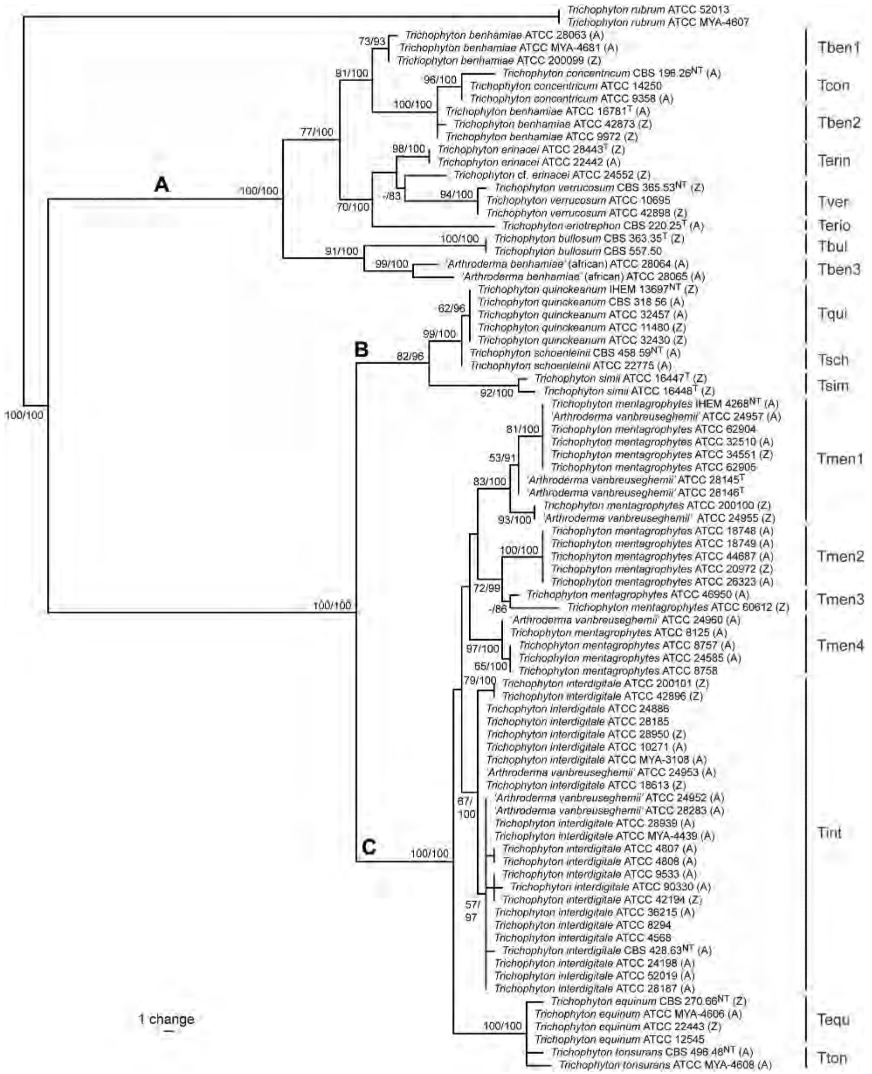
Procedure of MALDI-TOF MS sample preparation

Methods summarized from Suh *et al.* 2017 (Submitted to *Mycologia*)

T. mentagrophytes SC Study – Phylogeny

Multi-locus DNA sequence analysis

- 72 ATCC strains in the *T. mentagrophytes* SC
- 3 major lineages (A, B, C)
- 18 phylogroups
- >65% of the tested strains required taxonomic updates



Data from Suh *et al.* 2017 (Submitted to *Mycologia*)

ATCC

MALDI-TOF MS for *T. mentagrophytes* SC

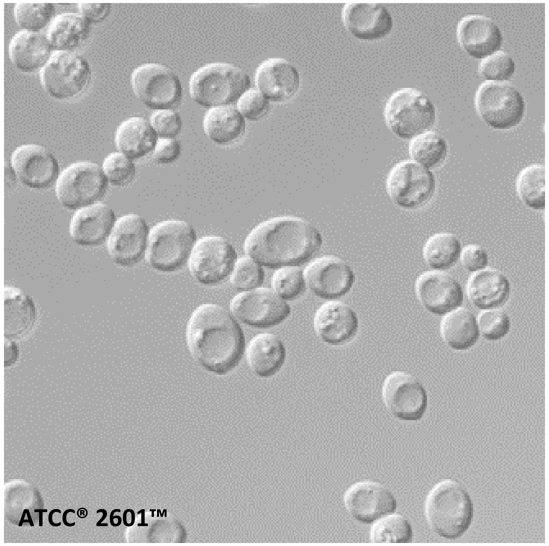
- Developed reference spectra for each phylogroup based on the new taxonomy in the species complex
- With the customized reference spectra, each species was identified correctly with high accuracy by MALDI-TOF mass spectrometry
- Two closely related species, *T. mentagrophytes* and *T. interdigitale,* were often cross-identified

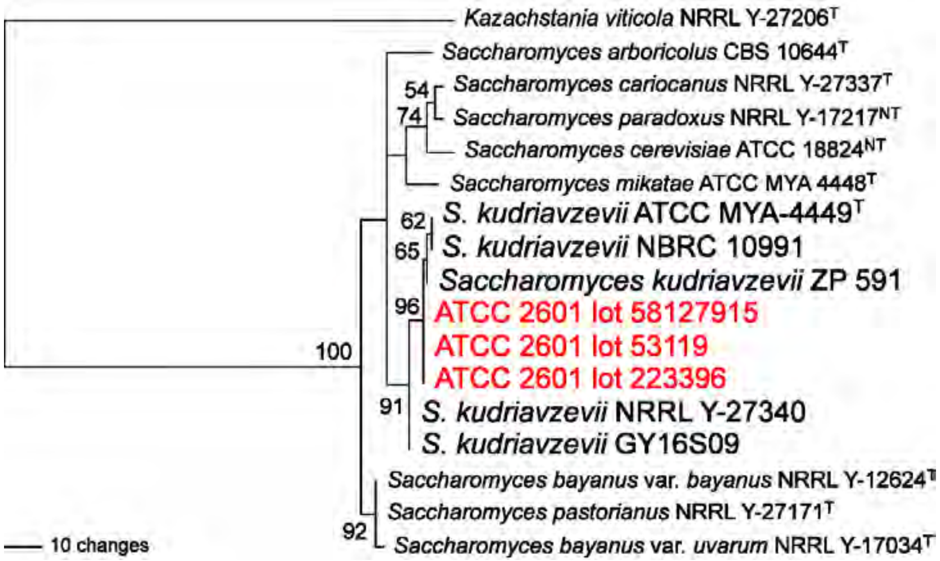
Species	Correct identification	Incorrect ID
T. benhamiae	94%	*T. concentricum*
T. concentricum	100%	
T. erinacei	100%	
‘*T. benhamiae*’ (African)	100%	
T. quinckeanum/T. schoenleinii	100%	
T. simii	100%	
T. mentagrophytes	91%	*T. interdigitale*
T. interdigitale	76%	*T. mentagrophytes* *T. equinum/T. tonsurans*
T. equinum/T. tonsurans	100%	

Data from Suh *et al.* 2017 (Submitted to *Mycologia*)

III. USP Drug-Testing Reference Strain ATCC® 2601™

- A reference strain designated by FDA and widely used in assays of amphotericin B fungizone and nystatin fungicidin according to U.S. Pharmacopeia General Chapters
- It has long been classified as *Saccharomyces cerevisiae* based on traditional taxonomic characterizations, but its barcode sequence indicated that it could be *S. kudriavzevii*





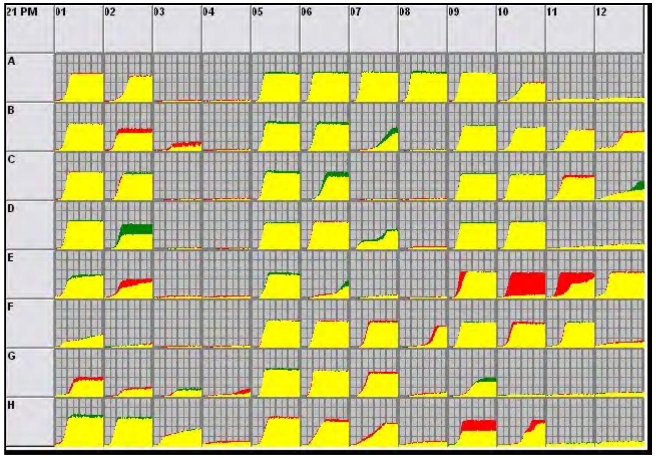
Data from Suh *et al.* PMF Newsletter19(2): 4-11, 2013

Polyphasic Approach for ATCC® 2601™

Multi-locus DNA sequence analyses

Genes	Sequence similarity	
	To *S. kudriavzevii*	To *S. cerevisiae*
ITS + D1D2 of LSU rDNA	99%	98%
Mt SSU rDNA	100%	90%
RPB1	99%	87%
RPB2	99%	87%
EF-1α	96%	98%

Chemical sensitivity assays by Biolog PM21D Microplates



Red, ATCC® 2601™ lot 58127915; Green, ATCC® 2601™ lot 223396; Yellow, overlapped

Results

- ATCC® 2601™ is *Saccharomyces kudriavzevii*, not *S. cerevisiae*
- From the investigation on drug-susceptibility of all the previous batches, the taxonomic change of this organism will not affect its application in antifungal drug testing assays

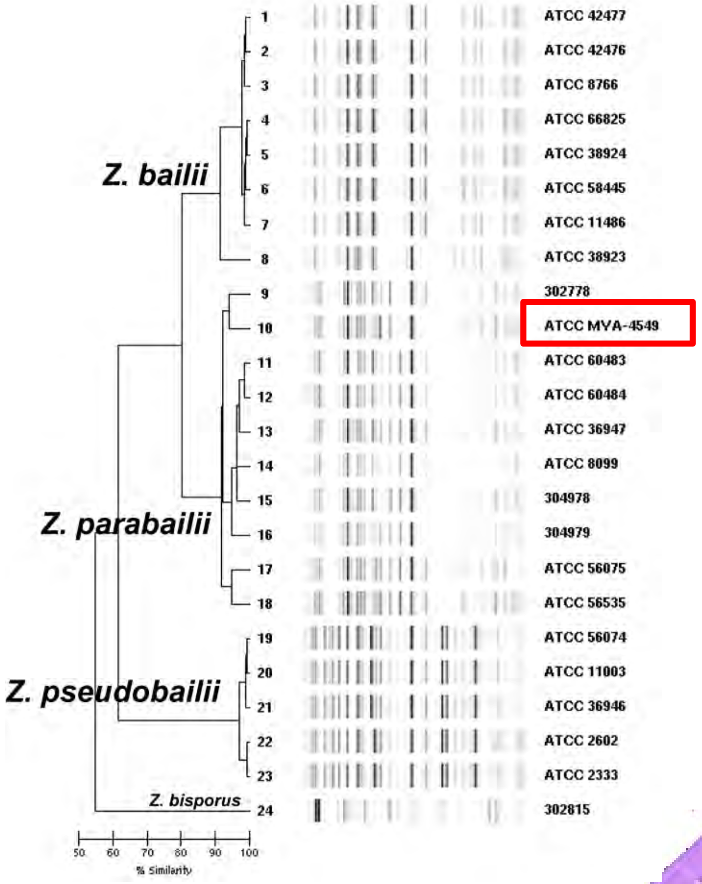
Data from Suh *et al.* PMF Newsletter19(2): 4-11, 2013

IV. Quality control strain ATCC® MYA-4549™

- Quality control strain for VITEK 2 yeast identification card for *Zygosaccharomyces bailii*
- DNA barcode sequence indicated that it is distinct from typical *Z. bailii*

Results

- Tested 23 ATCC strains that have been identified as *Z. bailii*
- Two novel species, *Z. pseudobailii* and *Z. parabailii*, were proposed based on the DNA sequence comparison and genome fingerprinting profiles
- ATCC® MYA-4549™ is *Z. parabailii*, not *Z. bailii*



Dendrogram and genome fingerprinting profiles of strains in *Z. bailii* and the two novel species generated by DiversiLab.
Data from Suh *et al.* IJSEM 63(5): 1922–1929, 2013

Future Topics

Next-generation sequencing

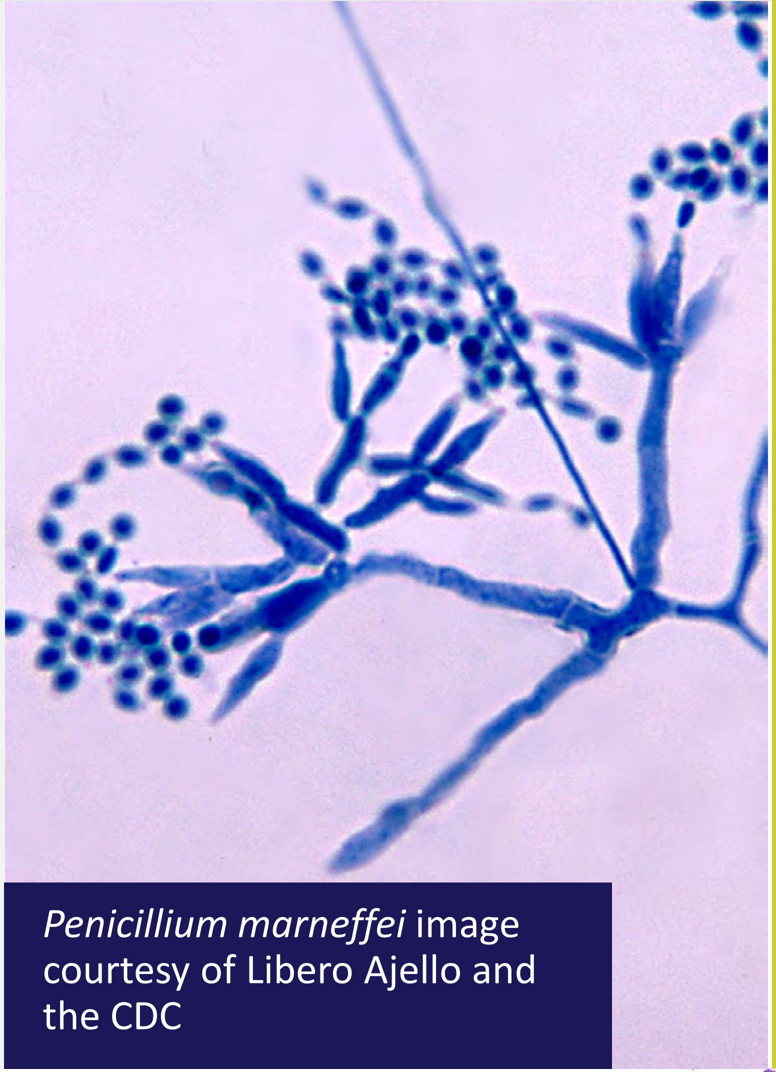
- Antifungal-resistant *Candida* spp.

MALDI-TOF MS

- *Cryptococcus gattii/C. neoformans* species complex

Summary

- Fungal systematics is currently undergoing a major revision due to the improvements in contemporary molecular techniques and the new ‘One fungus, One name’ rule for the nomenclature code
- The quality control procedures used to evaluate and characterize ATCC mycology products meet the high standard of industrial demands
- ATCC QC strains and reference materials are authenticated using a polyphasic approach
- Recent studies support MALDI-TOF mass spectrometry as a powerful rapid identification tool for yeasts and fungi



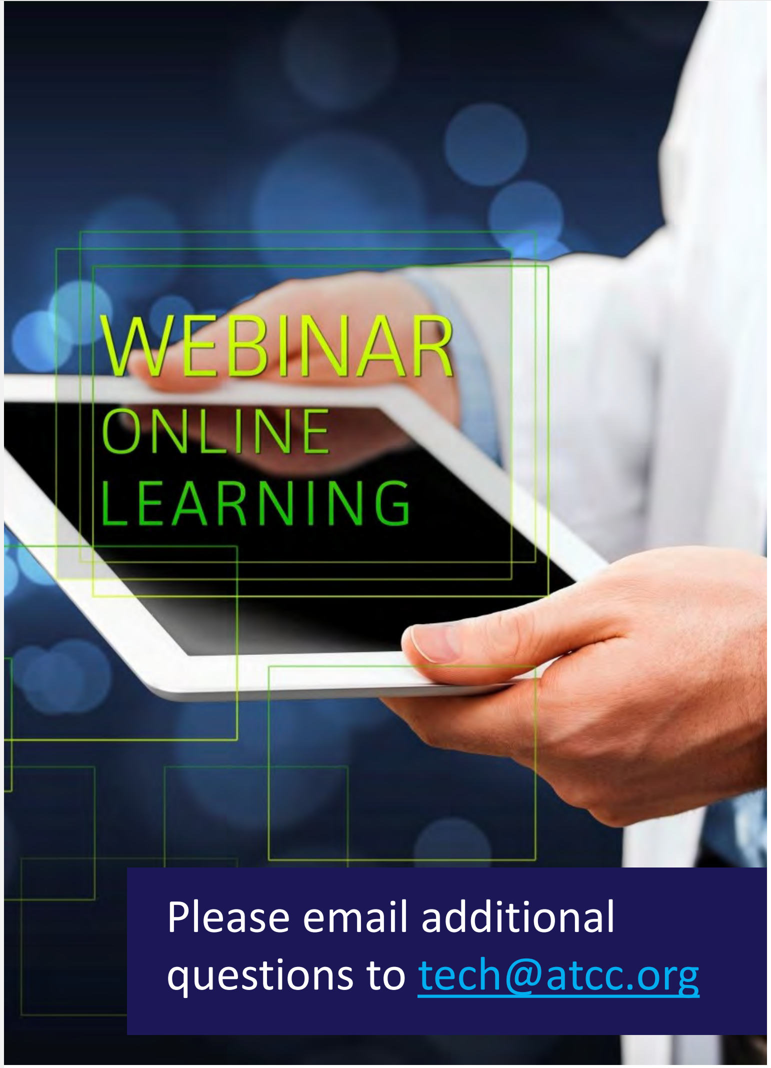
Penicillium marneffei image courtesy of Libero Ajello and the CDC

Disclaimers

Thank you for joining today!

Register for more ATCC webinars at www.atcc.org/webinars

Check out our Mycology Culture Guide at www.atcc.org/Guides



Please email additional questions to tech@atcc.org