

#### ASM Microbe 2024



Optimizing the Development and Accessibility of Biological Materials and Data Through Nextgeneration Technology

Credible Leads to Incredible™



#### Visit us at booth #912

# About ATCC®



- Founded in 1925, ATCC<sup>®</sup> is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - Media/reagents

- ATCC<sup>®</sup> collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 600+ employees, over onethird with advanced degrees



# Our offerings



#### Credible Collections

 The ATCC<sup>®</sup> collection of cell and microbial reference materials remain at the heart of incredible breakthroughs in scientific exploration.

#### Authentication Resources

 ATCC<sup>®</sup> offers a range of high-quality cell authentication testing services backed by nearly 60 years of experience in biomaterial management and authentication standards.



#### Offering Large Custom Solutions

 With an unmatched combination of extensive expertise, cutting edge technologies, best practices, and a world-renowned collection of cells and microbes, ATCC<sup>®</sup> is your ideal solutions partner.

#### Advance Cell Models

 Advanced biological models enable greater specificity and functionality to the researcher's toolkit.



#### **Quality Standards**

 ATCC<sup>®</sup> is a leader in the creation and maintenance of biological and published laboratory standards that protect public interests and provide quality reference material, education, accreditation, and certification services to the industry.

#### cGMP Manufacturing & Biorepository Services

Our longevity in the industry and reputation for quality ensures confidence for your Master and Working Mammalian Cell Banks and cGMPcompliant storage.



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# ATCC<sup>®</sup> collaborations



A long-term, multi-year strategic collaboration to jointly provide co-branded reference materials and reference standards that will serve to advance the quality and development of biologic medicines and therapies.



A strategic partnership to deliver best-in-class bioinformatics solutions for microbial genomics and microbiome analyses.



A multi-phase research collaboration to expand the use of non-model microorganisms and accelerate biomanufacturing and biotechnology R&D.



Collaboration to accelerate oncology research by generation of frozen, assay-ready tumor microtissues and tumor models.



Partnership to improve fungal identification systems and understand the impact of high passage count on a culture's genotype and phenotype.

Booth #502

ATCC

Booth #613

And dozens of other private and public partnerships!

# ATCC<sup>®</sup> & Biolog<sup>TM</sup>

Partnering to build new phenotypic identification and characterization data

F20 and

P10 P15

#### High-throughput phenotypic screening for characterizing WHO-listed fungal pathogens and monitoring phenotypic drift in bacteria

Max V. Cravener, <sup>1</sup>\* Shahin Ali,<sup>2</sup> Anthony Muhle,<sup>2</sup> Jonathan Jacobs,<sup>2</sup> and Victoria Knight-Connoni<sup>2</sup>

#### Introduction

#### Methods Continued

Cenotypic microbial analysis is often used as the standard baseline for defining new and emerging strains, however understanding the phenotypic profile of a given microbial strain is key to contextualizing the results of any study. Here, we generated baseline phenotypic profiles for eleven fungal pathogens recognized by the WH-O to be of special concern in the global health profilerans. Candida spo. (abicaris, auris, glubrats, paragalases, tropicals, and grown under nearly 2000 different conditions using Biologis Phenotype MicroArray (PM) plates that assay for carbon, ntrogen, sulfur, and phosphate utilization, pH and comotis stress resistance; and fung toleranse.

To investigate the occurrence of phenotypic drift in aboratory and production strains, we generated the same PM profiles for faster-growing bacteria: Escherichia coli, Streptococcus thermophilus, and Lactobacillus casel. We then simulated years of passages while monitoring for changes in each organism's henotypic profile. Once phenotypic drift was captured, affected generations were then sequenced to ascertain the occurrence of mutations within each strain.

Overall, this study demonstrated that high-throughput phenotypic screening can be a useful tool for both characterizing strains of interest and monitoring lab and production strains for phenotypic instability resulting from genetic changes after repeated passaging.

#### Methods

Phenotype MicroArrays (PM) from Biolog, used in conjunction with the Odin<sup>™</sup> family of instruments enables phenotypic screening with a large library of substrates and conditions against which to test your organisms.



WHO-listed fungal pathogen characterization: PM profiles were generated using standard Biolog procedures for fungi. Briefly, filamentous strains were grown for 7-10 days on 2% mait extract agar at 26°C until sufficient spore/contidal development: Veast strains were grown for 48 hours on Biolog Universal Yeast agar media at 26 °C prior to screening. Once sufficient growth was achieved, strains were transferred to Ffor YT inocultaing fluid and inoculated onto YT Microplates or PM1-0 and 21-25 and incubated in Odin L for 72 (YT) or 96 hours (FF) at 26°C and read every 20 minutes at 550 and 740mn.

Effordings K. Woodwork K., Walten N., Berkov LL, Jackon B., Chiller T., Valabheneli S. Candida auto: The recent emergence of a multiduperel Inorgianistingen effektive: 2019 and Intro(19-16). doi:10.0010/jmme/priorfdb-11.0016/jmme/priorfdb-11.0016/jmme/priorfdb-11001/jmme/priorfdb-100001200 Ordin and Efforting per tendension of Tolog, Inc. ATCC: In a registered trademick of the American Type Culture Collection Neutring and Illumina and Interinstant distantian of Thirton, Inc. Phenotypic drift monitoring: *E*. coli ATCC<sup>6</sup> [1775" (*E*.c.). S. thermophilus ATCC<sup>6</sup> 19258" (S.1), and *L*. case/ATCC<sup>8</sup> 393" (*L*.c.) were grown at 36"C on Biolog Universal Growth "5% sheep's blood, MT, and Lactobaelli MRS media (ATCC<sup>6</sup> Medium 416), respectively, with (S.t. & L.c.) or without (*E*.c.) 5% CO<sub>2</sub>. Passage 0 (PO) strains were streaked for single colonies and none colony was sub-ultured on that a fresh plate every 24 hr (*E*.c. and S.1) or 48 hr (*L*.c.) for up to 40 passages. PM profiles were generated using standard Biolog procedures with dye to measure metabolic activity (Figure 1). P0 strains were inoculated on plates PM 1-20 in triplicate, incubated, and read every 20 minutes in of ull. for 24 hrs at 36"C to establish a baselem. P15 *L*.c. and P20 *E*.c/S1 were then tested on PMI-20 and compared against respective P0 strains to identify altered phenotypes. A subset of PM plates with messureshe phenotypic drift were then replicated to ensure reproducibility. *E*.c. and S1. P40 strains were again tested on the respective PM subset in titriplicate to monitor for additional drift and/or stability of P20 phenotypes. Area Under the Curve (AUC) values were compared across triplicates using Sidal's multiple comparison test.

Phenotypic drift sequencing: We used the Illumina<sup>2</sup> DNA Prep Kit to prepare genomic DNA from P20 Ec. and P40 Ec, then performed whole-genome sequencing on Illumina<sup>4</sup> NextSeq<sup>4</sup>. Reads (150 bu) underwent Illutation with fasty 0.232, and sequence quality was assessed using FastCC v0.119. Reference-based assembles were conducted using the ATCC<sup>4</sup> TTS<sup>77</sup> reference sequence (P0 Ec.). Subsequently, reads from P20 Ec. and P40 Ec. (19 and 20 M, respectively) were mapped to the D6 E. reference sequence using how version 0.77.1418. Variants were called using loffeq, and a consensus sequence was generated using bothools 11.7 Variants with frequencies-95% were identified as the variant uncleotide.

Results: WHO-Listed Fungal Pathogens



(Figure 2).

Figure 2. Differentiation of major Candidia auria clades. Growth and metabolism senemonitored for 72 hours. Four C. auri strains from Clades A, B, C. and D. (MYA-5000, 5001, 5002, and 5003 respectively) for 72 hours on a YT Micropiate. Color Indicates Area Under the Curve for each substrate representing todal growth/metabolic output.

Candida auris is a well-known multi-drug resistant fungi which is cause for concern among medical researchers and clinicians globally<sup>1</sup>. We confirmed that C. auris was resistant to over 67% of the fungal inhibitor compounds in our PM library and susceptible tricolasm and thaliaun(1) actetate, and high concentrations of militefosine and lithium chloride (not shown). We profiled four C. auris strains representative of the 4 major clades using YT Microplates which test carbon source utilization, and our platform is able to differentiate the 4 clades using their unique metabolic and rowth characteristics ATCC

We simulated years of passaging without selective pressure in *E.c.*, *S.t.*, and *L.c.* and screened passage 0, 15/20, and 40 on PMI-20 plates. *E.c.* showed the most significant phenotypic drift with a total of 78 altered

biolog

Results: Phenotypic Drift

profiles followed by S.L. with 26, and L.c. with 20 (Table 1), Sensitivity phenotypes included both increased and decreased resistance for all three species (Figure 3). E.c. gained resistance to osfimetazede and lats resistance to Alchrettrazycline, S.L. lost, resistance to sofulum andre and increased its responsiveness to INT, and L.c. gained resistance to D.L-thoctic add and lost resistance to chromabuck (Figure 3).

*E.c.* showed significantly (p<0.0001) reduced ability to metabolize several amino acids (Table2) indicating a loss of fitness in nitrogen metabolism relative to P0.

 Using whole genome sequencing, we identified a total of 10 genetic variants in P20 E.c., comprising 7 SNPs and 3 deletions, when the reads were aligned to the P0 E.c. reference sequence.

 P40 E.c. exhibited 4 additional SNPs and 1 insertion. Among these variants there were four affected coding sequences: Actin cross-linking toxin VgrG1 Galactoside O-acetyltransferase, and two hypothetical proteins.

#### Conclusions

 Large-scale phenotypic profiles for emerging and important pathogens using our platform can allow quick identification beyond the species level.

Using Odin to monitor the phenotypic stability of production and research strains over time can complement genome sequencing to identify mutations.

It is necessary to monitor for phenotypic drift in strains after as few as 15 passages, as seen with *L. casei. E. coli* and *S. thermophilus* showing significant drift in metabolic and sensitivity phenotypes which worsened over time.

After 20 passages, we detected 10 genetic variations in E. coli and 27 phenotypic changes. This increased to an additional 55 phenotypic changes and 14 genetic variations affecting 4 genes by passage 40.

For bioproduction and experiments, use authenticated strains and minimize passaging to ensure reliable and reproducible results.

# **Session title:** Metabolism Enzyme Mechanics and Physiology

#### **Poster number:** MBP-FRIDAY-618

#### Time: Friday, June 14, 10 AM – 5 PM

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# Innovations at ATCC®

Much more than a culture collection!

ATCC<sup>®</sup> is more than culture management. New formats save time and money for our customers!



 Precisely quantitative DNA from bioproduction cell lines to control measurements of residual DNA. *Developed in partnership with USP®*!



High-titer, high-purity viruses



- Highly sensitive *Mycoplasma* testing kit
- CRM DNA controls for *Mycoplasma* testing

#### Other efforts to serve:

- Oncology
- Toxicology
- Bioprocessing
- Infectious Disease Diagnostics
- Research



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Improved and optimized quantitative formats



# ATCC<sup>®</sup> Genome Portal

A cloud-based platform that enables users to easily browse authenticated and traceable reference genomes and metadata.



Download whole-genome sequences and annotations from your browser or via our secure API.



Search for nucleotide sequences or genes within genomes.



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View genome assembly metadata and quality metrics.

### genomes.atcc.org

#### 4,500 Authenticated Microbial Reference Genomes

3,828 bacteria and archaea 362 viruses 306 fungi 4 protists New

New genomes released every quarter!

REST-API for bioinformatics applications available

# Today's speakers



#### Accessing the genetic potential of the biosphere Elise Ledieu-Dherbécourt, PhD Program Manager, Cultivarium



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# Uses of quantified microorganisms – challenges and opportunities

Nilay Chakraborty, PhD, MBA BioNexus Foundation Principal Scientist, ATCC



ASM Microbe 2024



# Accessing the genetic potential of the biosphere.

Elise Ledieu-Dherbécourt, PhD

PROGRAM MANAGER, CULTIVARIUM

# Develop open-source tools for life scientists to

**Expand** access to novel microorganisms

**Inspire** new research avenues

Push the frontiers of biotechnology

# We need the right microbe, with the right tools, for each job.

Microbes that scientist can actually engineer

> Today's challenges Degradation, Production

#### 1M+ species

All microbes in the environment

We need the right microbe, with the right tools, for each job.



# Scientific recipes for life scientists



# Halomonas elongata ATCC 33173

- Halophile
- Ectoine production
- Enzyme production
- Wide range of applications



#### Cultivarium Data

DOMAIN BACTERIA TAXONOMY ID 2746 of Halomonas elongata					
CULTURE					

#### Growth media

	MEDIA	TEMP. (*C)	MAX OD 4	DOUBLING TIME (HR)	TIME TO OD 0.2 (HR)	AUC	
٠	HCM.8	30	132 ± 0.02	2.57±0.07	11.05 ± 0.10	$0.0 \pm 0.01$	
٠	HBML8	30	1.30 ± 0.20	188 ± 0.42	9.50 ± 0.17	0.20 ± 0.07	
٠	HCM	30	1.22 ± 0.03	3.96 ± 0.17	19.00 ± 0.17	0.07 ± 0.00	
•	HCM 15	90	138 ± 0.06	2.93 ± 0.07	27.44 ± 125	000±000	
۰	HBM_15	30	133 ± 035	2.68 ± 0.39	2167 ± 2.17	0.07 ± 0.03	
•	MB	30	1.09 ± 0.18	237 ± 0.37	10.33 ± 0.00	0.15 ± 0.08	
٠	LB	30	ANTIBIOTICS				
•	HBM	90	The antibiotic screen was performed in the indicated liquid medium at 3PC.				
	HMLeHB	30	View is Profile -				



Antibiotics

#### Cultivarium Data

DOMAIN BACTERIA TAXONOMY ID 2746 (* Halomonas elon		
CULTURE	SEQUENCING	

Growth
media

		MEDIA	TEMP. (*C)	MAX OD 4	DOUBLING TIME (HR)	TIME TO OD 0.2 (HR)	AUC	
1		HCM.B	30	1.32 ± 0.02	$2.57 \pm 0.07$	11.05 ± 0.10	0.0 ± 0.01	
	٠	HBML8	30	1.30 ± 0.20	188 ± 0.42	9.50 ± 0.17	0.20 ± 0.07	
	•	HCM	30	1.22 ± 0.03	3.96 ± 0.17	19.00 ± 0.17	0.07 ± 0.00	
		HCM 15	30	118 ± 0.06	293±0.07	27.44 ± 1.25	0.02 ± 0.00	
		HBM.15	90	1J3 ± 0J5	2.68 ± 0.39	2167 ± 2.17	0.07 ± 0.03	
		MB	30	1.09 ± 0.18	2.37 ± 0.37	10.33 ± 0.00	015 ± 0.08	
		LB	90	ANTIBIOTICS				
	٠	HBM	90	The entitions screen was performed in the indicated liquid medium at 37°C.				
		HMI_RH8	30	View 15 Profile -				



Antibiotics



#### Cultivarium Data

DOMAIN BACTERIA TAXONOMY ID 2746 of Halomonas elon			
CULTURE	SEQUENCING	MOLECULAR	

Origin of replication

FUNCTIONAL

CONFIDENCE +

Growth
media

		MEDIA	TEMP. (*C)	MAX OD 4	DOUBLING TIME (HR)	TIME TO OD 0.2 (HR)	AUC	
ſ		HCM.8	30	132 ± 002	2.57±007	11.05 ± 0.10	0.0±0.01	
		HBML8	30	1.30 ± 0.20	1.88 ± 0.42	9.50 ± 0.17	0.20 ± 0.07	
	•	HCM	30	1.22 ± 0.03	3.96 ± 0.17	19.00 ± 0.17	0.07 ± 0.00	
		HCM 15	30	138 ± 0.06	293±0.07	27.44 ± 1.25	0.02 ± 0.00	
		HBM.15	30	1J3 a 0J5	2.68 ± 0.39	2167 ± 2.17	0.07 ± 0.03	
		MB	30	1.09 ± 0.18	2.37 ± 0.37	10.33 ± 0.00	015 ± 0.06	
		LB	30	ANTIBIOTICS				
	٠	HBM	30	The antibiotic screen was performed in the indicated liquid medium at 37°C.				
		HML_RHB	30	View & Profile -				



ORI RSF1010 Yes GenBank & Addgene C Gentamicin (20 µg/mL) GenBank 🛓 High **pBBRI** Yes GenBank 💩 Addgene 🗹 Spectinomycin (200 µg/mL) Genflank 🔬 High R5F1010 Yes High GenBank 🕁 Addgene 🗹 Chloramphenicol (8.5 µg/mL) GenBank 🛓 RSF1010 Yes High GenBenk 🚠 Addgene 🖒 Spectinomycin (200 µg/mL) GenBank & RSF1010 Yes High GenBank & Addgene 10 Tetracycline (2.5 µg/mL) GenBank 🛓 RSF1010 Yes High GenBank 🛓 Addgene 🗹 Kanamycin (200 µg/mL) GenBank 🕁 Kanamycin (200 µg/mL) GenBank 🛓 pBBRI Yes High GenBank 🛓 Addgene 🖻 GenBank & Anthenne M Snartinnmonin (2000 states) pBBR1-UP Yes Medium GenBank GENOME GenBack - Aj Yes Medium DBBR Sequenced using Illumina 4,017,490 bp GENOME SIZE Draft 23 CONTIGS genome CHECKM COMPLETENESS 99.86%

ORI PART PLASMID

ANTIBIOTIC

TRANSFORMATION PLASMID

Antibiotics

# Scientific recipes: H. elongata





# Scientific recipes for life scientists





# Scientific recipes for life scientists

**Molecular tools** 

## **POSSUM** Toolkit

Identify functional plasmids

#### **Computational tools**

### MicrobeMod

Methylation calling

### MACKEREL Toolkit

Identify functional promoters

### GenomeSPOT

Predict growth conditions





# How we can support your work

1. Nominate your organism

- 2. Collaborate on your organism
- 3. Investigate diversity

#### cultivarium.org



# 170,000+ species. One portal.



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Unlock access at **Booth 613**!

-

#### Cultivarium Team



# How we can support your work

1. Nominate your organism

- 2. Collaborate on your organism
- 3. Investigate diversity

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## Uses of Quantified Microorganisms: Challenges and Opportunities

Nilay Chakraborty, PhD, MBA BioNexus Foundation Principal Scientist, Cryobiology, ATCC

#### Credible Leads to Incredible™



# ATCC as a source of reference microorganisms

- Food and Drug Administration
- United States Pharmacopeia
- European Pharmacopeia
- World Health Organization for Standardization
- Clinical and Laboratory Standards Institute
- Pharmaceutical Inspection Co-operation Scheme
- And more

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Many of the microorganisms mentioned in guidelines are from- or can be obtained from ATCC



# Quality testing applications of quantified microbial reference strains across industries





### Challenges with reference microorganisms

**Process complexity** 



#### Strain identification

Limited resources and time



Not meeting required specifications





# Process complexity

- Rigid and regimented processing requirements regarding strain growth, quantification, and purification
- 2. Complex expansion criteria are often required to support conditions to be tested

#### Challenges



High-quality, easy-to-use reference materials that are easy to store and reduce processing complexity.

Solution



# Limited resources and time



#### Solution

- 1. Time-consuming and costly process
- 2. Skilled laboratory personnel required

### Challenges

Easy-to-use reference material that minimizes assay setup time, simplifies the workflow, and reduces dependency on operator skills.



# Strain identification

- 1. Complex strain identification
- 2. Genetic drift with advanced passages
- 3. Risk of contamination

#### Challenges

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Access to reference materials that are traceable and authenticated using a multifaceted approach that combines genotypic, phenotypic, and functional analyses.



# Not meeting required specifications





#### How ATCC is meeting these challenges



## Leverage meticulously authenticated materials

ATCC utilizes both classical and modern techniques

- Phenotypic analysis Colony morphology, cell attributes, biochemical analyses
- Genotypic analysis Sequencing conserved regions of the genome, whole-genome sequencing
- Proteotypic analysis MALTDI-TOF MS
- Functional analysis Serotype, drug resistance, virulence

No single method of identification is sufficient





## Gain insights with standardized bioinformatics data





# Generate results with tried- and true guidance



#### Supporting scientists with:

- Culture guides
- Product sheets
- Recorded trainings
- How-to guides
- And more



ATCC



# Gain research efficiencies leveraging innovation

#### Stability of *E. coli* in two different formulations



Proteomic characterization of *E. coli* lyophilized and stored in different buffer



- Our team is identifying new cryopreservation techniques, technologies, and formulations that reduce genetic drift, improve batch-tobatch quantification consistency, and maintain viability.
- We are investing our time and resources into developing the products you need to start your assays faster, reduce your costs, and shorten your time to market.



ATCC





## Connect with us







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#### Elise Ledieu-Dherbécourt, PhD

Program Manager Cultivarium

Booth #613

Nilay Chakraborty, PhD, MBA BioNexus Foundation Principal Scientist ATCC <u>nchakraborty@atcc.org</u> Booth #912





# Booth #912

Join our webinar with Biolog on September 19<sup>th</sup>





# Booth #613

Join our webinar with Cultivarium on October 3<sup>rd</sup>



# biolog Booth #502

Questions?



# Thank You

# CREDIBLE MODELS

INCREDIBLE OUTCOMES

