antifungal secondary metabolism has always been the prevalent source for drug development, exemplified by the echinocandins and polyene drug classes. Yet, the golden age discovery platforms were abandoned due to compound rediscovery and its pared economic cost.

Study: In an effort to retrace the original success stories, we combined the traditional approach of screening and assessing for antifungal secondary metabolites with modern advances in sequencing, genome mining, and metabolomics approaches, LC-MS, and NMR.

Solid bacteria and fungi were isolated through in vitro cultivation via the SipHop method. After application of the OSMAC approach, 389 broth were identified with activity against Candida albicans. To prioritize active strains, several criteria were set up; low to absent mammalian host cell toxicity, activity against a broad spectrum of fungal pathogens including wild-type reference strains, and established antifungal drug resistance strains and species identification of the producing strain. Continuing Lead hits were purified using bioactivity-based semi-preparative HPLC. The resulting pure fractions were analyzed by tandem LC-MS-MS, and proposed structures were later confirmed with NMR in vitro and in vivo validation of the purified compounds would be performed.

Additionally, aside from discovering a novel antifungal compound, another project goal is to gauge if impurities spectroscopy can provide an early indication regarding the mode of action of the present antifungal agent. For this, a PTC study was performed which showed that different antifungal drug classes provide distinct signature profiles by which they can be classified. As such, when active strains broths show unique impurity profiles, in comparison with the signature profiles of established antifungal drugs, it suggests that they work through a different mode of action.

Results: Several species were identified as producing antifungal secondary metabolites that are currently absent in the literature. Either the compound was novel or literature never described the species or a known producer, in case of a known antifungal compound. Moreover, several species are novel based on Binning sequence. Generally producing our current lead include bacteria: Pseudomonas, Teunamurrella, Parahalobacteriella, and fungi: Athelia, Penicillium. Within the collection, the Penicillium species appear to produce variants of the antifungal non-ribosomal polyether class.

S3.4d
The role of NLRP3 inflammasome in host defense during *Talaromyces marneffei* infection

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S3.4d.1 Animal oral paper session, September 21, 2022, 4:41 PM - 6:15 PM

*Talaromyces marneffei* (T. marneffei) is the only therapeutically important pathogen in Talaromyces. The pathogenesis of T. marneffei in mammals is not yet fully understood. *Infection of T. marneffei* cannot normally be cleared in murine models. The results of these studies are in the most critical characteristics of the present infection. Moreover, NLRP3 inflammasome activation in murine *T. marneffei*-infected immunocompromised mice is expected. Therefore, in the present study, we aimed to address the role played by the NLRP3 inflammasome in the murine *T. marneffei* infection in mice.

We established *T. marneffei* infected murine pulmonary model with two groups of mice, including the Nlrp3-/- mice and wild-type mice.

Objective: To determine if lung infected *T. marneffei* inflammasome activation and increased production of IL-1β upon pulmonary T. marneffei infection. Further, we demonstrated that T. marneffei caused activated the NLRP3 inflammasome both in mice and human macrophages. And T. marneffei induced IL-1β release by infected macrophages is NLRP3 inflammasome-dependent.

In vivo study, we found that NLRP3 contributes to the development of lethality in the early stage of pulmonary T. marneffei infection. However, Nlrp3-/- mice showed a similar lung weight to the WT in the middle stage of infection and the WT showed a number of lower lung weight than that of the NLRP3 mutation in the WT mice.

Moreover, NLRP3 contribution to pulmonary infection in *T. marneffei* infection is due to increased lung inflammation and pulmonary injury. So, in the present study, we demonstrated that the NLRP3 inflammasome is activated during *T. marneffei* infection. For NLRP3 inflammasome plays a dual role during the 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 stage of TSM deminimally contributes to the pathogenesis and suggests that targeting the inflammasome may be a therapeutic option to target *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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S3.4e.1 Animal oral paper session, September 21, 2022, 4:41 PM - 6:15 PM

DOG genes, encoding for 2-deoxyglucose-6-phosphate phosphohydrolase or low molecular weight phosphatases, with an axonemal biological function. In contrast to bacterialrbohydrates, these two DOG homologs, C. albicans only has one DOG gene. However, DOG plays an important role under osmotic or toxic stress by bioconverting glycerol which is known to be vital for bud formation and virulence of this pathogen, yet via a novel alternative pathway.

The known classical pathway of glycerol production begins when the glycerol intermediate dihydroxyacetone phosphate (DHAP) is converted into glycerol-3-phosphate (G-3-P) by a pair of glycerol-3-phosphate dehydrogenase, Gpd1 and Gpd2. Gpd1 or Gpd2 is further dephosphorylated into glycerol by glycerol-3-phosphate phosphatase, Gpd1 and Gpd2. However, an alternative pathway, where DHAP is dephosphorylated into DHG, which is subsequently converted into glycerol has been proposed, but the enzymes involved in this process have not yet been described. We recently found that in Saccharomyces cerevisiae, the DOG1 and DOG2 are involved in the production of DHG from DHAP, thereby allowing the synthesis of glycerol in the absence of the classical pathway. Overexpression of the DOG genes reversed the osmotic tolerance of the gpd1Δ gpd2Δ and gpd1Δ gpd2Δ Δgal1Δ strains, which are otherwise lethal. Our findings also confirmed the relevance of all variants for both fungal pathogens (Can et al., submitted).

Since DOG1 has a potential role in bioconverting glycerol via an unconventional route, we are interested to determine its contribution in influencing virulence and biofilm formation in Candida albicans. This pathogenic pathway has been overlooked for the past two decades, leaving behind an evident knowledge gap. We have now generated multiple deletion strains, using CRISPR-Cas9 for the C. albicans counterparts of the GPD1 and GPD2, and DOG genes as well as multiple DOG1 overexpression strains in which we observed the restoration of osmotic stress tolerance phenotypically and via growth curves. We also have NMR data showing the accumulation of various metabolites of central metabolism in these strains. Additionally, we have determined the influence of the role of DOG1 and DOG2 in biofilm formation or virulence as well as on stress, the latter with our carboxylated biofilm substrates mouse model system. We also linked DOG1 and its role in glycerol metabolism to the survival of C. albicans inside macrophages. Finally, we would be setting up a high-throughput small compound screening for this phasitogen as a potential antifungal target.
Human pythiosis

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Most the expert session, September 22, 2022, 8:00 AM - 9:00 AM

Human pythiosis is a rare, life-threatening infection, which is generally caused by Pythium insidiosum, a fungal-like organism. Four forms of human pythiosis are described: 1) vascular pythiosis affecting the patient’s arteries causing amput- ate, thrombosis, and gangrene; 2) ocular pythiosis, mainly causing infections of the cornea; 3) skin and soft tissue infections (cutaneous and subcutaneous pythiosis); and 4) disseminated pythiosis. Vascular pythiosis is associated with high mortality and has a mortality rate of 50%-90%, which attributes to difficulties in diagnosis of the infection and the lack of effective standard treatment. Risk factors and patients with chalcosiosis are at risk for vascular infection. Early diagnosis of Pythium infection requires a high index of clinical suspicion and is essential for good treatment results. Macroscopic morphology of Pythium spp. resembles other non-septate hyphae, such as agents of mucormycosis. Definite laboratory diagnosis includes te- nase cultivation with zoospore induction, polymerase chain reaction (PCR), and detection of Pythium antibodies. Radical surgery together with antimicrobial therapy, and antifungal agents (e.g., terbinafine and itraconazole) have previously been used for the treat- ment of human pythiosis. However, patients with incomplete surgical resection had almost 100% mortality. A novel therapeutic approach and reliable biomarkers are needed to improve patient outcomes.

There are several studies demonstrating excellent in vitro activity of antibacterial agents, especially macrozoles, tetracyc- clines, and oxazolecarboximides against P. insidiosum. There are also evidences of synergy among antibacterial classes such as tetracyclines and macrolides. In 2019, Susongrat, et al. reported successful treatment of two patients, who had inoperable intraabdominal vascular pythiosis, with adjunctive antibacterial agents as salvage therapy. The medications included itraconazole in combination with doxycycline and amphotericin or clotrimazole. This case report also confirmed the result of a previous study by Worasilchai, et al. (2018) that serum 1,3-beta-D-glucan (BDG) is useful for monitoring disease activity. The ongo- ing multicenter prospective study is conducted to evaluate the use of antibacterial agents (macrolides and azalides) with itraconazole and surgery in vascular pythiosis and to evaluate the alternative markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Torvorapanit, et al. (2021) reported preliminary results of 10 patients, which demonstrated favorable outcomes. It was shown that 2/4 patients with the residual disease could successfully be treated with medications. ESR and CRP declined over time after treatment and Spearman’s correlation of ESR and BDG was 0.65, and between CRP and BDG was 0.4. The study aims to recruit 50 patients, which should assess the usefulness of antibacterial agents and these markers in patients with vascular pythiosis.

S4.1a Fusarium: MICs, mono versus combination therapy and 3osanogepic
Martin Hoernigl
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S4.1 Treatment of rare mold infections in 2022: the role of new and old antifungals, September 22, 2022, 10:30 AM - 12:00 PM

Penicillium is one of the most clinically prevalent rare molds causing superficial infections such as keratitis in immuno- compromised hosts and severe disseminated infections frequently presenting as fungemia in the immunocompromised. These fungi are ubiquitous in nature and are found in soil and air. Only a few of the > 70 Penicillium spp. are opportunistic pathogens in humans.