Biolog for Functional Cellular Insights

ATCC®
Credible leads to Incredible

biolog

High-Throughput Phenotypic Screening to Characterize Fungal Pathogens and Monitor Phenotypic Drift in Bacterial Production Strains

Shahin Ali, PhD, Sr. Scientist, ATCC
Max Cravener, PhD, Field Applications Scientist, Biolog

Shahin Ali, PhD

Senior Scientist, ATCC Collections

Shahin is a Senior Scientist at ATCC with over 15 years of experience in the field of fungal biology and plant-pathogen interactions. Before joining ATCC, Shahin worked for the USDA-ARS at Beltsville Agricultural Research Center, Maryland. He obtained his PhD from University College Dublin, Ireland in 2013.

Max Cravener, PhD

Field Applications Scientist: Biolog

Max received his PhD in 2022 from University of Georgia in Microbiology with a focus on fungal genetics where he worked with Candida albicans studying natural variation among clinical isolates and how gene expression and regulatory differences can impact their ability to form biofilms on implanted medical devices.

ATCC – Life science innovations that touch people

- Founded in 1925 — we have been supplying scientists with essential scientific resources, services, and standards for nearly a century
- ATCC is ISO 9001 and ISO 13485 certified and ISO/IEC 17025 and ISO 17034 accredited
- Leading global supplier of authenticated cell models and viral and microbial standards
- An innovative R&D company that provides better models
  - Gene editing, microbiome, NGS, primary cells, and advanced cell models
- Services provider
  - Customer base in diagnostics, drug discovery, and applied markets; cGMP and Biorepository Services
- Patent repository consists of >90% of all USA bio-patents

bsi. ISO 9001 Quality Management

bsi. ISO 13485 Medical Devices Quality Management

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

ATCC

The value of a diverse collection

Case Study: Zika Virus

① HISTORY

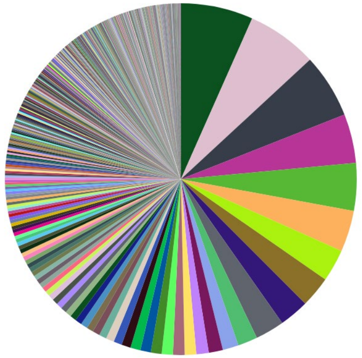
Discovered in 1947

- Human infection in 1954
- Major outbreak in 2013
- 1.3 million affected in Brazil

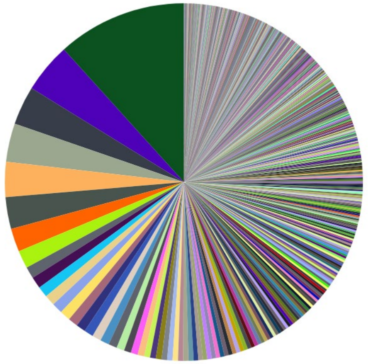
ATCC® VR-1838™ — Isolated from the blood of a rhesus monkey that became infected while stationed as a sentinel in forest near Entebbe, Uganda. Deposited in 1947.

ATCC®'s comprehensive collection of microbes

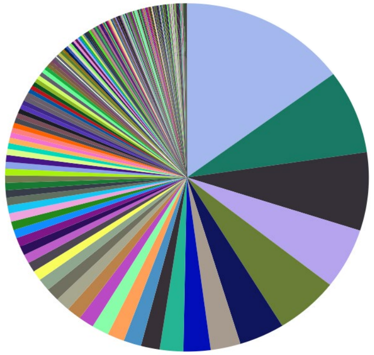
- Comprehensive microbial collection with enhanced authentication
  - 70,000+ bacteria, fungi, viruses, and protozoa
  - Over 1,300 microbial type strains
- Brand recognition
  - Organizations and regulatory agencies specify ATCC® cultures in their standards and guidelines
  - USP, ISO, FDA, CLSI, USDA, ASTM, AOAC, WHO
  - Over 475 reference strains recommended for use in quality control
- ATCC® has live microbes and derivatives, including inactivated materials and nucleic acids
- ATCC® uses a variety of advanced techniques to characterize and authenticate biomaterials—no single method of identification is sufficient



Bacteriology
1226 genera



Mycology
1864 genera

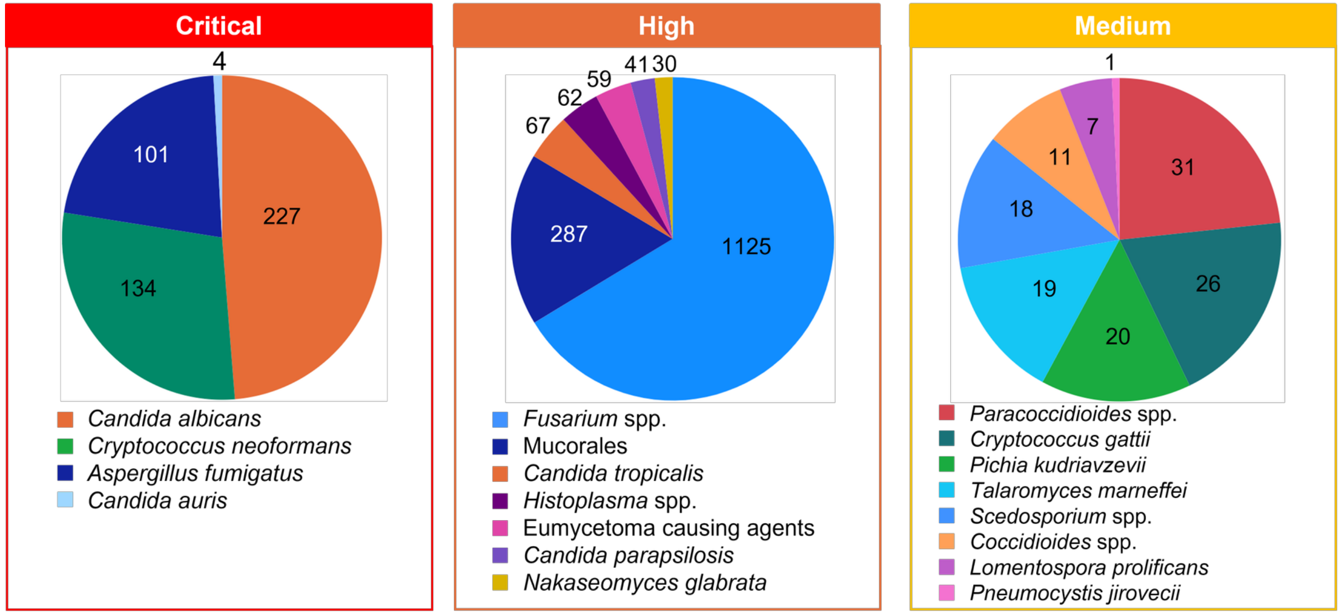


Virology
200 genera

The economic burden of fungi: Human pathogens

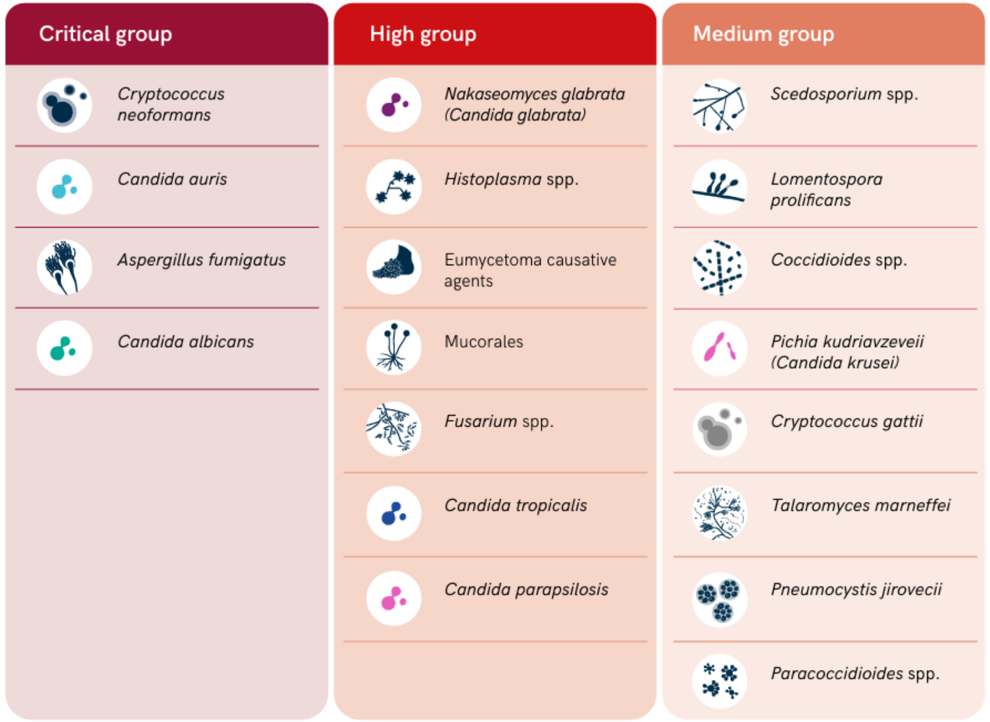
- The economic burden of fungal diseases in the US alone is around $11.5 billion.
- WHO released fungal priority pathogens list to guide research, development and public health action.

ATCC® Items on the WHO Priority Fungal Pathogens List



**Pneumocystis jirovecii* is provided as synthetic DNA (unculturable)

https://www.atcc.org/blogs/2022/who-releases-priority-fungal-pathogens-list



Critical group	High group	Medium group
Cryptococcus neoformans	*Nakaseomyces glabrata (Candida glabrata)*	*Scedosporium* spp.
Candida auris	*Histoplasma* spp.	*Lomentospora prolificans*
Aspergillus fumigatus	Eumycetoma causative agents	*Coccidioides* spp.
Candida albicans	Mucorales	*Pichia kudriavzevii (Candida krusei)*
	Fusarium spp.	*Cryptococcus gattii*
	Candida tropicalis	*Talaromyces marneffei*
	Candida parapsilosis	*Pneumocystis jirovecii*
		Paracoccidioides spp.

https://www.who.int/publications/i/item/9789240060241

GLOBAL HEALTH

WHO Releases Priority Fungal Pathogens List

A first-of-its-kind report to drive research on fungal pathogens

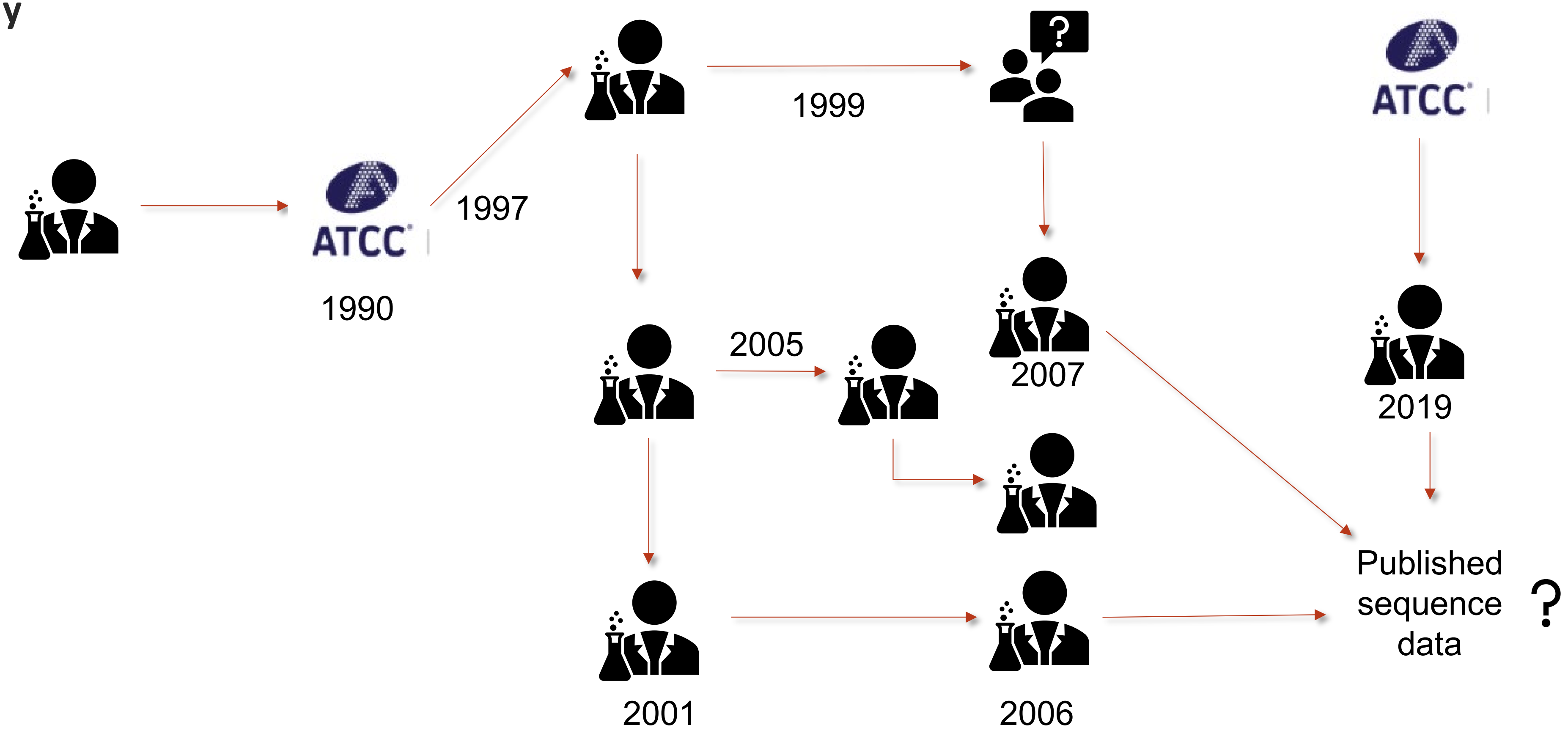
November 16, 2022 | Shahin Ali, PhD, Rebecca Bradford, MBA, MS, PMP, and Victoria Knight-Connoni, PhD

In October 2022, the World Health Organization (WHO) released a list of fungal priority pathogens to drive research on species with public health importance. This list identifies 19 fungi representing serious health threats due to their ability to cause severe invasive disease and their emerging resistance to antifungal drugs.

Traceability and reproducibility crisis

Laboratory passages can lead to various genetic and phenotypic changes.

Case study



Traceability and reproducibility crisis

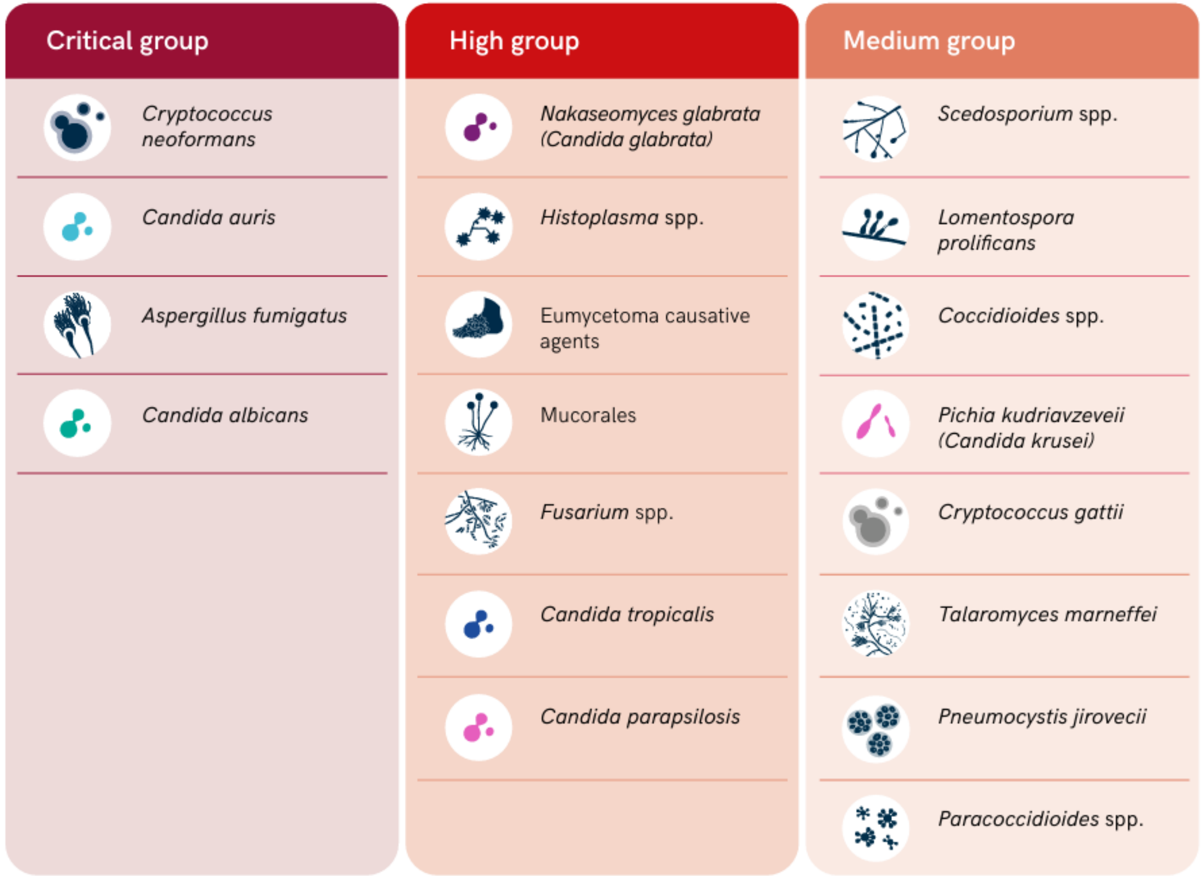
Evaluation of genome sequences from public databases

Product	NCBI existing reference genomes	NCBI assembly level (plasmids)	Sequencing technology and coverage	# of SNPs	# of indels	Average coverage (variants)
Acinetobacter baumannii (ATCC® 17978™)	GCA_001593425.2	Complete Genome	Illumina (300.0x)	14	5	210.1
	GCA_000015425.1*	Complete Genome (2)	Not available	118	656	152.7
	GCA_014672775.1	Complete Genome (1)	PacBio (399.24x)	15	87	170.4
	GCA_013372085.1	Complete Genome (2)	Illumina, Nanopore (80x)	14	2	210.2
	GCA_004797155.2	Complete Genome (2)	PacBio (247.19x)	28	62	162.1
	GCA_001077675.1	Complete Genome (1)	Illumina, PacBio (153x)	15	6	135.9
	GCA_011067065.1	Complete Genome (2)	PacBio (231.08x)	60227	2486	165.6
Candida albicans (ATCC® 10231™)	GCA_015227795.1	3, 081 Contigs	NovaSeq (16x)	10174	1573	265.6
	GCA_002276455.1	2,219 Scaffolds	HiSeq (95x)	13408	2390	274.6
Meyerozyma guilliermondii (ATCC® 6260™)	GCF_000149425.1	9 RefSeq Scaffolds	Not available	505	1973	278.2
	GCA_006942155.1	9 Contigs	ONT+MiSeq (240x)	74	386	223.3
Clavispora lusitaniae (ATCC® 42720™)	GCF_000003835.1	9 RefSeq Scaffolds	Not available	587	2336	265.6
	GCA_003675505.1	109 Scaffolds	NextSeq (182x)	102	5142	236.9

Phenotypic Identification and Characterization

WHO fungal priority pathogens list

- Serves to inform clinicians, researchers, and governments on fungal pathogen
  - Priority
  - Public health importance
  - Unmet research and development needs



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	Candida tropicalis	*Talaromyces marneffei*
	Candida parapsilosis	*Pneumocystis jirovecii*
		Paracoccidioides spp.

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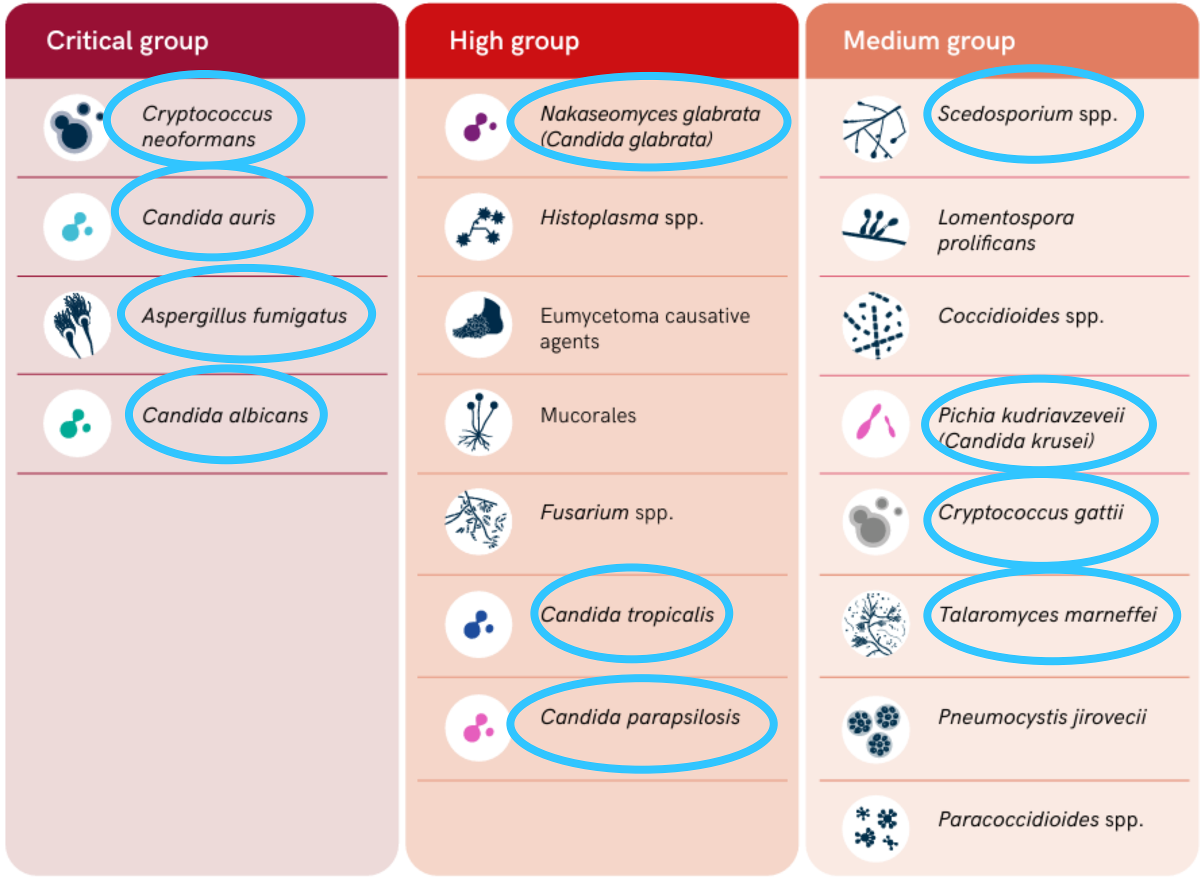
WHO fungal priority pathogens list

- Chose 11 species including
  - 4 Critical Priority Group
    - *Cryptococcus neoformans, Candida auris, Candida albicans, Aspergillus fumigatus*
  - 3 High Priority Group
    - *Candida glabrata, Candida tropicalis, Candida parapsilosis*
  - 4 Medium Priority Group
    - *Scedosporium prolificans, Talaromyces marneffei, Candida krusei, Cryptococcus gattii*

- Two isolates of each used to generate phenotypic ID profiles



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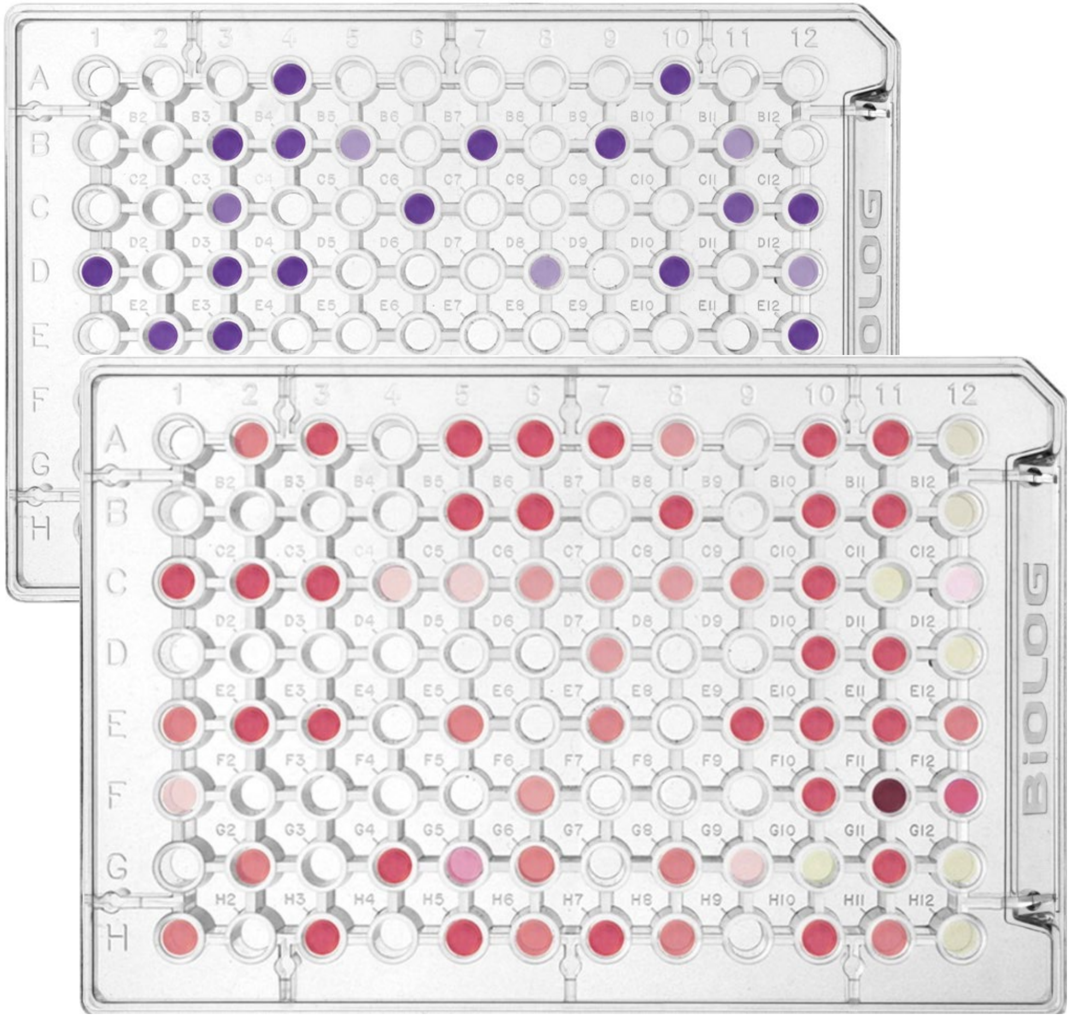
The FF and YT Microplate

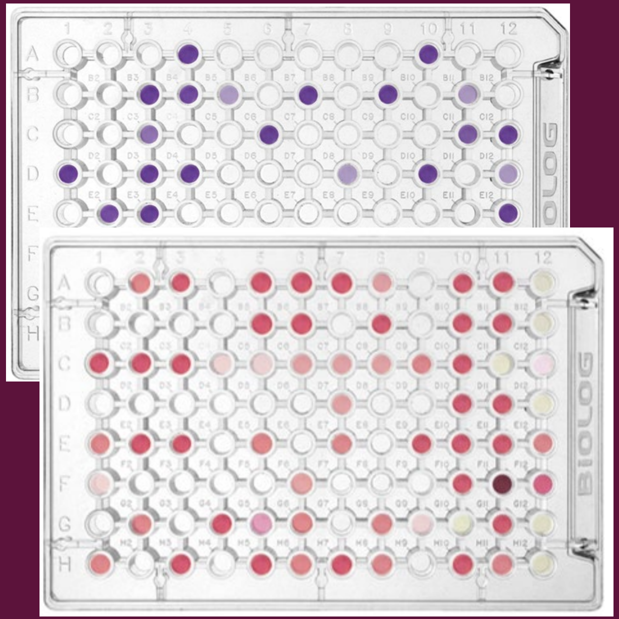


- Designed for Sporulating Fungi (FF) and Yeast (YT)
- Cells are collected directly from agar solid media
- Spores/mycelia/yeast suspended in Biolog inoculation fluid and inoculated onto FF or YT Microplates
  - No DNA extraction needed
- Incubation and kinetic reading every 20 minutes for up to 96 hours
  - Many ID in 24 hours

Biolog’s systems for microbial identification

Biolog YT
MicroPlate

Biolog FF
MicroPlate



- Biolog uses a unique single dye, single color chemistry
- This chemistry can be used to measure metabolism of any nutrient substrate
- Analysis of both color development (OD_{490} or OD_{590}) and turbidity (OD_{740}) provides for accurate identifications to the species level in one to three days
- The Odin™ database contains the unique metabolic patterns for over 2900 unique taxa comprising more than 1500 aerobes, 360 anaerobes, 260 yeast, and 700 filamentous fungi

Adding the pathogens to the Biolog Phenotypic ID Database

11 Fungal pathogens

- 2-4 unique strains per species

Run on 5 YT or FF Microplates

- To capture day-to-day variation

Compile ID profiles and test

- Ensures robust, reliable profiles

New ID profiles available on Odin™

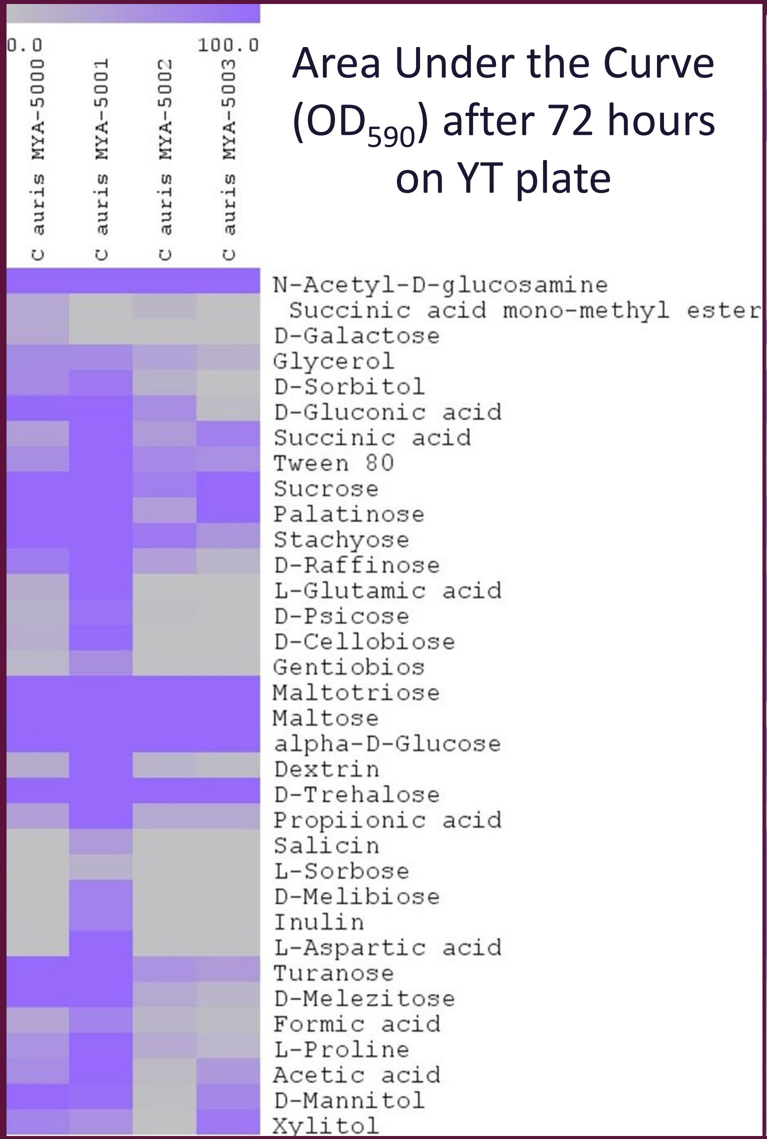
- Species and some strain-level ID

Differentiating *Candida auris* strains

Odin can differentiate the 4 major clades of *C. auris*

- *Candida auris* is a naturally multidrug-resistant pathogen that has garnered significant attention.
- It is notoriously difficult to ID to the strain level.
- We can differentiate the 4 major clades with this phenotypic method!

Metabolism



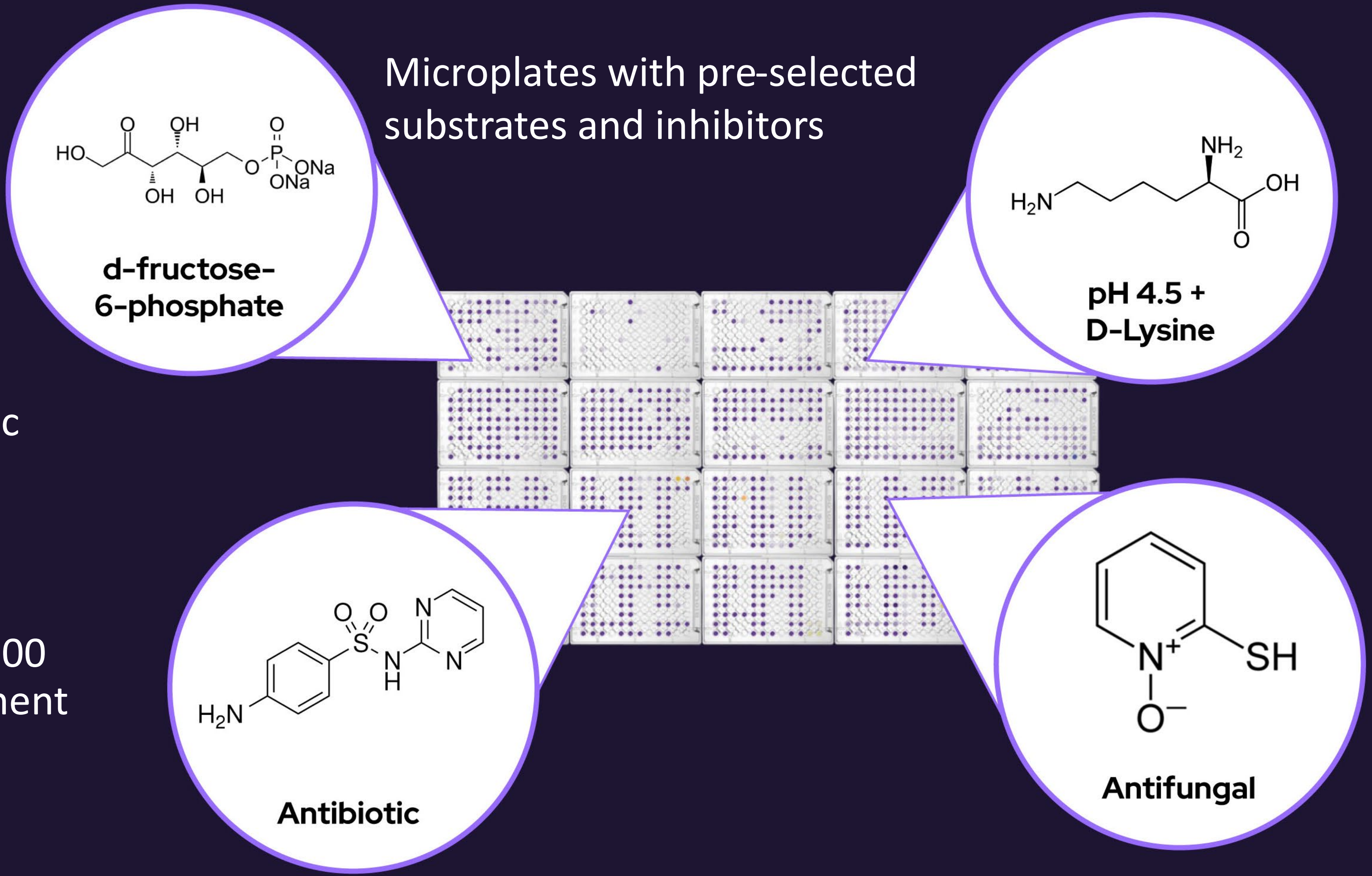
Area Under the Curve (OD_{590}) after 72 hours on YT plate

Growth

PM analyses of WHO pathogens

Comprehensive panels for phenotypic characterization

Microplates with pre-selected substrates and inhibitors



Each well probes a specific biochemical pathway or sensitivity

Odin™ monitors up to 4,800 conditions in one experiment

Metabolism of C-sources produces an electron flow

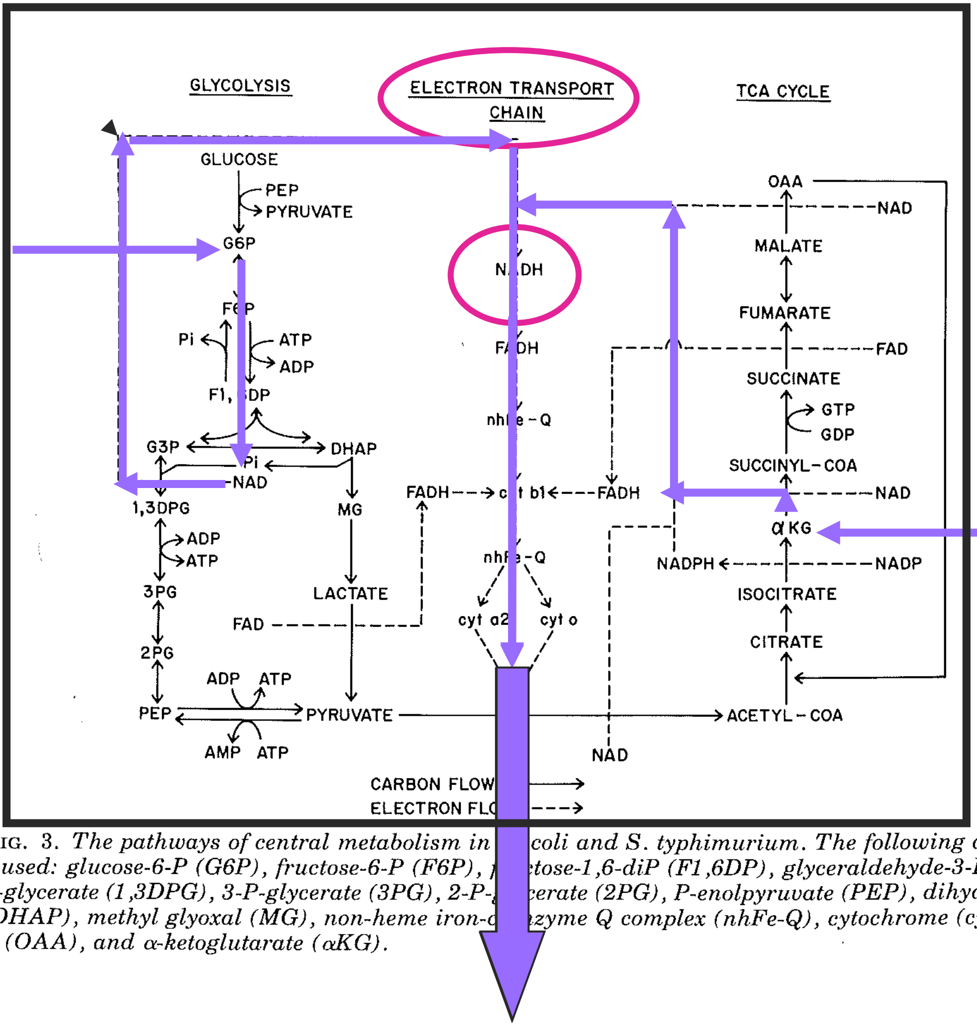


FIG. 3. *The pathways of central metabolism in coli and S. typhimurium. The following abbreviations are used: glucose-6-P (G6P), fructose-6-P (F6P), ctose-1,6-diP (F1,6DP), glyceraldehyde-3-P (G3P), 1,3-diP-glycerate (1,3DPG), 3-P-glycerate (3PG), 2-P- cerate (2PG), P-enolpyruvate (PEP), dihydroxyacetone-P (DHAP), methyl glyoxal (MG), non-heme iron-c zyme Q complex (nhFe-Q), cytochrome (cyt), oxaloacetate (OAA), and α-ketoglutarate (αKG).*

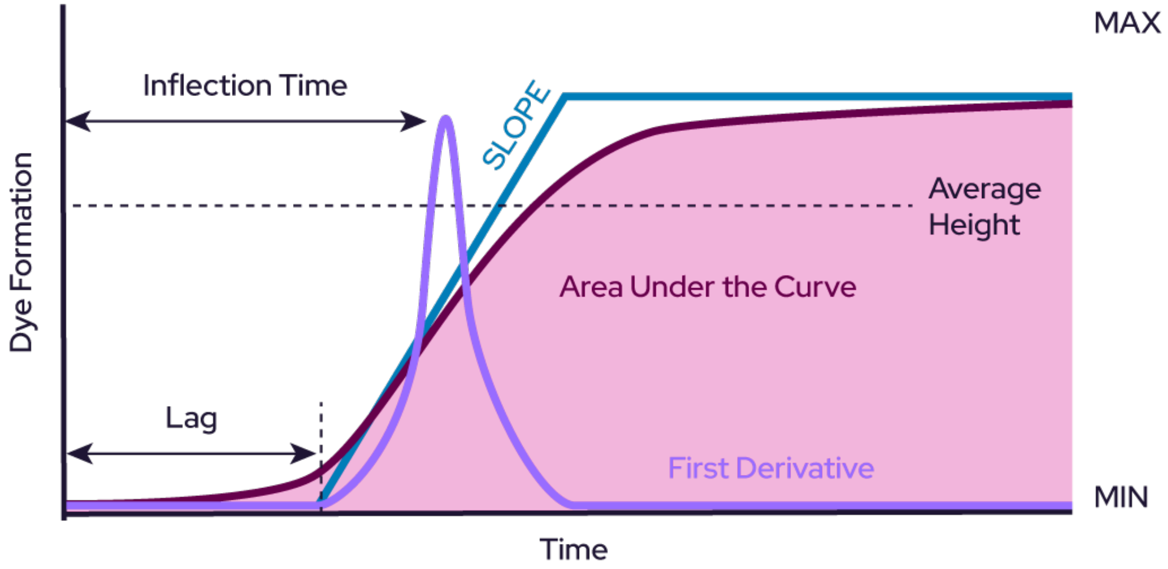
fructose

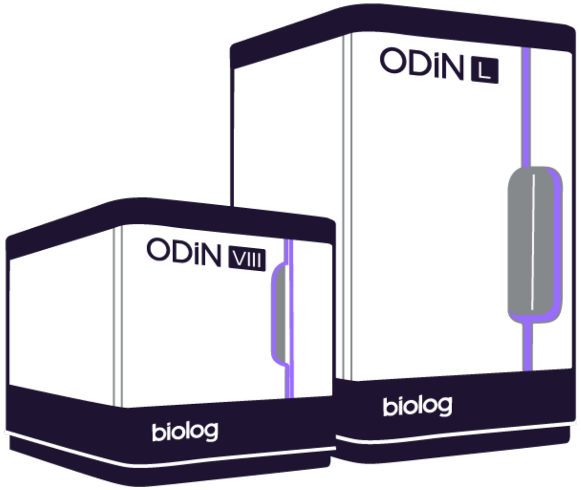
glutamine

Growth = Functional Readout

Testing sub-circuits within the cell's master circuitry biolog

Additional info from the kinetic reading in Odin™





- Each well exhibits a different rate of dye formation, so single endpoint reads for an entire plate are not ideal
- Odin™ software computes multiple parameters for phenotypic characterization and comparison

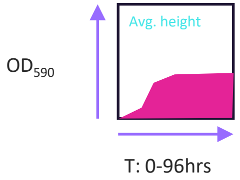
Phenotype microarray setup

- PM plates 1-10 & 21-25
  - Composed of C, N, S, and P substrates; pH and osmotic stress; antifungal resistance
- Measuring growth (no dye needed)
  - OD_{590} read every 20 minutes
  - *S. prolificans, T. marneffei*, and *A. nidulans*
    - 26°C for 96 hours
  - *C. albicans, C. auris, C. neoformans*
    - 30°C for 72 hours

Summary of results

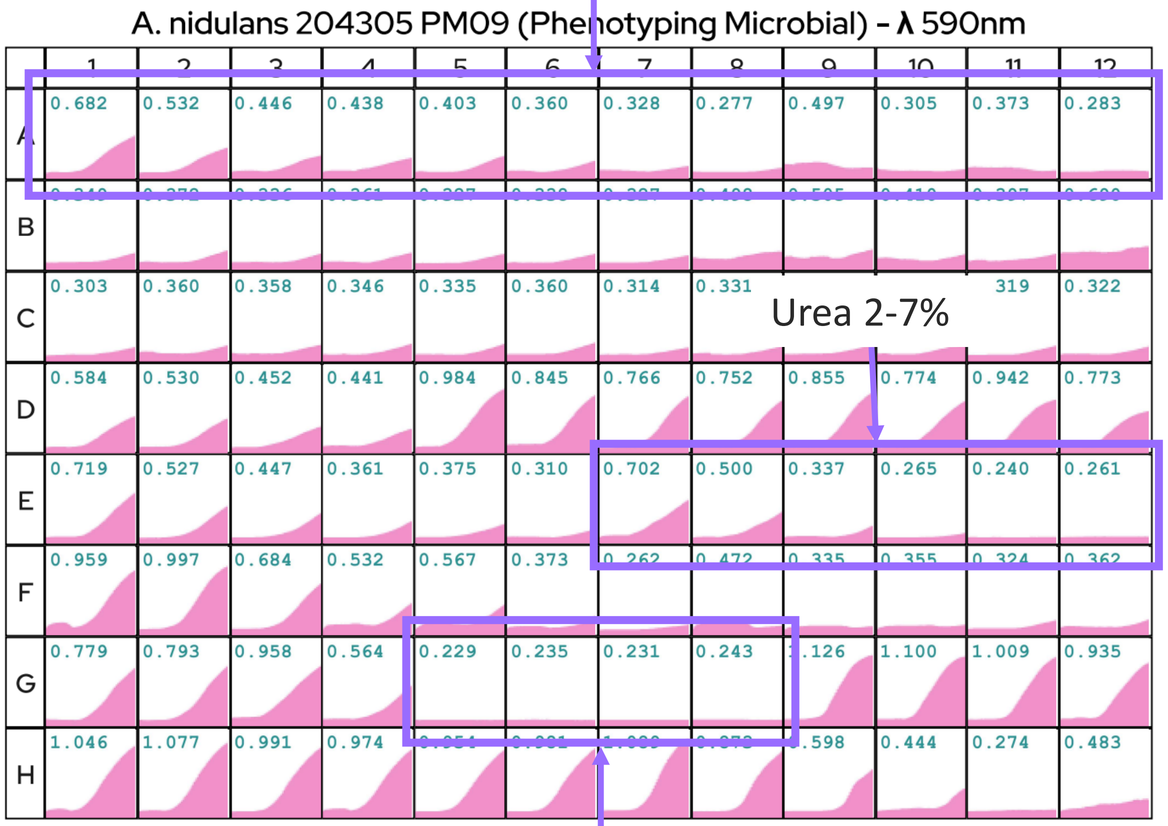
- Growth was measured for each of the 6 species on at least one plate
- Numerous nutritional substrates were identified as preferred for each species
- Each species demonstrated unique susceptibility profiles to various antifungal drugs and stress conditions

Aspergillus nidulans: stress resistance



NaCl 1-10%

Tobramycin
0.05-1.5 mM

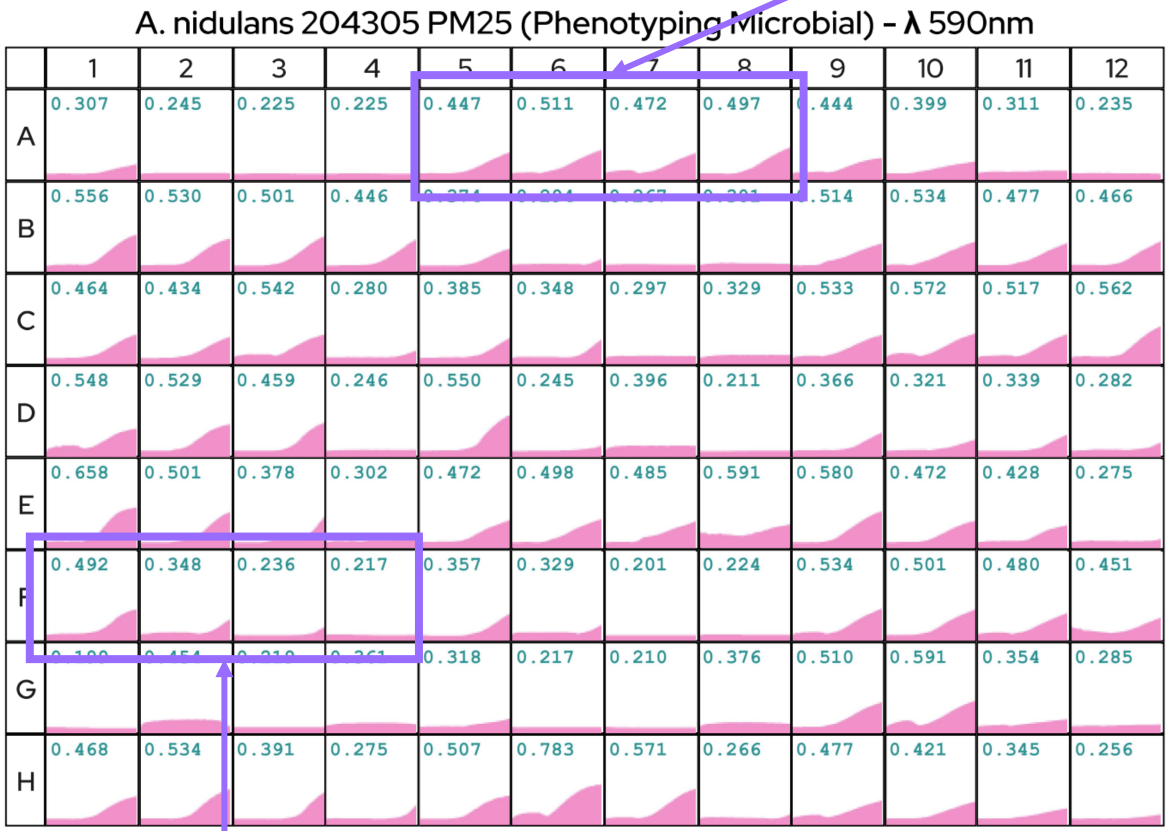


A. nidulans 204305 PM09 (Phenotyping Microbial) - λ 590nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.682	0.532	0.446	0.438	0.403	0.360	0.328	0.277	0.497	0.305	0.373	0.283
B	0.340	0.372	0.336	0.361	0.327	0.330	0.327	0.408	0.505	0.410	0.307	0.600
C	0.303	0.360	0.358	0.346	0.335	0.360	0.314	0.331			319	0.322
D	0.584	0.530	0.452	0.441	0.984	0.845	0.766	0.752	0.855	0.774	0.942	0.773
E	0.719	0.527	0.447	0.361	0.375	0.310	0.702	0.500	0.337	0.265	0.240	0.261
F	0.959	0.997	0.684	0.532	0.567	0.373	0.262	0.472	0.335	0.355	0.324	0.362
G	0.779	0.793	0.958	0.564	0.229	0.235	0.231	0.243	.126	1.100	1.009	0.935
H	1.046	1.077	0.991	0.974	0.954	0.981	1.009	0.973	.598	0.444	0.274	0.483

Urea 2-7%

Sodium benzoate pH 5.2
20mM-100 mM



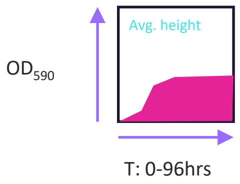
A. nidulans 204305 PM25 (Phenotyping Microbial) - λ 590nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.307	0.245	0.225	0.225	0.447	0.511	0.472	0.497	.444	0.399	0.311	0.235
B	0.556	0.530	0.501	0.446	0.374	0.294	0.267	0.301	.514	0.534	0.477	0.466
C	0.464	0.434	0.542	0.280	0.385	0.348	0.297	0.329	0.533	0.572	0.517	0.562
D	0.548	0.529	0.459	0.246	0.550	0.245	0.396	0.211	0.366	0.321	0.339	0.282
E	0.658	0.501	0.378	0.302	0.472	0.498	0.485	0.591	0.580	0.472	0.428	0.275
F	0.492	0.348	0.236	0.217	0.357	0.329	0.201	0.224	0.534	0.501	0.480	0.451
G	0.199	0.454	0.210	0.261	0.318	0.217	0.210	0.376	0.510	0.591	0.354	0.285
H	0.468	0.534	0.391	0.275	0.507	0.783	0.571	0.266	0.477	0.421	0.345	0.256

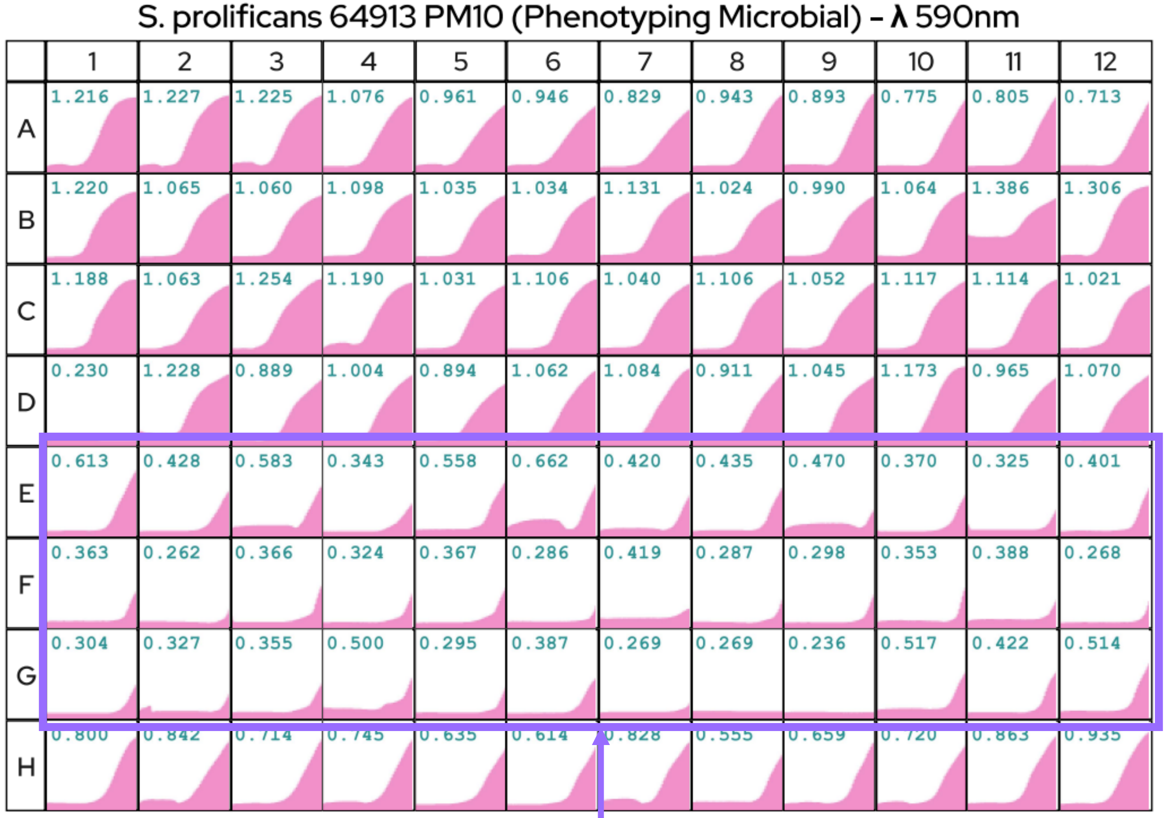
Malic acid
1.6mM-44 mM

Values indicate Average Curve Height

Scedosporium prolificans: stress resistance



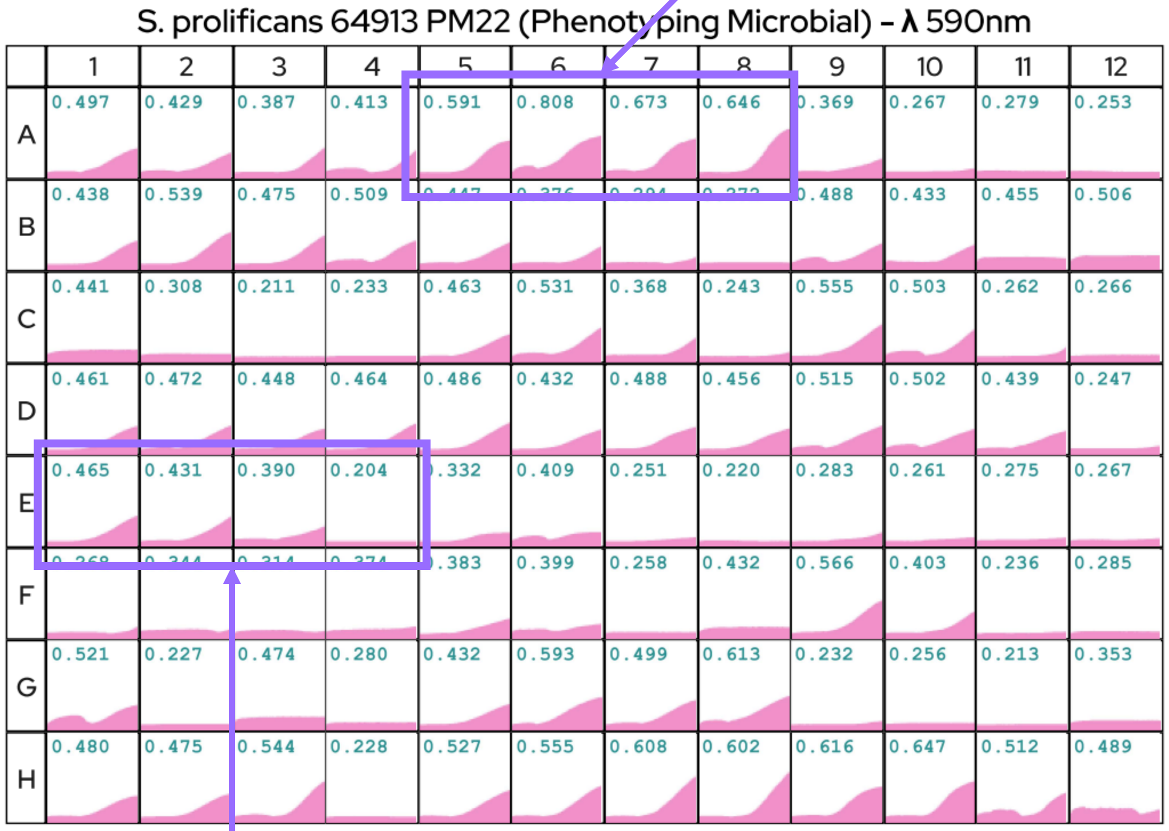
Sodium metavanadate
0.19-5.3 mM



S. prolificans 64913 PM10 (Phenotyping Microbial) – λ 590nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.216	1.227	1.225	1.076	0.961	0.946	0.829	0.943	0.893	0.775	0.805	0.713
B	1.220	1.065	1.060	1.098	1.035	1.034	1.131	1.024	0.990	1.064	1.386	1.306
C	1.188	1.063	1.254	1.190	1.031	1.106	1.040	1.106	1.052	1.117	1.114	1.021
D	0.230	1.228	0.889	1.004	0.894	1.062	1.084	0.911	1.045	1.173	0.965	1.070
E	0.613	0.428	0.583	0.343	0.558	0.662	0.420	0.435	0.470	0.370	0.325	0.401
F	0.363	0.262	0.366	0.324	0.367	0.286	0.419	0.287	0.298	0.353	0.388	0.268
G	0.304	0.327	0.355	0.500	0.295	0.387	0.269	0.269	0.236	0.517	0.422	0.514
H	0.800	0.842	0.714	0.745	0.635	0.614	0.828	0.555	0.659	0.720	0.863	0.935

Alkaline pH (9.5) causes growth to be stalled



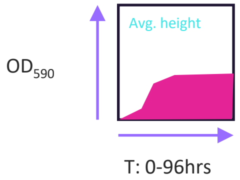
S. prolificans 64913 PM22 (Phenotyping Microbial) – λ 590nm

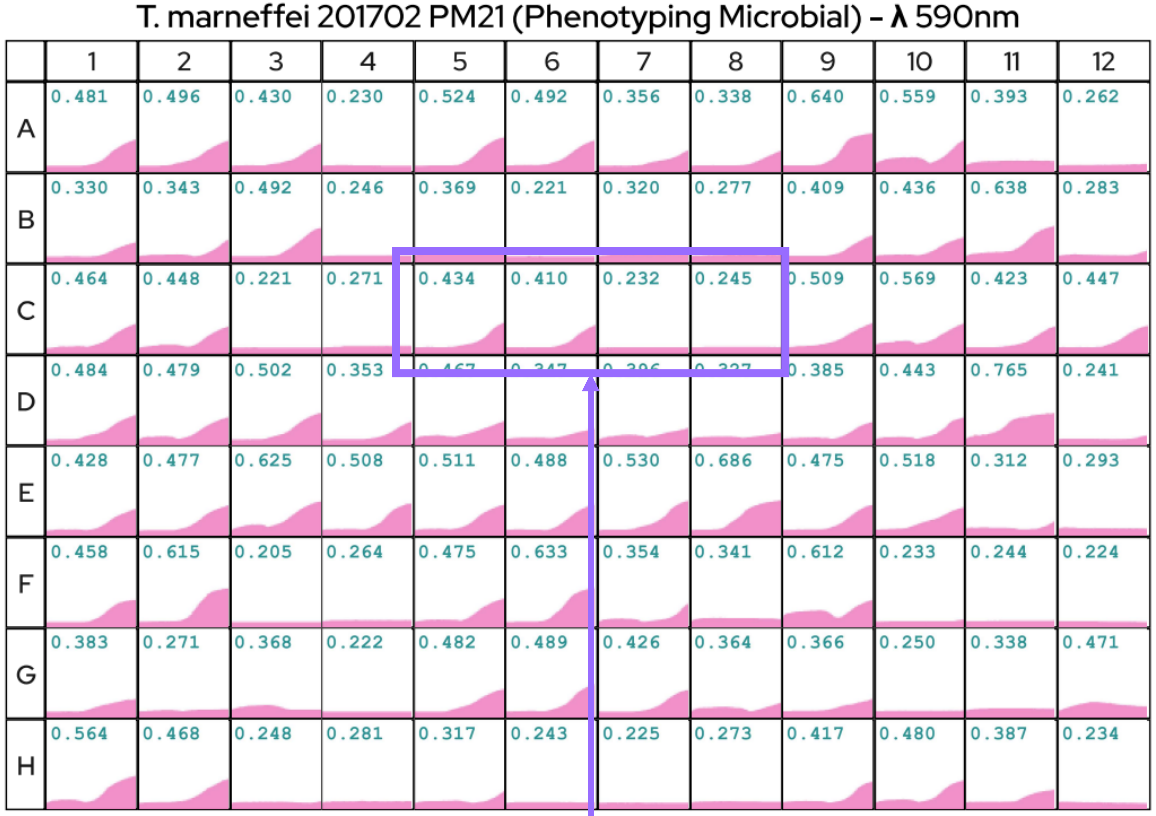
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.497	0.429	0.387	0.413	0.591	0.808	0.673	0.646	0.369	0.267	0.279	0.253
B	0.438	0.539	0.475	0.509	0.447	0.376	0.294	0.272	0.488	0.433	0.455	0.506
C	0.441	0.308	0.211	0.233	0.463	0.531	0.368	0.243	0.555	0.503	0.262	0.266
D	0.461	0.472	0.448	0.464	0.486	0.432	0.488	0.456	0.515	0.502	0.439	0.247
E	0.465	0.431	0.390	0.204	0.332	0.409	0.251	0.220	0.283	0.261	0.275	0.267
F	0.268	0.344	0.314	0.374	0.383	0.399	0.258	0.432	0.566	0.403	0.236	0.285
G	0.521	0.227	0.474	0.280	0.432	0.593	0.499	0.613	0.232	0.256	0.213	0.353
H	0.480	0.475	0.544	0.228	0.527	0.555	0.608	0.602	0.616	0.647	0.512	0.489

CCCP
0.0023-0.061 mM

Values indicate Average Curve Height

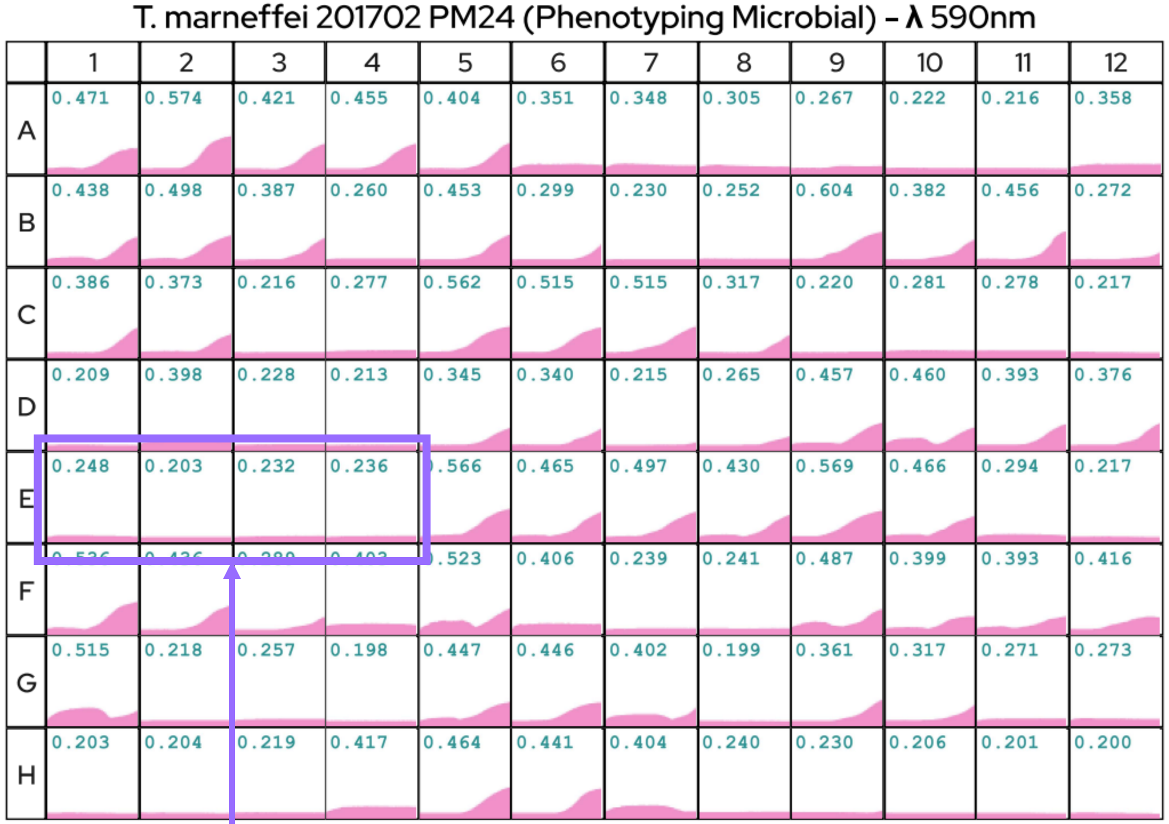
Talaromyces marneffei: stress resistance





T. marneffei 201702 PM21 (Phenotyping Microbial) – λ 590nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.481	0.496	0.430	0.230	0.524	0.492	0.356	0.338	0.640	0.559	0.393	0.262
B	0.330	0.343	0.492	0.246	0.369	0.221	0.320	0.277	0.409	0.436	0.638	0.283
C	0.464	0.448	0.221	0.271	0.434	0.410	0.232	0.245	0.509	0.569	0.423	0.447
D	0.484	0.479	0.502	0.353	0.467	0.347	0.306	0.327	0.385	0.443	0.765	0.241
E	0.428	0.477	0.625	0.508	0.511	0.488	0.530	0.686	0.475	0.518	0.312	0.293
F	0.458	0.615	0.205	0.264	0.475	0.633	0.354	0.341	0.612	0.233	0.244	0.224
G	0.383	0.271	0.368	0.222	0.482	0.489	0.426	0.364	0.366	0.250	0.338	0.471
H	0.564	0.468	0.248	0.281	0.317	0.243	0.225	0.273	0.417	0.480	0.387	0.234



T. marneffei 201702 PM24 (Phenotyping Microbial) – λ 590nm

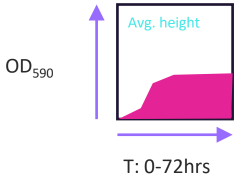
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.471	0.574	0.421	0.455	0.404	0.351	0.348	0.305	0.267	0.222	0.216	0.358
B	0.438	0.498	0.387	0.260	0.453	0.299	0.230	0.252	0.604	0.382	0.456	0.272
C	0.386	0.373	0.216	0.277	0.562	0.515	0.515	0.317	0.220	0.281	0.278	0.217
D	0.209	0.398	0.228	0.213	0.345	0.340	0.215	0.265	0.457	0.460	0.393	0.376
E	0.248	0.203	0.232	0.236	0.566	0.465	0.497	0.430	0.569	0.466	0.294	0.217
F	0.536	0.436	0.280	0.403	0.523	0.406	0.239	0.241	0.487	0.399	0.393	0.416
G	0.515	0.218	0.257	0.198	0.447	0.446	0.402	0.199	0.361	0.317	0.271	0.273
H	0.203	0.204	0.219	0.417	0.464	0.441	0.404	0.240	0.230	0.206	0.201	0.200

Domiphen bromide
0.0005-0.0147 mM

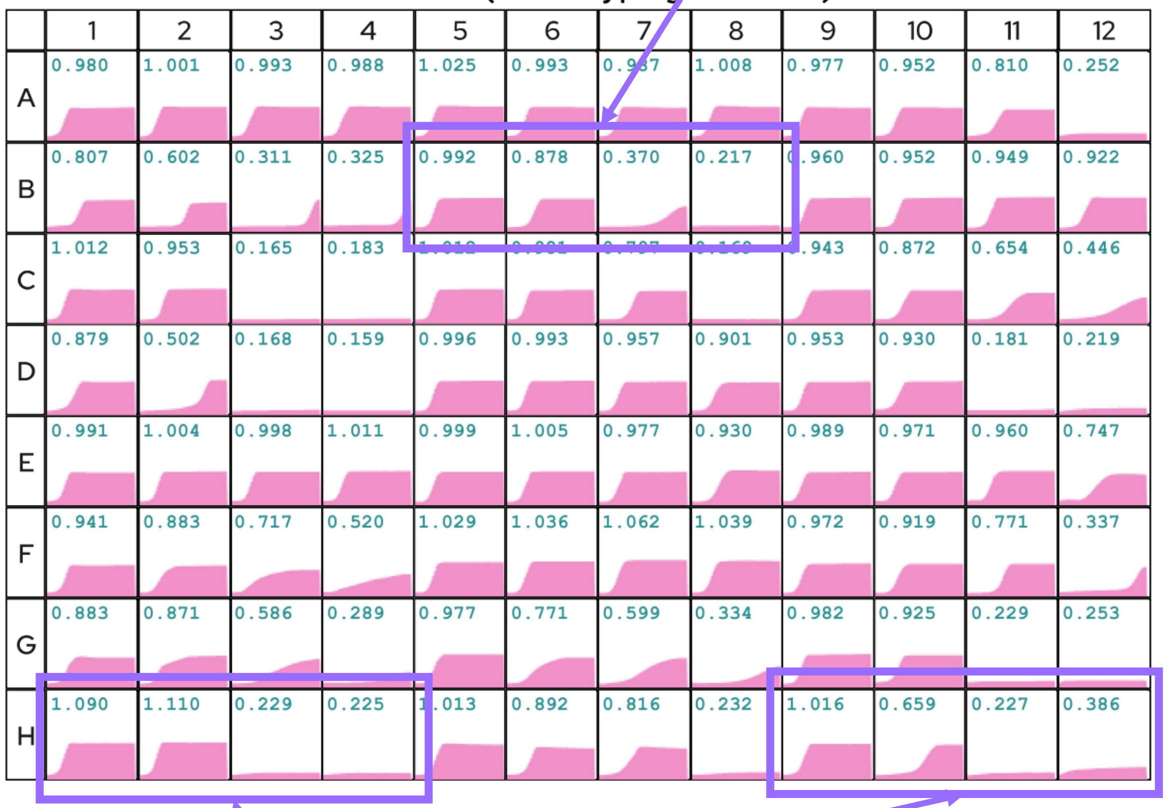
Bereberine
0.01-0.269 mM

Values indicate Average Curve Height

Candida albicans: stress resistance



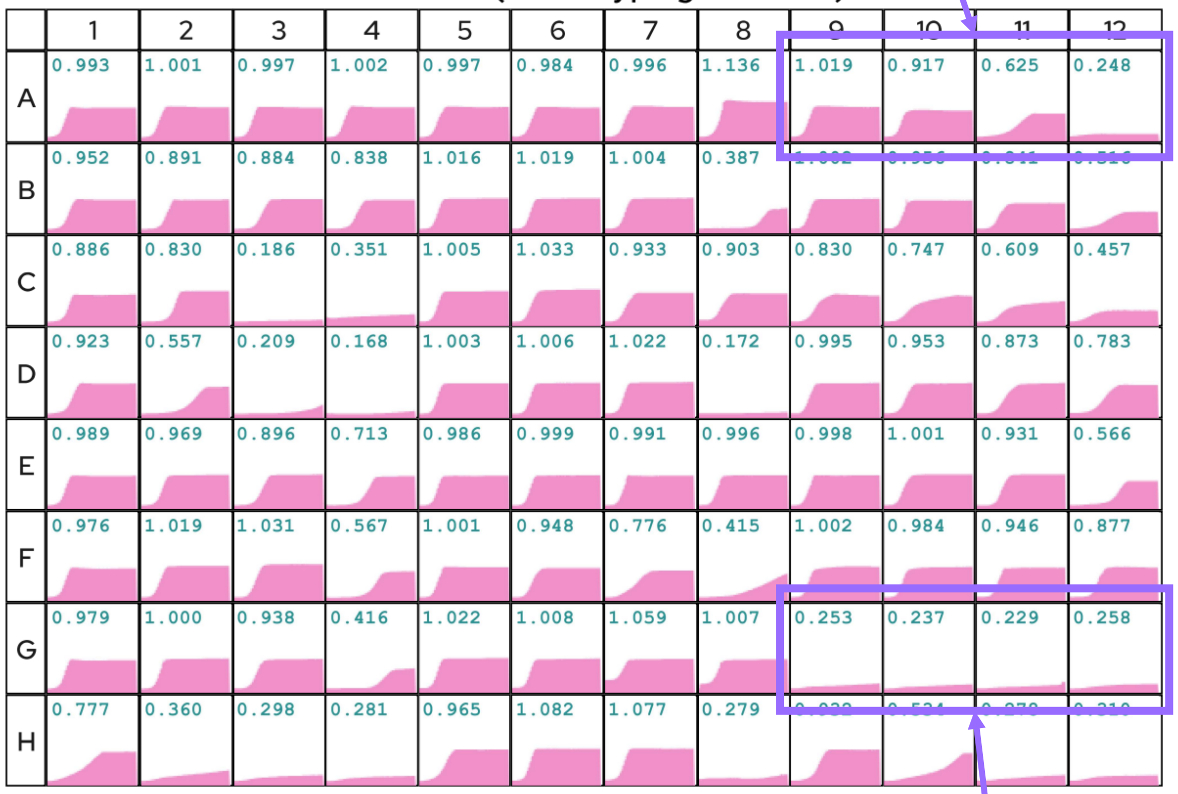
Dodecyltrimethyl ammonium bromide
0.02-0.54 mM



C albicans 2876 PM21 (Phenotyping Microbial) – λ 590nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.980	1.001	0.993	0.988	1.025	0.993	0.987	1.008	0.977	0.952	0.810	0.252
B	0.807	0.602	0.311	0.325	0.992	0.878	0.370	0.217	0.960	0.952	0.949	0.922
C	1.012	0.953	0.165	0.183	1.012	0.881	0.707	0.169	0.943	0.872	0.654	0.446
D	0.879	0.502	0.168	0.159	0.996	0.993	0.957	0.901	0.953	0.930	0.181	0.219
E	0.991	1.004	0.998	1.011	0.999	1.005	0.977	0.930	0.989	0.971	0.960	0.747
F	0.941	0.883	0.717	0.520	1.029	1.036	1.062	1.039	0.972	0.919	0.771	0.337
G	0.883	0.871	0.586	0.289	0.977	0.771	0.599	0.334	0.982	0.925	0.229	0.253
H	1.090	1.110	0.229	0.225	1.013	0.892	0.816	0.232	1.016	0.659	0.227	0.386

Zaragozic acid A
0.0003-0.0079 mM



C albicans 2876 PM24 (Phenotyping Microbial) – λ 590nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.993	1.001	0.997	1.002	0.997	0.984	0.996	1.136	1.019	0.917	0.625	0.248
B	0.952	0.891	0.884	0.838	1.016	1.019	1.004	0.387	1.002	0.956	0.841	0.516
C	0.886	0.830	0.186	0.351	1.005	1.033	0.933	0.903	0.830	0.747	0.609	0.457
D	0.923	0.557	0.209	0.168	1.003	1.006	1.022	0.172	0.995	0.953	0.873	0.783
E	0.989	0.969	0.896	0.713	0.986	0.999	0.991	0.996	0.998	1.001	0.931	0.566
F	0.976	1.019	1.031	0.567	1.001	0.948	0.776	0.415	1.002	0.984	0.946	0.877
G	0.979	1.000	0.938	0.416	1.022	1.008	1.059	1.007	0.253	0.237	0.229	0.258
H	0.777	0.360	0.298	0.281	0.965	1.082	1.077	0.279	0.932	0.534	0.278	0.310

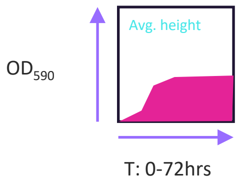
Diamide
0.05-1.3 mM

Zinc chloride
0.65-17.4 mM

Fluconazole
.0016-0.042 mM

Values indicate Average Curve Height

Candida auris: nutrition

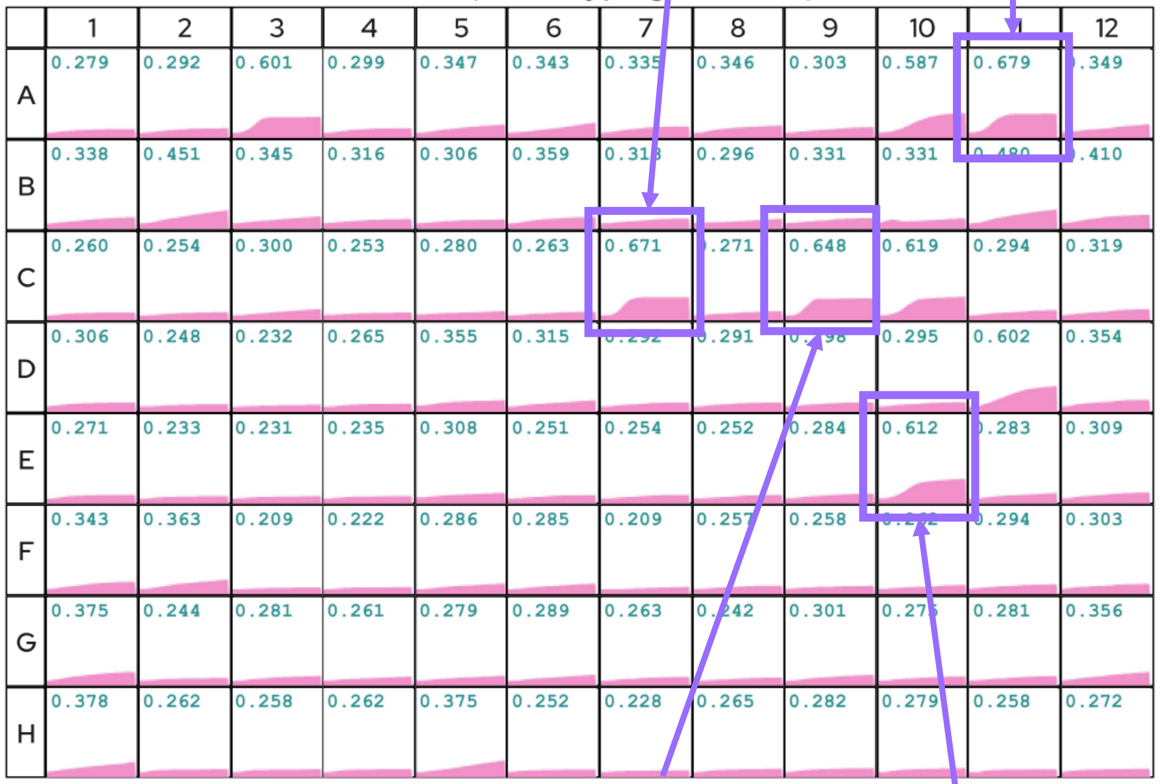


D-Fructose

D-Mannose

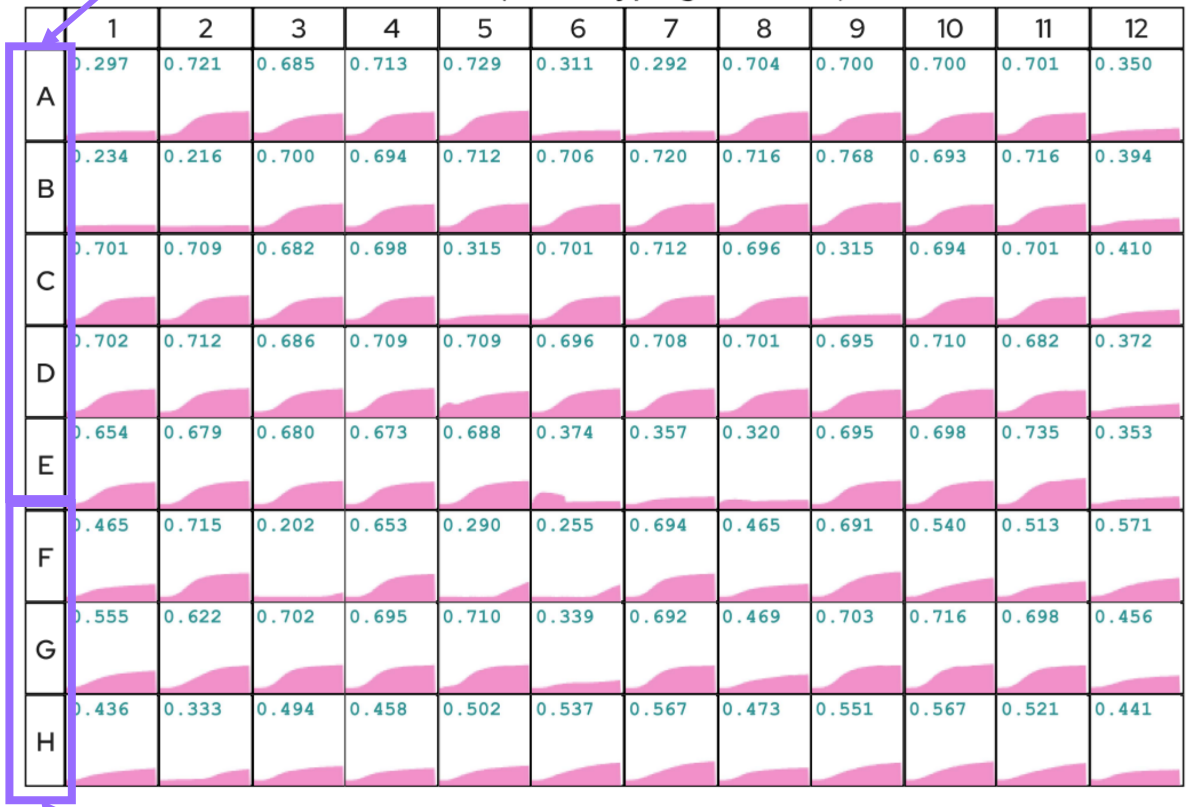
Phosphourus compounds

C auris 5000 PM01 (Phenotyping Microbial) – λ 590nm



	1	2	3	4	5	6	7	8	9	10	[illegible]	12
A	0.279	0.292	0.601	0.299	0.347	0.343	0.335	0.346	0.303	0.587	0.679	.349
B	0.338	0.451	0.345	0.316	0.306	0.359	[illegible]	0.296	0.331	0.331	0.480	.410
C	0.260	0.254	0.300	0.253	0.280	0.263	0.671	.271	0.648	0.619	0.294	0.319
D	0.306	0.248	0.232	0.265	0.355	0.315	0.292	0.291	[illegible]	0.295	0.602	0.354
E	0.271	0.233	0.231	0.235	0.308	0.251	0.254	0.252	0.284	0.612	.283	0.309
F	0.343	0.363	0.209	0.222	0.286	0.285	0.209	0.257	0.258	[illegible]	.294	0.303
G	0.375	0.244	0.281	0.261	0.279	0.289	0.263	0.242	0.301	[illegible]	0.281	0.356
H	0.378	0.262	0.258	0.262	0.375	0.252	0.228	0.265	0.282	0.279	0.258	0.272

C auris 5000 PM04 (Phenotyping Microbial) – λ 590nm



	1	2	3	4	5	6	7	8	9	10	11	12
A	.297	0.721	0.685	0.713	0.729	0.311	0.292	0.704	0.700	0.700	0.701	0.350
B	.234	0.216	0.700	0.694	0.712	0.706	0.720	0.716	0.768	0.693	0.716	0.394
C	.701	0.709	0.682	0.698	0.315	0.701	0.712	0.696	0.315	0.694	0.701	0.410
D	.702	0.712	0.686	0.709	0.709	0.696	0.708	0.701	0.695	0.710	0.682	0.372
E	.654	0.679	0.680	0.673	0.688	0.374	0.357	0.320	0.695	0.698	0.735	0.353
F	.465	0.715	0.202	0.653	0.290	0.255	0.694	0.465	0.691	0.540	0.513	0.571
G	.555	0.622	0.702	0.695	0.710	0.339	0.692	0.469	0.703	0.716	0.698	0.456
H	.436	0.333	0.494	0.458	0.502	0.537	0.567	0.473	0.551	0.567	0.521	0.441

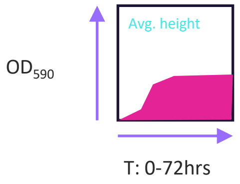
α-D-Glucose

Maltotriose

Sulfur compounds

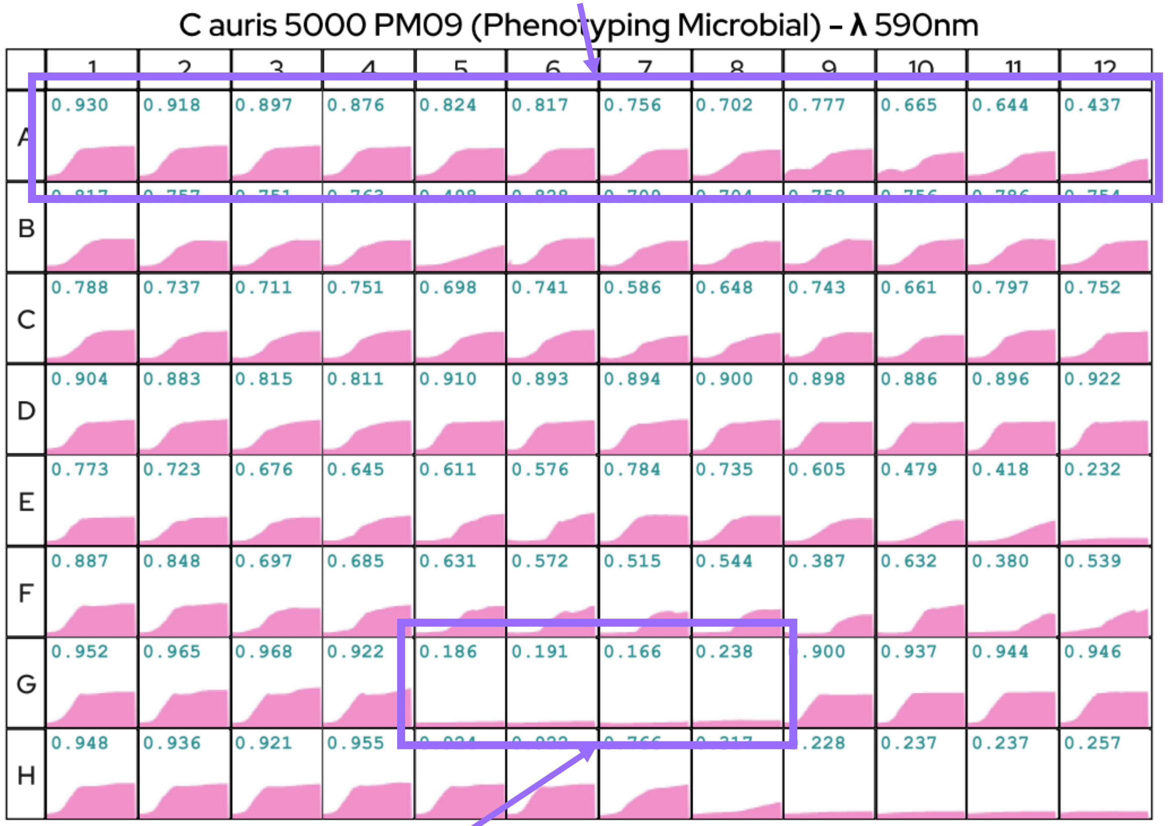
Values indicate Average Curve Height

Candida auris: stress resistance



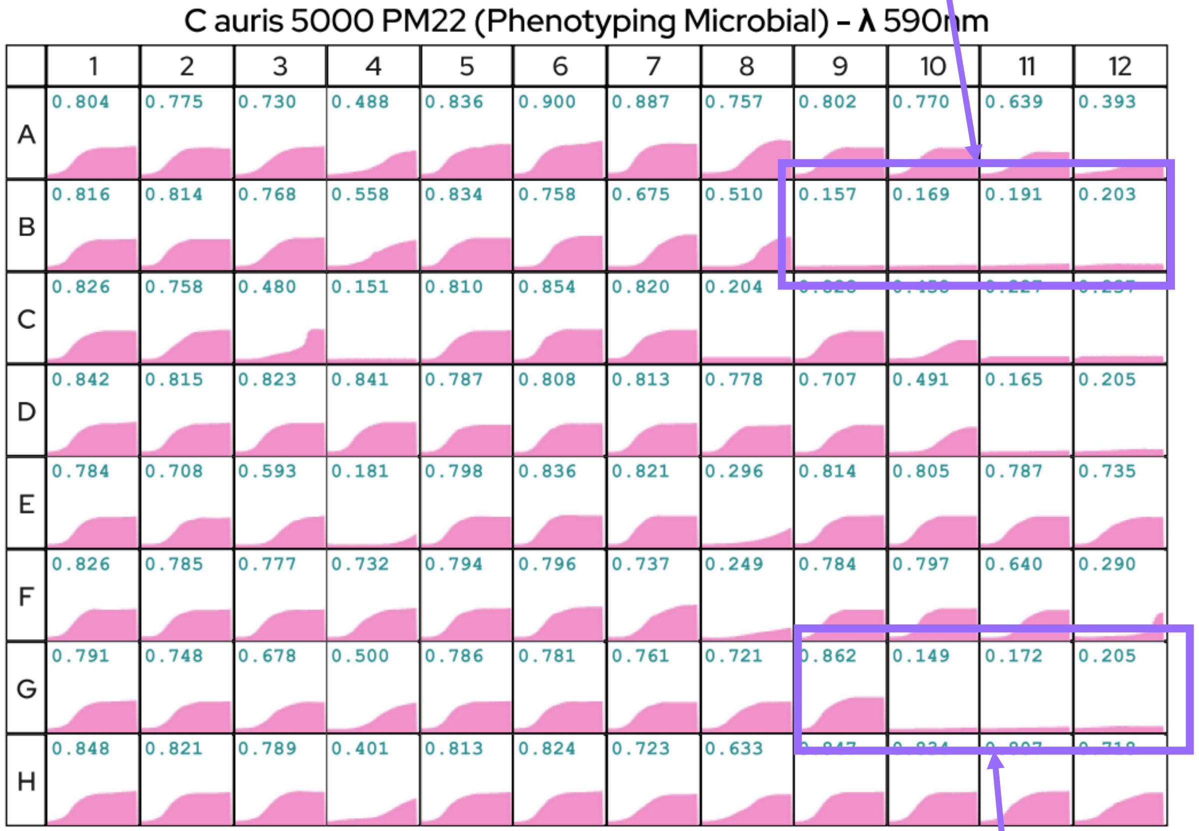
Decreased growth with increasing pH (3.5-10)

Triclosan
Range of 0.038-1.036mM



C auris 5000 PM09 (Phenotyping Microbial) – λ 590nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.930	0.918	0.897	0.876	0.824	0.817	0.756	0.702	0.777	0.665	0.644	0.437
B	0.817	0.757	0.751	0.763	0.408	0.828	0.709	0.704	0.758	0.756	0.786	0.754
C	0.788	0.737	0.711	0.751	0.698	0.741	0.586	0.648	0.743	0.661	0.797	0.752
D	0.904	0.883	0.815	0.811	0.910	0.893	0.894	0.900	0.898	0.886	0.896	0.922
E	0.773	0.723	0.676	0.645	0.611	0.576	0.784	0.735	0.605	0.479	0.418	0.232
F	0.887	0.848	0.697	0.685	0.631	0.572	0.515	0.544	0.387	0.632	0.380	0.539
G	0.952	0.965	0.968	0.922	0.186	0.191	0.166	0.238	.900	0.937	0.944	0.946
H	0.948	0.936	0.921	0.955	0.824	0.822	0.766	0.217	.228	0.237	0.237	0.257



C auris 5000 PM22 (Phenotyping Microbial) – λ 590nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.804	0.775	0.730	0.488	0.836	0.900	0.887	0.757	0.802	0.770	0.639	0.393
B	0.816	0.814	0.768	0.558	0.834	0.758	0.675	0.510	0.157	0.169	0.191	0.203
C	0.826	0.758	0.480	0.151	0.810	0.854	0.820	0.204	0.828	0.456	0.227	0.237
D	0.842	0.815	0.823	0.841	0.787	0.808	0.813	0.778	0.707	0.491	0.165	0.205
E	0.784	0.708	0.593	0.181	0.798	0.836	0.821	0.296	0.814	0.805	0.787	0.735
F	0.826	0.785	0.777	0.732	0.794	0.796	0.737	0.249	0.784	0.797	0.640	0.290
G	0.791	0.748	0.678	0.500	0.786	0.781	0.761	0.721	.862	0.149	0.172	0.205
H	0.848	0.821	0.789	0.401	0.813	0.824	0.723	0.633	0.847	0.834	0.807	0.718

Sodium Benzoate
pH 5.2 20mM-200mM

Thallium acetate

Values indicate Average Curve Height

Take home message:

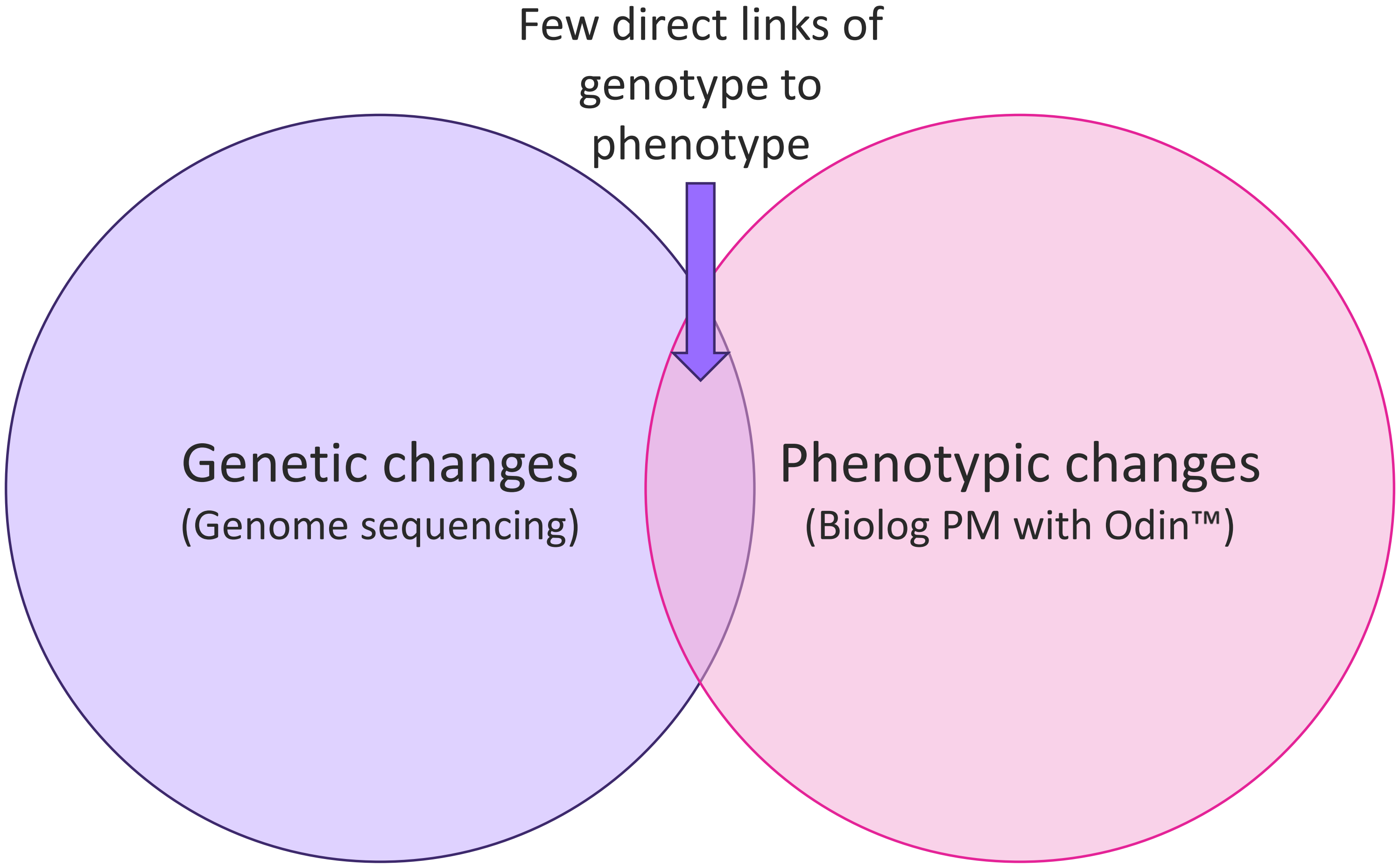
Comprehensive phenotypic profiles for important and emerging fungal pathogens with Odin™ can allow for fast characterization and identification beyond the species level.

Monitoring Phenotypic Drift in Bacterial Production Strains with Odin™

Monitoring drift is a challenge

- Genetic monitoring is time consuming and often expensive
- Phenotypic monitoring can be prohibitive as dozens to hundreds of traits must be assessed
- Biolog Phenotype MicroArrays with Odin™ allow early and rapid phenotypic testing of >2000 unique conditions testing growth or metabolic output

Monitoring both genotype and phenotype is necessary for the whole story of how organisms change over time



Few direct links of genotype to phenotype

Genetic changes
(Genome sequencing)

Phenotypic changes
(Biolog PM with Odin™)

Overview

- We know that genetic drift occurs over time as mutations accumulate at measurable rates; however, it is unclear how this translates to phenotypic drift as strains are continually passaged.

- Hypothesis: If bacterial strains are passaged many times, we will be able to detect phenotypic variation relative to the original strain using Odin™ L and Phenotype Microarrays PM 1-20.

- Experiment: passage *Escherichia coli* (ATCC® 11775™), *Streptococcus thermophilus* (ATCC® 19258™), and *Lactocaseibacillus casei* (ATCC® 393™) on solid media every 24 hours. Samples will be taken at P0, P20, and P40 and screened for phenotypic drift and genomes sequenced.

PM experimental setup

- *E. coli* (ATCC® 11775™) streaked on BUG+ 5% blood
    - Incubate 24 hours 36°C
    - Pick colony for subculture and repeat
- *S. thermophilus* (ATCC® 19258™)
    - M17 + 0.5% lactose incubated with 5% CO_2
    - Passaged every 24 hours
- *L. casei* (ATCC® 393™)
    - Medium #416 incubated with 5% CO_2
    - Passaged every 48 hours

Escherichia coli

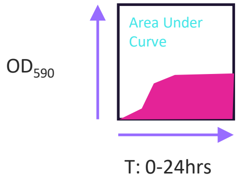
(ATCC® 11775™)

Results from passaging *E. coli*

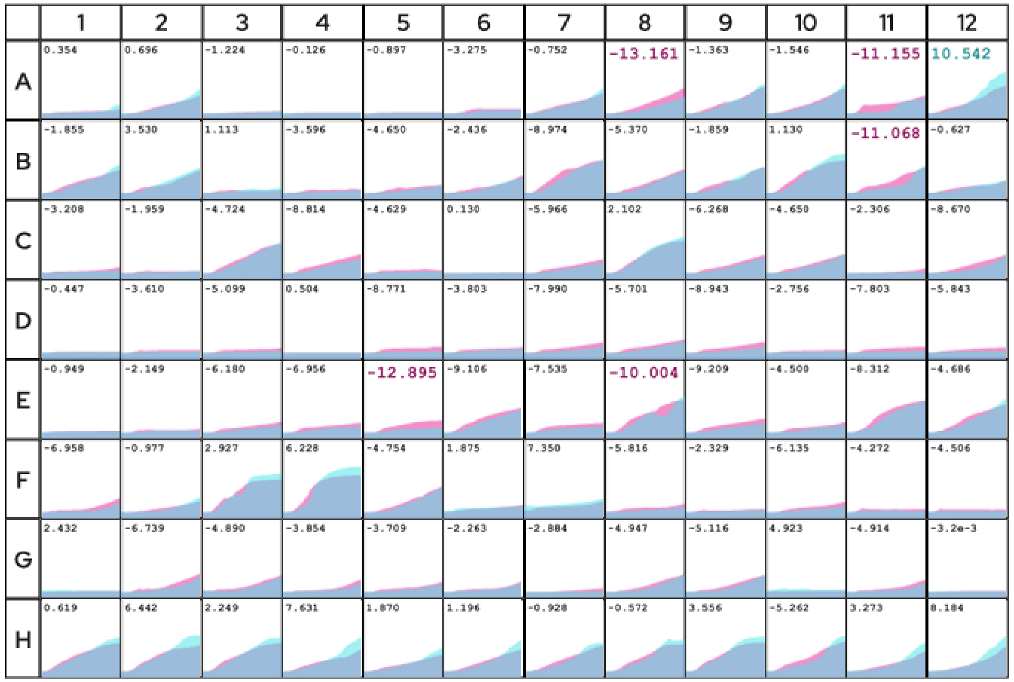
- Passage 0 (P0) and P20 strains were assessed with PM1-20 with dye and incubated in Odin™ for 24 hours at 36°C and reads were taken every 20 minutes
  - Significant phenotypes on PM 3, 10, 11, 12, 14, &15 (triplicate verified)
- P40 strain screened on PM3, 10, 11, 12, 14, &15 to assess stability of phenotypes (triplicate verified)

When phenotype appeared	**# of significantly changed substrates**	
	Metabolics	Sensitivities
P20 and increased in P40	1	2
P20 and preserved in P40	4	20
P40	18	27

Comparison of metabolism in *E. coli* strains



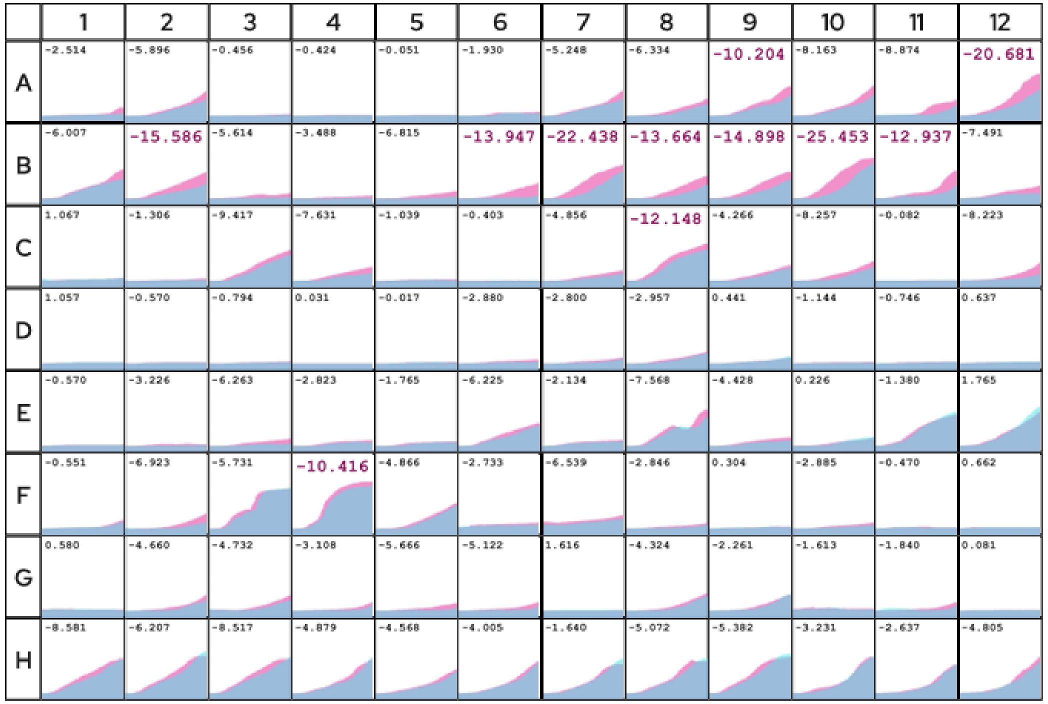
- *E. coli* strain P20 and P40 showed decreasing abilities to metabolize a variety of nitrogen sources
  - Amino acids and amines
  - Pink in both indicates worsening phenotype



■ P20 ■ P0

E coli 11775 P20 to E coli 11775 P0 PM03 (Phenotyping Microbial) – λ 590nm – Max OD 3.2

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.354	0.696	-1.224	-0.126	-0.897	-3.275	-0.752	-13.161	-1.363	-1.546	-11.155	10.542
B	-1.855	3.530	1.113	-3.596	-4.650	-2.436	-8.974	-5.370	-1.859	1.130	-11.068	-0.627
C	-3.208	-1.959	-4.724	-8.814	-4.629	0.130	-5.966	2.102	-6.268	-4.650	-2.306	-8.670
D	-0.447	-3.610	-5.099	0.504	-8.771	-3.803	-7.990	-5.701	-8.943	-2.756	-7.803	-5.843
E	-0.949	-2.149	-6.180	-6.956	-12.895	-9.106	-7.535	-10.004	-9.209	-4.500	-8.312	-4.686
F	-6.958	-0.977	2.927	6.228	-4.754	1.875	7.350	-5.816	-2.329	-6.135	-4.272	-4.506
G	2.432	-6.739	-4.890	-3.854	-3.709	-2.263	-2.884	-4.947	-5.116	4.923	-4.914	-3.2e-3
H	0.619	6.442	2.249	7.631	1.870	1.196	-0.928	-0.572	3.556	-5.262	3.273	8.184



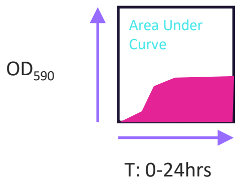
■ P40 ■ P20

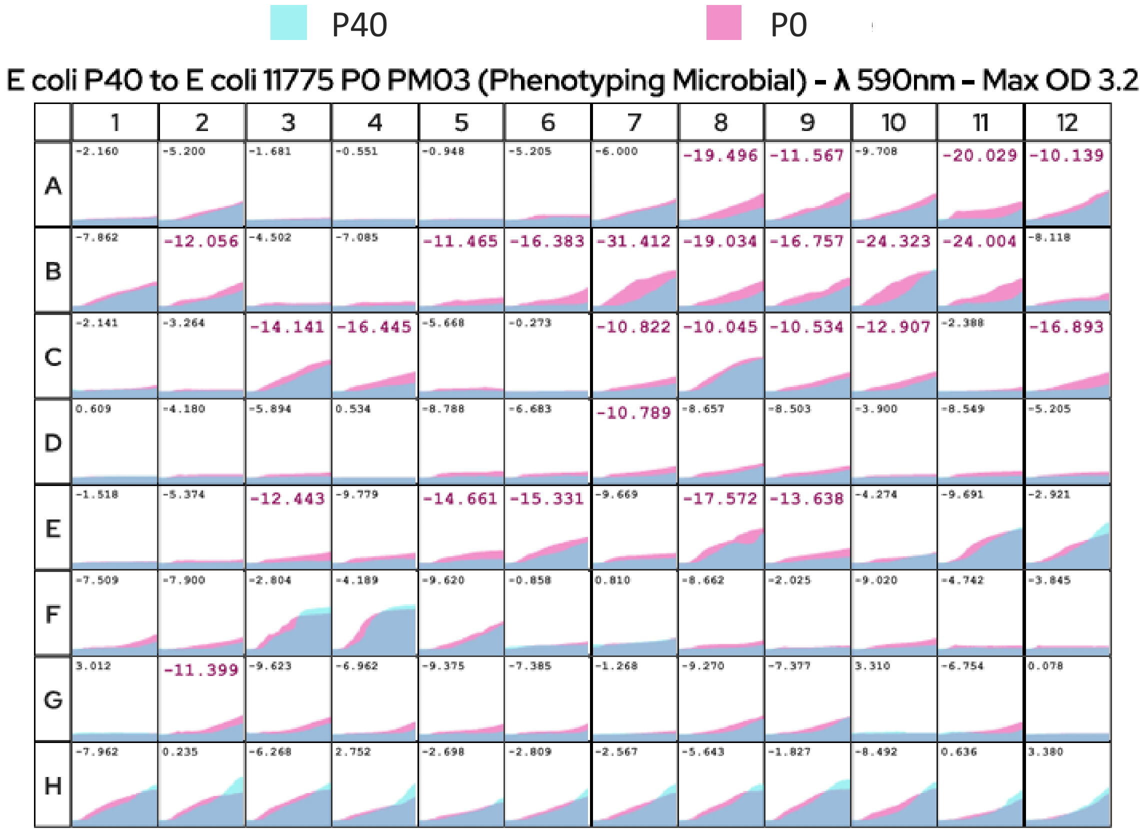
E coli P40 to E coli 11775 P20 PM03 (Phenotyping Microbial) – λ 590nm – Max OD 3.2

	1	2	3	4	5	6	7	8	9	10	11	12
A	-2.514	-5.896	-0.456	-0.424	-0.051	-1.930	-5.248	-6.334	-10.204	-8.163	-8.874	-20.681
B	-6.007	-15.586	-5.614	-3.488	-6.815	-13.947	-22.438	-13.664	-14.898	-25.453	-12.937	-7.491
C	1.067	-1.306	-9.417	-7.631	-1.039	-0.403	-4.856	-12.148	-4.266	-8.257	-0.082	-8.223
D	1.057	-0.570	-0.794	0.031	-0.017	-2.880	-2.800	-2.957	0.441	-1.144	-0.746	0.637
E	-0.570	-3.226	-6.263	-2.823	-1.765	-6.225	-2.134	-7.568	-4.428	0.226	-1.380	1.765
F	-0.551	-6.923	-5.731	-10.416	-4.866	-2.733	-6.539	-2.846	0.304	-2.885	-0.470	0.662
G	0.580	-4.660	-4.732	-3.108	-5.666	-5.122	1.616	-4.324	-2.261	-1.613	-1.840	0.081
H	-8.581	-6.207	-8.517	-4.879	-4.568	-4.005	-1.640	-5.072	-5.382	-3.231	-2.637	-4.805

Values reported Reference – Test Area Under the Curve (AUC)

Comparison of metabolism in *E. coli* strains





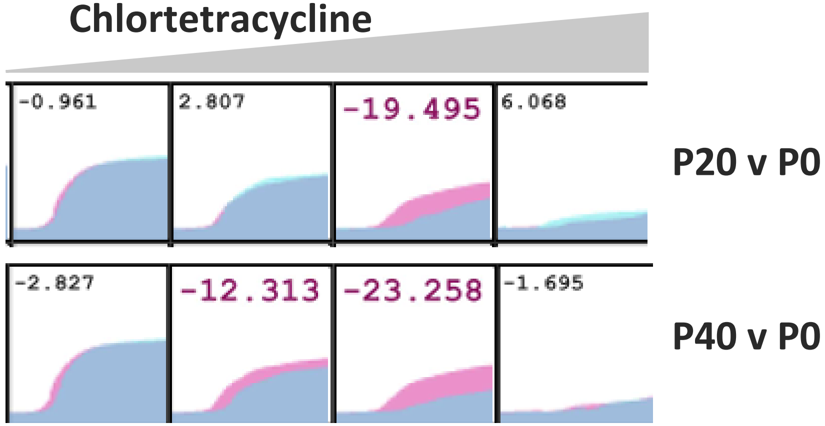
E coli P40 to E coli 11775 P0 PM03 (Phenotyping Microbial) - λ 590nm - Max OD 3.2

- Overall decrease in ability to metabolize nitrogen sources is statistically significant (Sidak's MCT) by passage 40

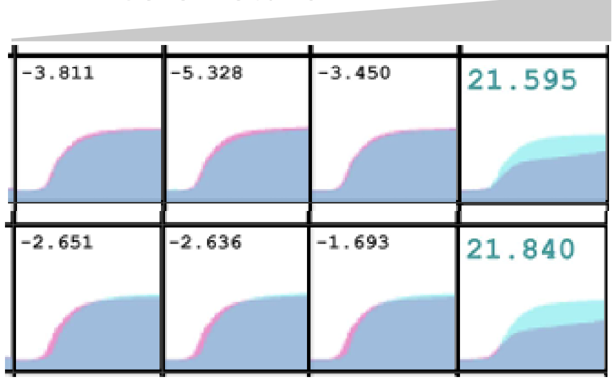
Well	Substrate	Diff. AUC	p-val
A8	L-arginine	-19.468	<0.0001
A9	L-asparagine	-11.567	<0.0001
A11	L-cysteine	-20.029	<0.0001
B2	Glycine	-12.056	<0.0001
B5	L-leucine	-11.465	<0.0001
B6	L-lysine	-16.383	<0.0001
B7	L-methionine	-31.412	<0.0001
B8	L-phenylalanine	-19.034	<0.0001
B9	L-proline	-16.757	<0.0001
B10	L-serine	-24.323	<0.0001
B11	L-threonine	-24.004	<0.0001

Changes in sensitivities (Response to inhibitory compounds)

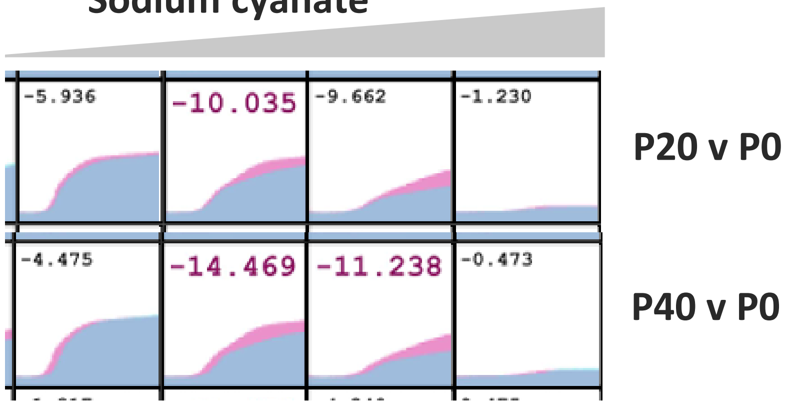
Chlortetracycline



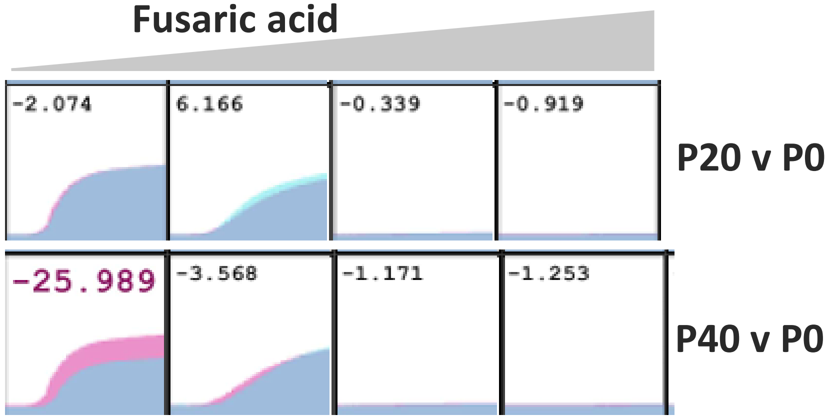
Cefemetazol



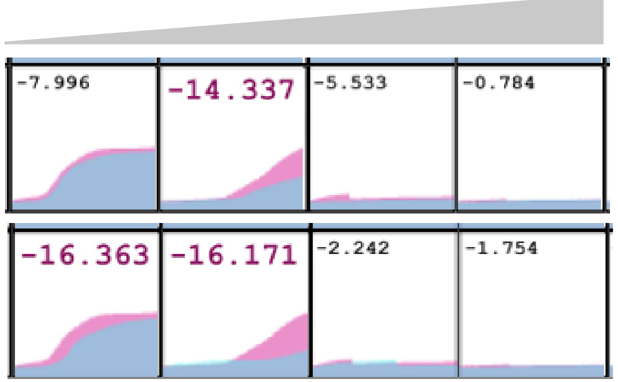
Sodium cyanate



Fusaric acid



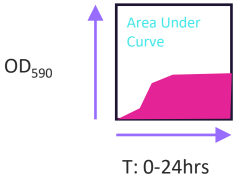
2-nitroimidizol



Legend:

P0

P20/P40



- All values reported are Area Under the Curve (AUC)
- Values are averaged from triplicate runs

Monitoring genetic drift by sequencing

	Variant	P20 Frequency	P40 Frequency	Affected proteins
E. coli	C>A	56.65%	60.30%	type VI secretion system tip protein TssI/ VgrG
	A>T	7.49%	7.69%	galactoside O-acetyltransferase
	G>T	33.62%	33.56%	16S ribosomal RNA

Top 3 Variants

- Using whole-genome sequencing, we identified a total of 10 genetic variants in P20 that comprised 7 SNPs and 3 deletions when the reads were aligned to the P0 reference sequence.
- P40 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 for *E. coli*

- Phenotypic drift occurs more rapidly than expected in only 20 passages (or less)

- While some changes are stable once they appeared, others get more exaggerated

When phenotype appeared	**# of significantly changed substrates**	
	Metabolics	Sensitivities
P20 and increased in P40	1	2
P20 and preserved in P40	4	20
P40	18	27

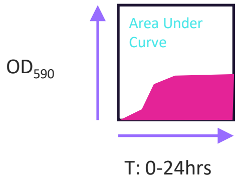
Streptococcus thermophilus

(ATCC® 19258™)

Results from passaging *S. thermophilus*

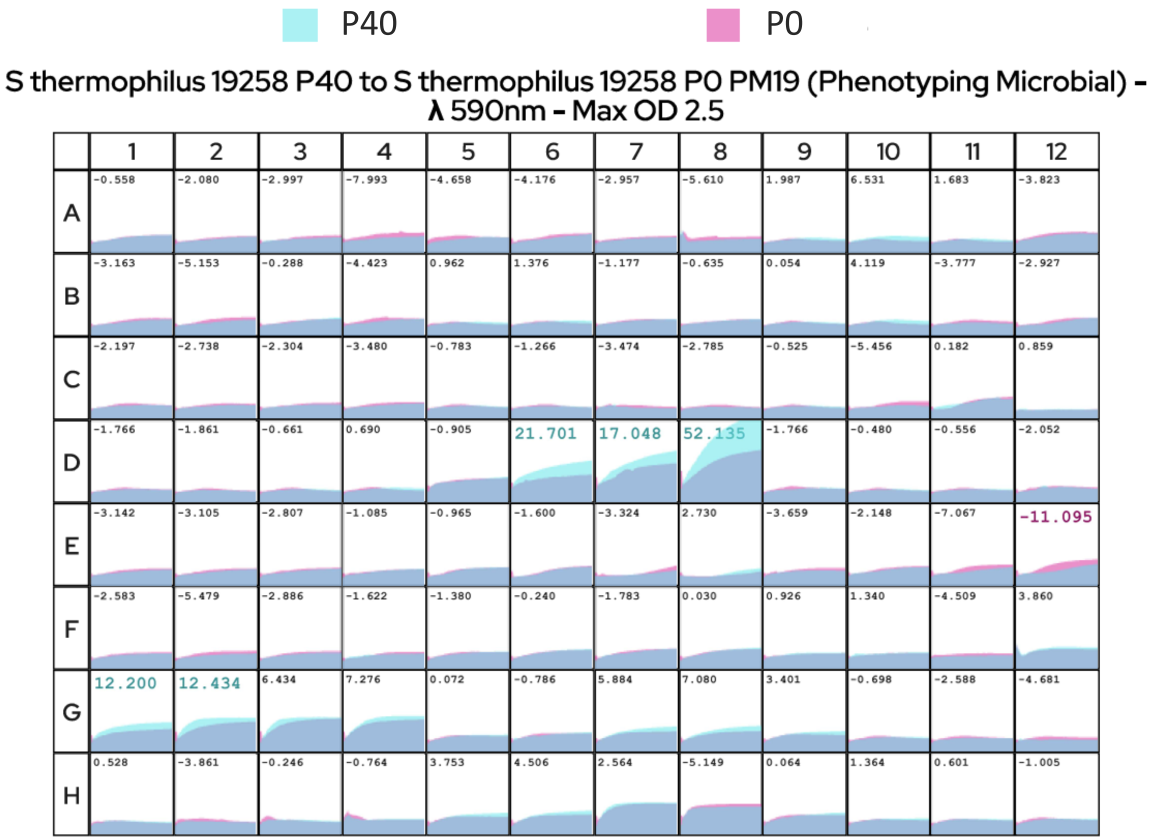
- Passage 0 (P0) and P20 strains were assessed with PM1-20 with dye and incubated in Odin™ for 24 hours at 36°C and reads were taken every 20 minutes
    - Significant phenotypes on PM20, 19, 18, 17, 16, 15, 14, 10, & 9
- P40 strain screened on PM 9, 10, & 14-20 to assess stability of phenotypes (triplicate verified)

When Phenotype appeared	# of Sensitivity phenotypes
P20	14
P40	12



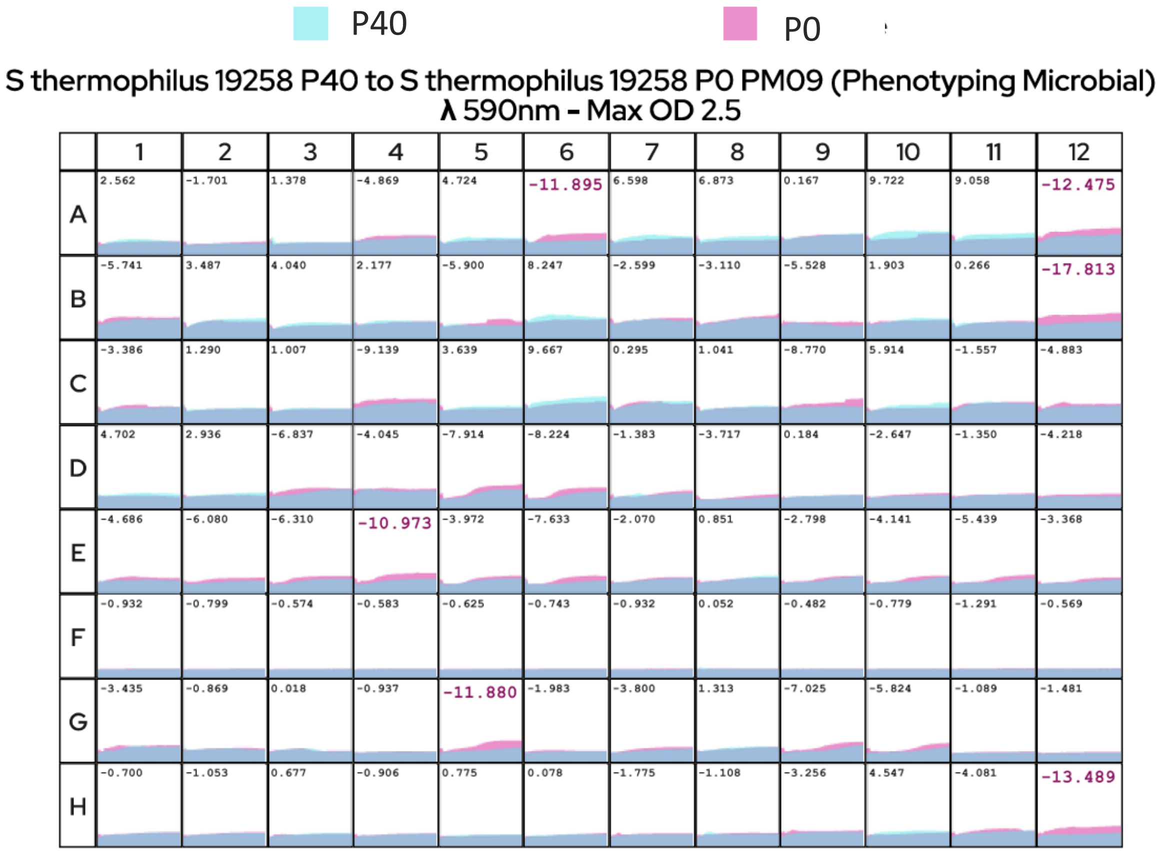
General trends for *S. thermophilus*

- *S. thermophilus* appeared to gather phenotypes rather than exacerbating existing ones
- Typically, no significant changes in phenotypes identified in P20



S thermophilus 19258 P40 to S thermophilus 19258 P0 PM19 (Phenotyping Microbial) - λ 590nm - Max OD 2.5

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.558	-2.080	-2.997	-7.993	-4.658	-4.176	-2.957	-5.610	1.987	6.531	1.683	-3.823
B	-3.163	-5.153	-0.288	-4.423	0.962	1.376	-1.177	-0.635	0.054	4.119	-3.777	-2.927
C	-2.197	-2.738	-2.304	-3.480	-0.783	-1.266	-3.474	-2.785	-0.525	-5.456	0.182	0.859
D	-1.766	-1.861	-0.661	0.690	-0.905	21.701	17.048	52.135	-1.766	-0.480	-0.556	-2.052
E	-3.142	-3.105	-2.807	-1.085	-0.965	-1.600	-3.324	2.730	-3.659	-2.148	-7.067	-11.095
F	-2.583	-5.479	-2.886	-1.622	-1.380	-0.240	-1.783	0.030	0.926	1.340	-4.509	3.860
G	12.200	12.434	6.434	7.276	0.072	-0.786	5.884	7.080	3.401	-0.698	-2.588	-4.681
H	0.528	-3.861	-0.246	-0.764	3.753	4.506	2.564	-5.149	0.064	1.364	0.601	-1.005



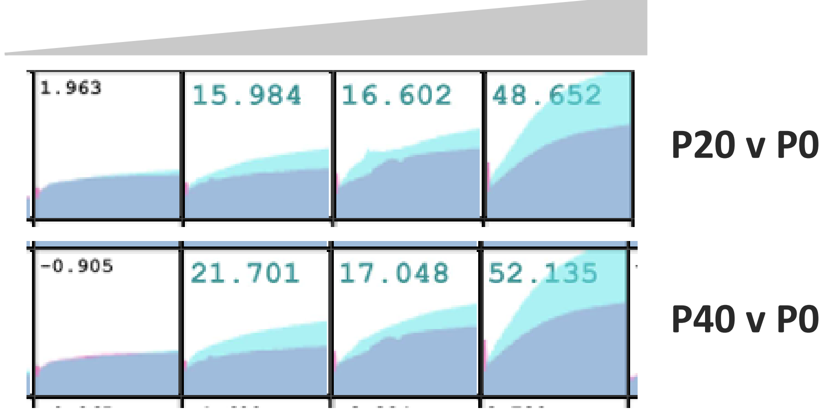
S thermophilus 19258 P40 to S thermophilus 19258 P0 PM09 (Phenotyping Microbial) λ 590nm - Max OD 2.5

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.562	-1.701	1.378	-4.869	4.724	-11.895	6.598	6.873	0.167	9.722	9.058	-12.475
B	-5.741	3.487	4.040	2.177	-5.900	8.247	-2.599	-3.110	-5.528	1.903	0.266	-17.813
C	-3.386	1.290	1.007	-9.139	3.639	9.667	0.295	1.041	-8.770	5.914	-1.557	-4.883
D	4.702	2.936	-6.837	-4.045	-7.914	-8.224	-1.383	-3.717	0.184	-2.647	-1.350	-4.218
E	-4.686	-6.080	-6.310	-10.973	-3.972	-7.633	-2.070	0.851	-2.798	-4.141	-5.439	-3.368
F	-0.932	-0.799	-0.574	-0.583	-0.625	-0.743	-0.932	0.052	-0.482	-0.779	-1.291	-0.569
G	-3.435	-0.869	0.018	-0.937	-11.880	-1.983	-3.800	1.313	-7.025	-5.824	-1.089	-1.481
H	-0.700	-1.053	0.677	-0.906	0.775	0.078	-1.775	-1.108	-3.256	4.547	-4.081	-13.489

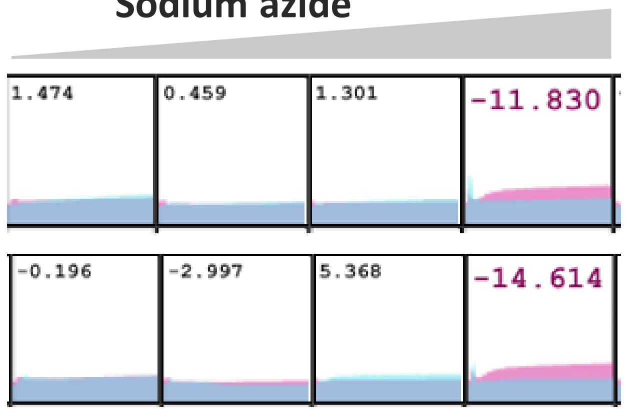
Values reported Reference – Test Area Under the Curve (AUC)

Changes in sensitivities (Response to inhibitory compounds)

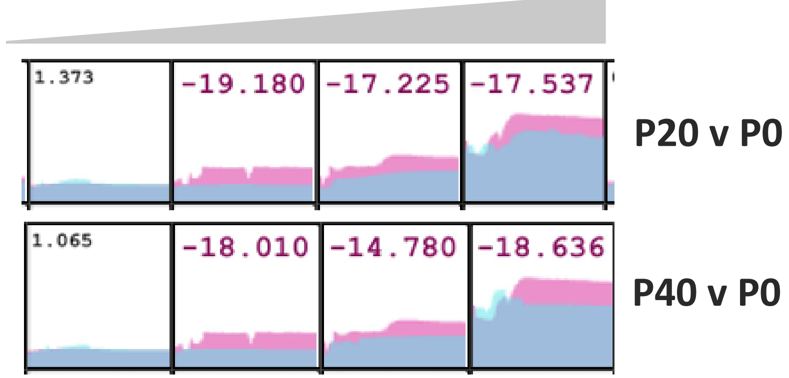
Iodonitrotetrazolium (INT)



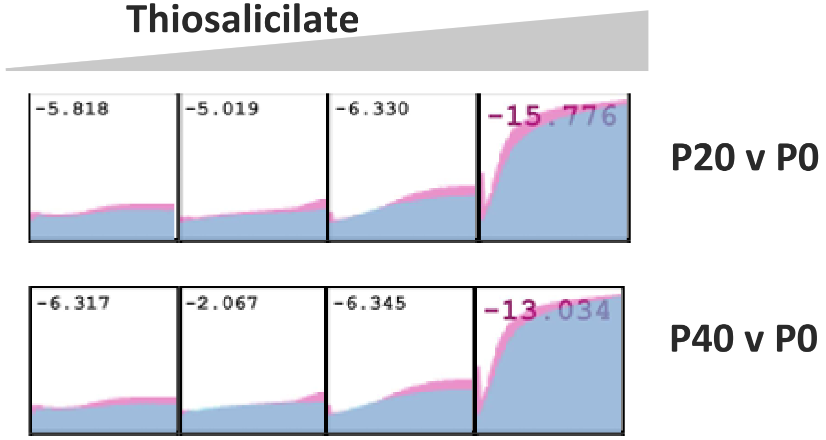
Sodium azide



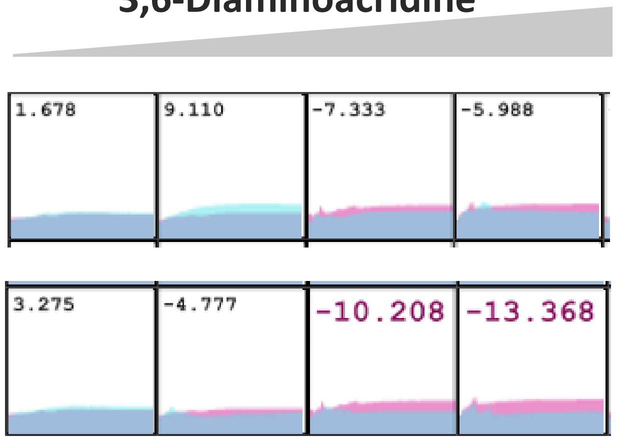
Ferric chloride



Thiosalicilate



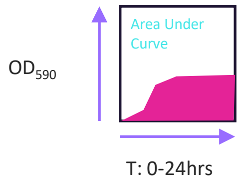
3,6-Diaminoacridine



Legend:

🟪 P0

🟦 P20/P40



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Summary of phenotypic changes in *S. thermophilus*

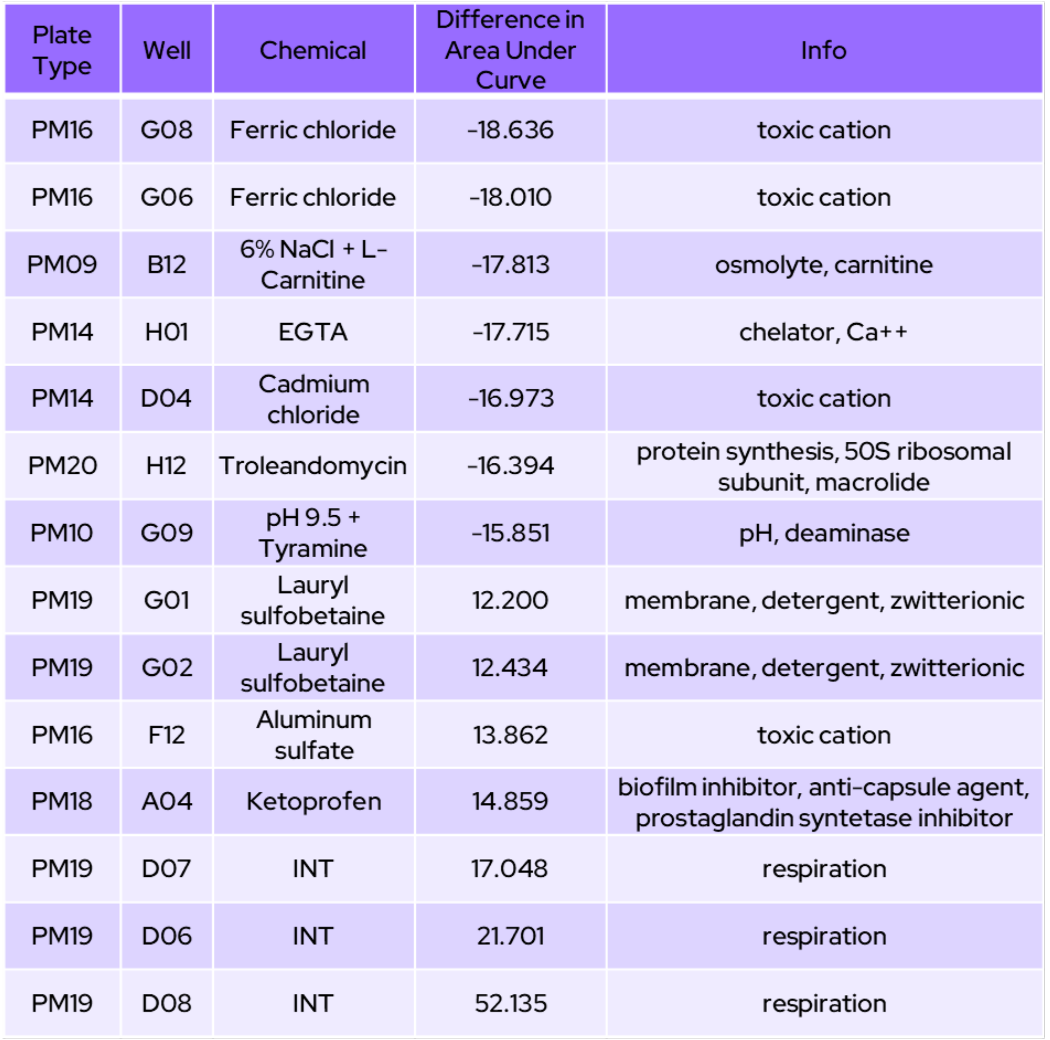
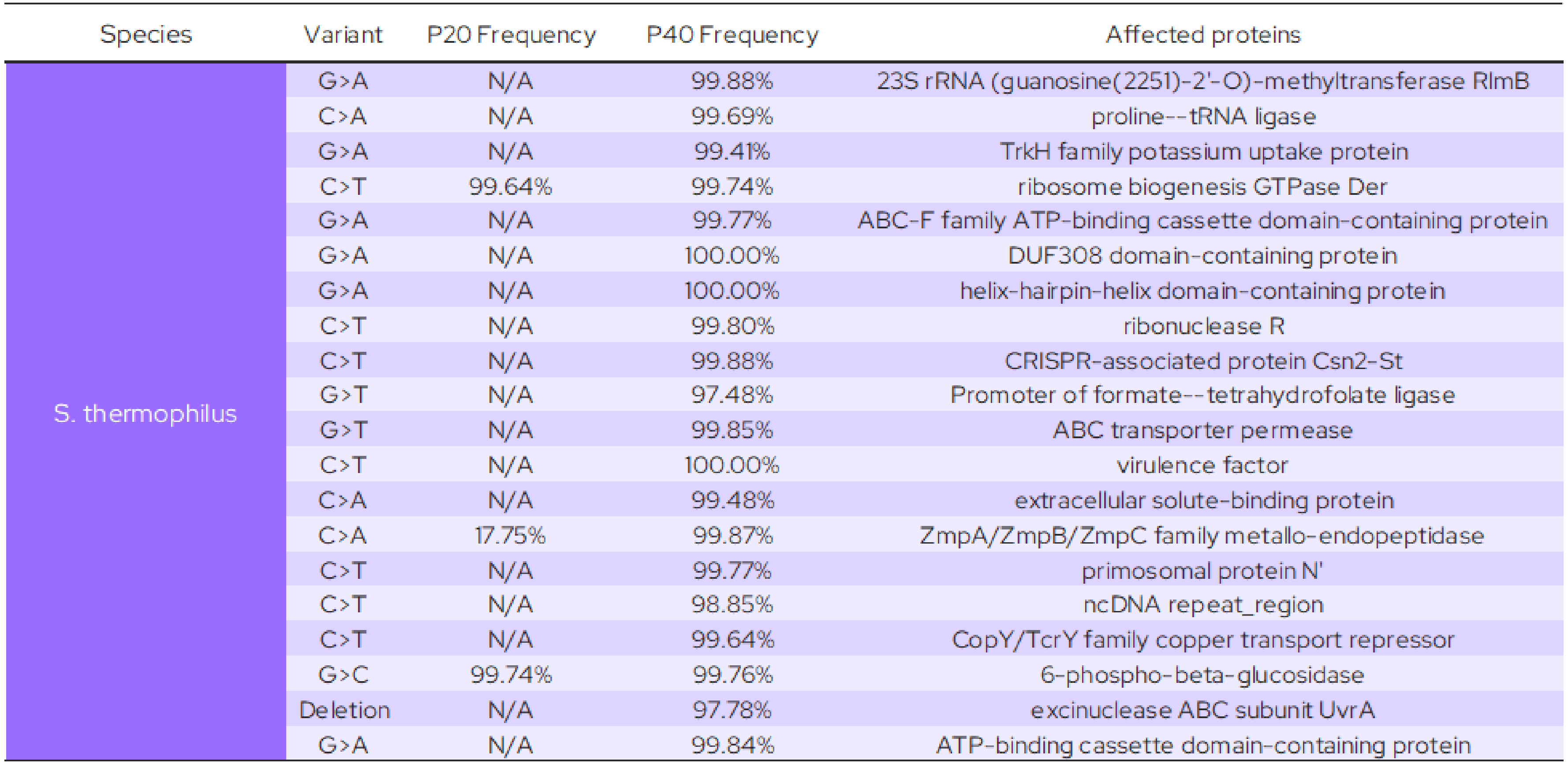


Plate Type	Well	Chemical	Difference in Area Under Curve	Info
PM16	G08	Ferric chloride	-18.636	toxic cation
PM16	G06	Ferric chloride	-18.010	toxic cation
PM09	B12	6% NaCl + L-Carnitine	-17.813	osmolyte, carnitine
PM14	H01	EGTA	-17.715	chelator, Ca++
PM14	D04	Cadmium chloride	-16.973	toxic cation
PM20	H12	Troleandomycin	-16.394	protein synthesis, 50S ribosomal subunit, macrolide
PM10	G09	pH 9.5 + Tyramine	-15.851	pH, deaminase
PM19	G01	Lauryl sulfobetaine	12.200	membrane, detergent, zwitterionic
PM19	G02	Lauryl sulfobetaine	12.434	membrane, detergent, zwitterionic
PM16	F12	Aluminum sulfate	13.862	toxic cation
PM18	A04	Ketoprofen	14.859	biofilm inhibitor, anti-capsule agent, prostaglandin syntetase inhibitor
PM19	D07	INT	17.048	respiration
PM19	D06	INT	21.701	respiration
PM19	D08	INT	52.135	respiration

S. thermophilus P40 has gathered quite a few significant phenotypes.

- Most extreme seem to be related to cation resistance and a shift away from respiration (INT)

Genetic drift



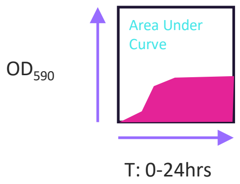
Species	Variant	P20 Frequency	P40 Frequency	Affected proteins
S. thermophilus	G>A	N/A	99.88%	23S rRNA (guanosine(2251)-2'-O)-methyltransferase RlmB
	C>A	N/A	99.69%	proline--tRNA ligase
	G>A	N/A	99.41%	TrkH family potassium uptake protein
	C>T	99.64%	99.74%	ribosome biogenesis GTPase Der
	G>A	N/A	99.77%	ABC-F family ATP-binding cassette domain-containing protein
	G>A	N/A	100.00%	DUF308 domain-containing protein
	G>A	N/A	100.00%	helix-hairpin-helix domain-containing protein
	C>T	N/A	99.80%	ribonuclease R
	C>T	N/A	99.88%	CRISPR-associated protein Csn2-St
	G>T	N/A	97.48%	Promoter of formate--tetrahydrofolate ligase
	G>T	N/A	99.85%	ABC transporter permease
	C>T	N/A	100.00%	virulence factor
	C>A	N/A	99.48%	extracellular solute-binding protein
	C>A	17.75%	99.87%	ZmpA/ZmpB/ZmpC family metallo-endopeptidase
	C>T	N/A	99.77%	primosomal protein N'
	C>T	N/A	98.85%	ncDNA repeat_region
	C>T	N/A	99.64%	CopY/TcrY family copper transport repressor
	G>C	99.74%	99.76%	6-phospho-beta-glucosidase
	Deletion	N/A	97.78%	excinuclease ABC subunit UvrA
	G>A	N/A	99.84%	ATP-binding cassette domain-containing protein

- Using whole-genome sequencing, we identified a total of 5 SNPs in P20 when the reads were aligned to the P0 reference sequence.
- P40 exhibited 20 additional SNPs and 1 deletion. These variants affected 19 protein coding genes and 5 ribosomal RNAs.

Lactobacillus casei

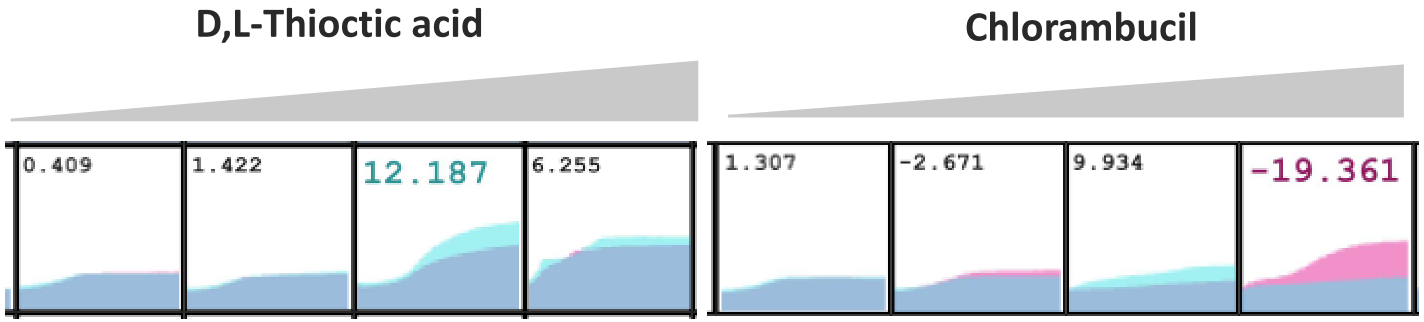
(ATCC® 393™)

L. casei



- Passage 0 (P0) and P15 strains were assessed with PM1-20 anaerobically without dye and incubated in Odin for 24 hours at 36°C and reads were taken every 20 minutes

When phenotype appeared	# of significantly changed substrates	
	Metabolics	Sensitivities
P15	0	8



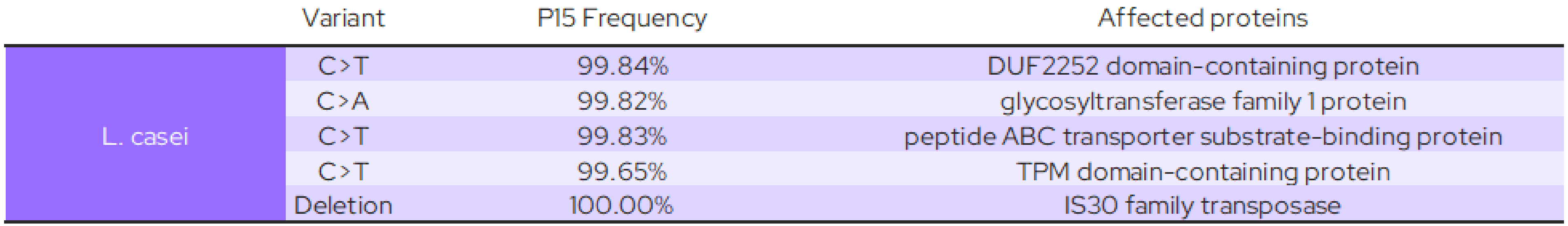
P15 v P0

Legend:

P0 **P20/P40**

- All values reported are Area Under the Curve (AUC)
- Values are averaged from triplicate runs

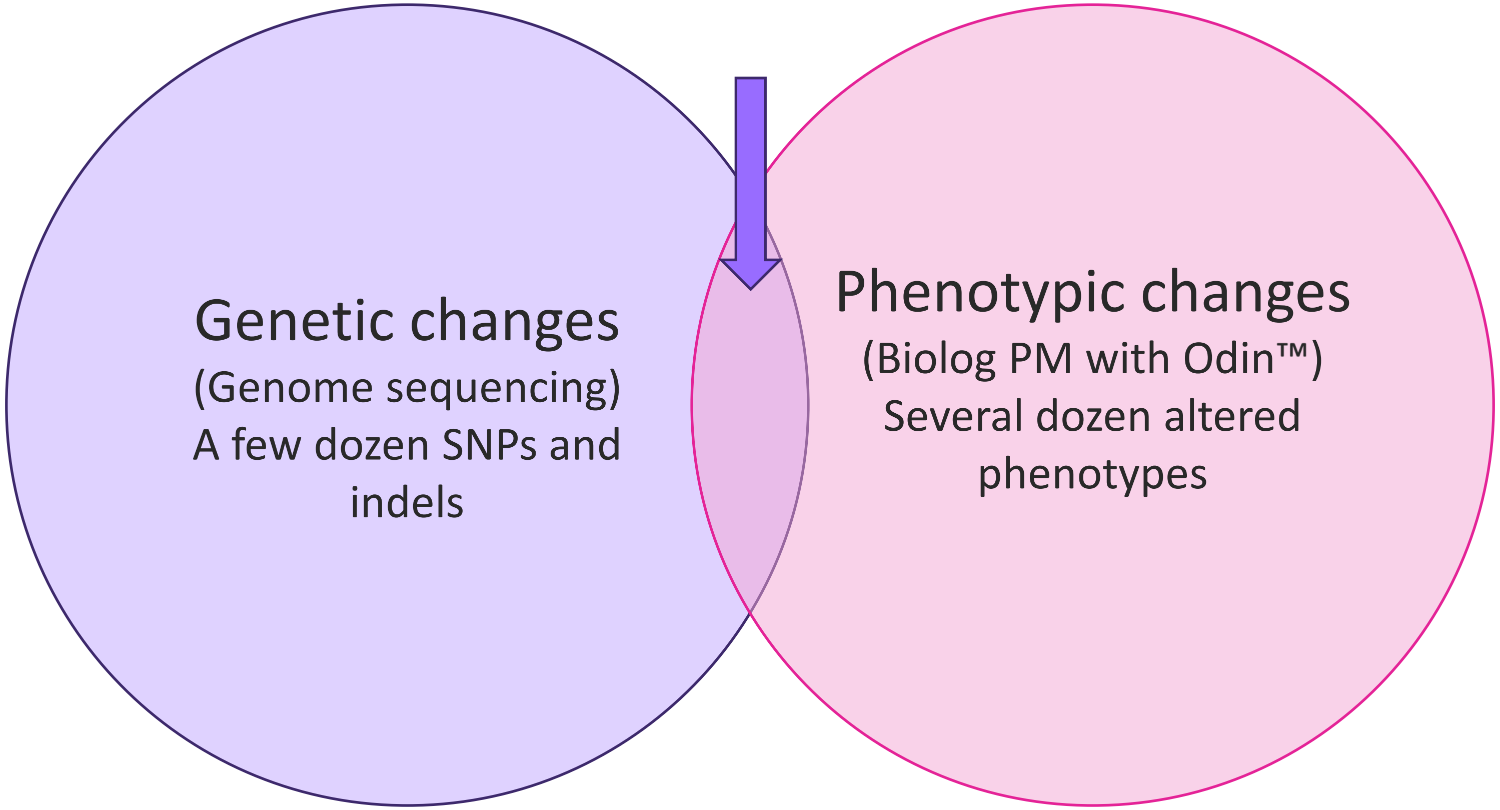
Genetic drift



	Variant	P15 Frequency	Affected proteins
L. casei	C>T	99.84%	DUF2252 domain-containing protein
	C>A	99.82%	glycosyltransferase family 1 protein
	C>T	99.83%	peptide ABC transporter substrate-binding protein
	C>T	99.65%	TPM domain-containing protein
	Deletion	100.00%	IS30 family transposase

- P15 exhibited 7 SNPs and a deletion mutation compared to P0. These variants affected 5 protein coding genes.

Monitoring both genotype and phenotype revealed unique changes in all three production strains



Only a few mutations in genes related to detected phenotypes

Genetic changes
(Genome sequencing)
A few dozen SNPs and indels

Phenotypic changes
(Biolog PM with Odin™)
Several dozen altered phenotypes

Phenotypic and genotypic drift monitoring is critical for reproducible and consistent results

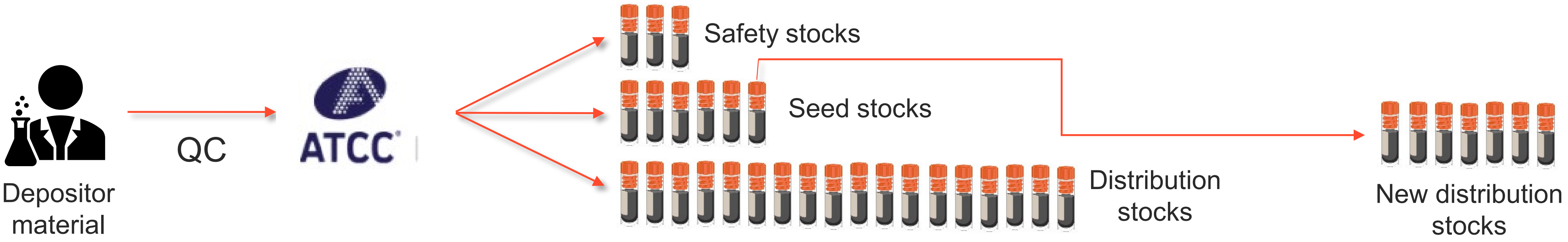
- Strains are passaged frequently as part of normal lab operations
- Drift in genome sequence happens at predictable rates
- Phenotypic drift can be independent of mutations and occur randomly
- Monitoring for both while minimizing passages reduces risk

Establish a baseline for important strains

- Whether WHO-listed fungal pathogens, or high value production strains, having a solid phenotypic foundation is critical
- Biolog Phenotype MicroArrays with Odin™ can streamline screening by screening >2000 unique conditions simultaneously
- ATCC® microbial stocks provide traceable and reliable points of reference

Authenticated physical material

Always start your research and bioproduction with reliable authenticated material



Depositor material

QC

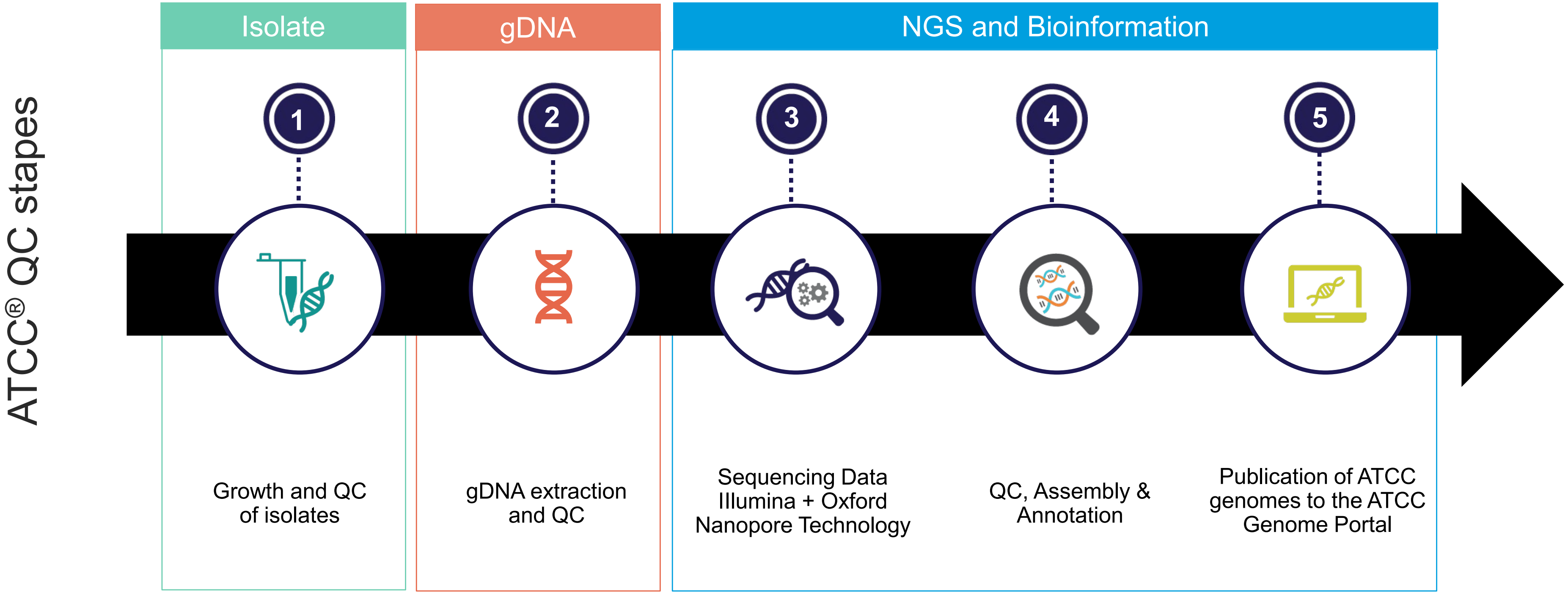
ATCC

Safety stocks

Seed stocks

Distribution stocks

New distribution stocks



ATCC® QC stapes

Isolate

1. Growth and QC of isolates

gDNA

2. gDNA extraction and QC

NGS and Bioinformation

3. Sequencing Data Illumina + Oxford Nanopore Technology
4. QC, Assembly & Annotation
5. Publication of ATCC genomes to the ATCC Genome Portal

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