antifungal secondary metabolites have always been the prevalent source of drug development, exemplified by the echinocandins and polypeptide drug classes. Yet, the golden age discovery platforms were abandoned due to compound rediscovery and its economic cost.

Study: In an effort to retrace the original success stories, we combined the traditional approach of screening and cataloging for antifungal secondary metabolites with modern advances in sequencing, genomics, and high-throughput screening platforms.

Materials and methods: The screening was performed by focused enrichment of secondary metabolic pathways in Talaromyces. The primary screening was performed by cultivating Talaromyces in media containing either 0.5 or 1 mM glycerol. The resulting fungal biomass was then screened for antifungal activity.

Results: Several species were identified as producing antifungal secondary metabolites that are currently absent in the literature. Further, the compound was retested in relevant bioassays to validate the species as a producer of a known or a variant of a known antifungal compound. Moreover, several novel species are novel based on BLAST analysis. Generally producing our current lead include: Bionemastax, Teonematella, Parahalobate, and fungi. Arthroleptis, Penicillium. Within the collection, the Penicillium species appear to produce variants of the antifungal structurally privileged class.

S3.4d The role of NLRP1 inflammasome in host defense during Talaromyces marneffei infection

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S3.4f Oral paper oral paper, September 21, 2022, 4:45 PM - 6:15 PM

Talaromyces (Penicillium) marneffei is the only therapeutically important pathogen in Talaroecyt. The pathogenesis of T. marneffei is not yet fully understood. Infection of T. marneffei is mostly associated with primary susceptibility, such as in children, newborns, and immunocompromised patients. Therefore, in the present study, we aimed to address the role played by the NLRP1 inflammasome during the T. marneffei systemic infection in mice.

We established T. marneffei infected mice and performed the NLRP1 mouse model with two groups of mice, including the Nlrp1-–/- mice and wild-type mice.

We found that infected mice deleted NLRP1 inflammasome activation and increased production of IL-1β upon pulmonary T. marneffei infection. Further, we demonstrated that T. marneffei can activate the NLRP1 inflammasome both in mice and human macrophages. And T. marneffei macrophages induced IL-1β release by infected macrophages is NLRP1 inflammasome-dependent. In vivo study, we found that NLRP1 contributes to the development of lethality in the early stage of pulmonary T. marneffei infection. However, Nlrp1-–/- mice showed similar survival to wild type in the middle stage of infection. That is a unique feature of the role of NLRP1 in T. marneffei systemic infection. Moreover, NLRP1 contributes to the pathogenesis of T. marneffei systemic infection in mice.

So, in the present study, we demonstrated that the NLRP1 inflammasome is activated during T. marneffei infection. For NLRP1 inflammasome plays a dual role during pathogenic T. marneffei early inflammatory response inducing a proinflammatory environment, and a subsequent excessive damaging inflammatory response that contributes to pathogenesis and mortality. This study identifies for the first time that activation of the inflammasome in the later stages of T. marneffei dramatically contributes to pathogenesis and suggests that targeting the inflammasome may be a therapeutic option to treat pathogenic T. marneffei infection.

S3.4e Unraveling the role of DOG genes in a novel alternative pathway of glycerol biosynthesis in Candida albicans and its influence on virulence

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DOG genes, encoding for 2-deoxy-D-xylulose-5-phosphate synthase for low molecular weight phosphoglycerates, with an anchor in biological function. In contrast to bacteriochlorophylls which have two DOG homologues, C. albicans only has one DOG gene. We hypothesized that DOG plays an important role under osmotic or toxic stress by bioenergetic glycerol biosynthesis, which is known to be related to virulence and virulence of this pathogen, via a novel alternative pathway.

The known classical pathway of glycerol production begins when the glycerol 3-phosphate dehydrogenase (G3PD) is converted into glycerol-3-phosphate (G-3-P) by a pair of glycerol-3-phosphate dehydrogenase Gpd1 and Gpd2. GPD1 is further dehydrogenated into glycerol by glycerol-3-phosphate dehydrogenase Gpd1 and Gpd2. However, an alternative pathway, where DEPS is dehydrogenated into DHX, which is subsequently converted into glycerol has been proposed, but the enzymes involved in this process have not yet been described. We recently showed in Saccharomyces cerevisiae, the dog enzymes are involved in the production of DHX from DEPS, thereby allowing the synthesis of glycerol in the absence of the classical pathway. Overexpression of the DOG genes restored the normal-growth of the gpd1 and gpd2 double deletion strain. Further, the DEPS and DHX enzymes also can convert DHX to glycerol (Anwul et al., submitted).

Since DOG1 has a potential role in bioenergetic glycerol via an unconventional route, we are interested to determine its contribution in influencing virulence and biofilm formation in Candida albicans. This pathway has been overlooked for the past two decades, leaving behind an evidence knowledge gap. We have now generated multiple deletion strains, using CRISPR-Cas9 for the C. albicans counterparts of the GPD1 and DOG genes as well as multiple DOG overexpression strains in which we observed the restoration of osmotic stress tolerance phenotypes and biofilm growth. We also have NMR data showing the accumulation of various metabolites of central metabolism in these strains. Additionally, we have determined the possible role of DOG genes in biofilm formation or as well as in virulence, the latter with our carbonyl-based high throughput screen model system. We also linked DOG1 and its role in glycerol metabolism with the survival of the in vivo mice model. Furthermore, we would be setting up a high throughput small compound screening for this phasor as a potential antifungal drug target.