antifungal secondary metabolites have 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 redundancy and its economic burden.

Study: In an effort to revisit the original success stories, we combined the traditional approach of screening for antifungal secondary metabolites with modern advances in sequencing, genomics, and metabolicomics, to isolate novel antifungal candidates.

Results: Secondary metabolites were isolated from a fungal strain identified during a screening campaign that targets the NLRP3 inflammasome-dependent bacterial clearance pathway. Several compounds, including a known antifungal compound, were identified. Moreover, a novel antifungal compound was identified and shown to exhibit antifungal activity against a panel of fungal species.

Conclusion: This study highlights the potential of revisiting traditional screening approaches to discover novel antifungal candidates.

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

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A Porcine oral paper was submitted, September 21, 2022, 4:41 PM - 6:15 PM

Talaromyces (Penicillium) marneffei is the only thermally dimorphic pathogenic Talaromyces. The pathogenesis of T. marneffei in mammals is not yet fully understood. Inhibition of T. marneffei consilia without normal clearance may result in consilia dissemination throughout the body and lead to disseminated infection. In TSM patients, studies have shown that the ratio of G:18 is very high and was consequently associated with the severity of the response and outcome of post-treatment. Therefore, in the present study, we aim to address the role played by the NLRP3 inflammasome in the pathogenicity of T. marneffei infection in mice.

We established T. marneffei infected mice pulmonary models with two groups of mice, including the Nlrp3−/− mice and wild-type mice. We found that infected mice displayed NLRP3 inflammasome activation and increased production of IL-1β upon pulmonary T. marneffei infection. Further, we demonstrated that T. marneffei consilia activated the NLRP3 inflammasome both in mice and human macrophages. And T. marneffei consilia induced IL-1β released 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 fungal load to the WT in the middle stage of infection, and NLRP3−/− mice had a lower number of infected WT mice could be seen in the late stage of infection. Moreover, NLRP3 contributes to pathogenic inflammation in pulmonary T. marneffei infection and contributes to neutrophil infiltration 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 pathogenic T. marneffei infection, an early inflammatory response inducing a protective 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 late stages of TSM detrimentally contributes to pathogenesis and suggests that targeting the inflammasome may be a therapeutic option to target pathogenic T. marneffei infection.

S3.4e Unraveling the role of DOG genes in a novel alternative pathway of glyceral biosynthesis in Candida albidi- canae and its influence on virulence

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DOG genes, encoding for 2,3-dihydroxy-6-phosphogluconate dehydratase, for low molecular weight phosphogluconates, with an amino- terminal histidine kinase. In contrast to Buchnera cyanovorins which have two DOG homologs, C. albidi canae only harbor one DOG gene. We hypothesized that DOG plays an important role under osmotic or toxic stress by biosynthesizing glycerol, which is known to be useful for biofilm formation and virulence of this pathogen. Yet, via a novel alternative pathway.

The known classical pathway of glycerol production begins when the glycerol intermediate molecule dihydroxyacetone phosphate (DHAP) is conserved into glyceral-3-phosphate (G-3-P) by a pair of glyceral-3-phosphate-dehydrogenase, Gpd1 and Gpd2. However, an alternative pathway, where DHAP is dehydroxyphosphorylated into DHAG, which is subsequently converted into glycerol has been proposed, but the steps involved in this process have not yet been described. We recently showed that in Buchnera cyanovorins, the DOG domain are involved in the production of DHAG from DHAP, thereby allowing the synthesis of glycerol in the absence of the classical pathway. Overexpression of the DOG genes restored the osmoregulation of the glyp1 and glyp2 mutants, further demonstrating that DOG plays an important role in glycerol production and compensating for the DHAP dehydroxyphosphorylation pathway.

Since DOG has a potential role in biosynthesizing glycerol via an unconventional route, we are interested to determine its contribution in influencing virulence and biofilm formation in Candida albidi canae. This 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. albidi canae counterparty of the C. GPD, 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 influence of the role of DOG in albidi canae, biofilm formation in vivo as well as in vitro, the latter with our carbon-based biofilm substrates mouse model system. We also linked DOG and its role in glycerol synthesis to the survival of the albidi canae in mouse macrophages. Finally, we would be setting up a high throughput small compound screening for this potential antifungal drug target.
M1.2
Human pythiosis

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Meet 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 arteritis, 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 10%-40%, which attributes to difficulties in diagnosis of the infection and the lack of effective standard treatment. Rice farmers and patients with diabetes 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 tissue cultivation with zoospore induction, polymerase chain reaction (PCR), and detection of Pythium antibodies. Radical surgery together with immunotherapy, and antifungal agents (e.g., terbinafine and itraconazole) have previously been used for the treatment 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 macrolides, tetracyclines, and oxazolidinones against P. insidiosum. There are also evidences of synergy among antibacterial classes such as tetracyclines and macrolides. In 2018, Susaengrat, 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 antibiotics or clarithromycin. This case report also confirmed the result of a previous study by Worrachai, et al. (2018) that serum 1,3-beta-D-glucan (BDG) is useful for monitoring disease activity. The ongoing multicenter prospective study is conducted to evaluate the use of antibacterial agents (amphotericin and doxycycline) with itraconazole and surgery in vascular pythiosis and to evaluate the alternative markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Tavoseongnat, et al. (2021) reported preliminary results of 10 patients, which demonstrated favorable outcomes. It was shown that BDG, ESR, and CRP declined over time after treatment and Spearman’s correlation between ESR and BDG was 0.65, and between CRP and BDG was 0.6. The study aims to recruit 50 patients, which should confirm the usefulness of antibacterial agents and these markers in patients with vascular pythiosis.

S4.1a
Fusariosis: MICs, mono versus combination therapy and feomaneurigose

Martin Hornigl
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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

Pneumatocystis is one of the most clinically prevalent rare molds causing superficial infections such as keratitis in immunocompromised 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 Pneumocystis spp. are opportunistic pathogens in humans.