4-2020

Mitochondria as a Potential Antifungal Target for Isocyanide Compounds

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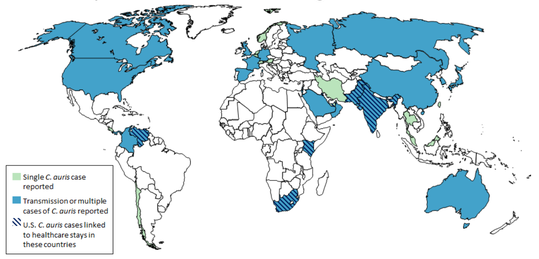
Background

- Antibacterial and antifungal resistance has created a need for new antimicrobial compounds with different mechanisms of action relative to established drugs.
- Fungi and mammals are both eukaryotes, so it is difficult to find a compound that is effective in fungi and is not toxic to mammals.
- Natural isocyanide such as the fungal-natural product Xanthocillin have antimicrobial properties[1] and are generally non-toxic in mammalian cell culture models.
- Para-nitrophenyl isocyanide (p-NPIC), was synthesized as a "model" isocyanide and tested for its ability to inhibit microbial growth.



1. Lim FY, Won TH, Raffa N, Baccile JA, Wisecaver J, Rokas A, … Keller NP. Fungal Isocyanide Synthases and Xanthocillin Biosynthesis in Aspergillus fumigatus. *mBio*. 2018; 9:e00785-18.
2. Center for Disease and Control. Countries from which Candida auris cases have been reported, as of May 31, 2019. https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html#world. July 18, 2019.

Xanthocillin and Para-nitrophenyl Isocyanide Structure

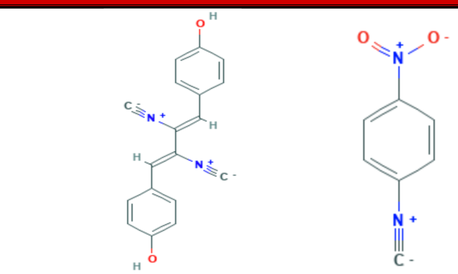


Figure 1: The chemical structure of the natural compound, Xanthocillin (left), produced by *Penicillium notatum*. The synthetic compound, para-nitrophenyl isocyanide (p-NPIC) was synthesized by Dr. David Berkowitz from the Department of Chemistry at the University of Nebraska–Lincoln (right).

Research Questions

- Is para-nitrophenyl isocyanide an effective anti-microbial compound?
- If so, what is the minimum inhibitory concentration of para-nitrophenyl isocyanide to deletion mutants in solid and liquid media?
- What is the molecular mechanism of action of para-nitrophenyl isocyanide?

Testing p-NPIC on *S. cerevisiae* Mutants on Solid Medium

Determining MIC on *S. cerevisiae* and *C. albicans* on Solid Medium

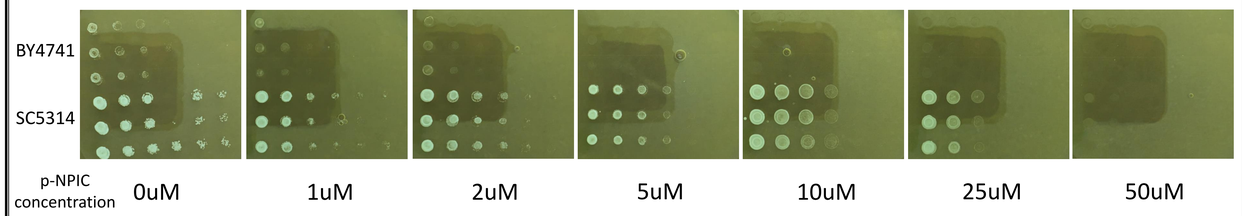
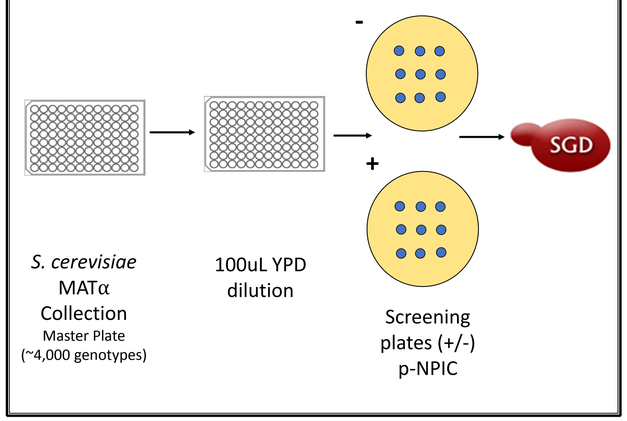


Figure 4: Determining the MIC of *Saccharomyces cerevisiae* strain (BY4741) and *Candida albicans* strain (SC5314) on solid YPD media containing various concentrations of p-NPIC. Cultures were serial diluted 5-fold and transferred using a 48 pin multi-blot replicator. Plates were grown at 30ºC for 24 hours.

Genetic Screening on Solid Media Methods

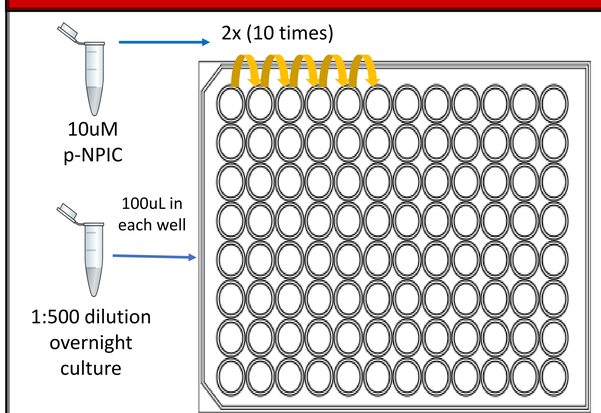


Hypersensitive Deletion Mutants on Solid Media

Gene Classes	# of Hypersensitive Mutant Strains	List of Affected Genes
Mitochondrial function	60	AIM10, MRPL, RPO41, …
Vacuolar membrane ATPase	10	VMA4, VMA6, VPH2, …
Iron-sulfur and copper binding	7	FRE2, GRX5, SCO1, …
Cytochrome C function	5	COX7, OXA1, QCR2, …
Ribosomal function	4	RPS23B, RPP2B, BUD21, …
Translation factors	3	HCR1, TIF3, MRN1
Other	76	

Testing p-NPIC on *S. cerevisiae* Mutants in Liquid Medium

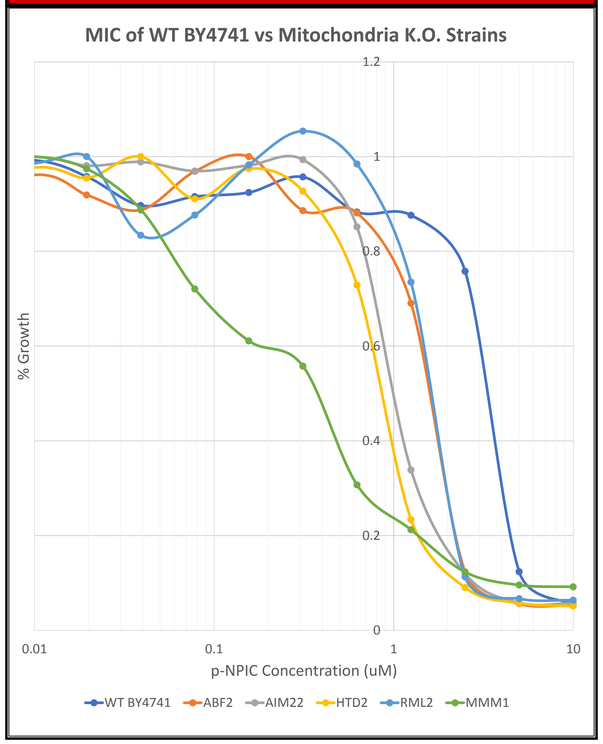
p-NPIC Serial Dilution Method



Hypersensitive Deletion Mutants in Liquid Media

Gene Classes	# of Hypersensitive Mutant Strains	Top 3 Most Affected Genes
Mitochondrial function	21	GCV3, HTD2, OAR1
Vacuolar membrane ATPase	7	VMA6, VMA10, VMA16
Iron-sulfur and copper binding	3	NFU1, GRX5, IBA57
Cytochrome C function	2	QCR2, COX19
Translation factors	1	TIF3
Other	13	HMF1, MOT3, NUP133

MIC of Hypersensitive Mitochondria Deletion Mutants in Liquid Media



Conclusions

- Disrupting genes involved in mitochondrial function, iron and copper homeostasis, and the vacuolar membrane ATPase cause hypersensitivity to this compound.
- We hypothesize that p-NPIC and other isocyanides are disrupting metal homeostasis and the function of Complex IV, leading to cell growth arrest.

Future Questions

- Does p-NPIC disrupt metal homeostasis?
- Is p-NPIC an enhancer of ROS production at Complex IV?
- Do other synthetic isocyanide compounds share the same mechanism of action?

Acknowledgments

This work was supported by the UNL UCARE Research program and an NIH Jumpstart seed award from the UNL Office of Research.