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 rediscovery and its economic return.

Study: In an effort to retrieve the original success stories, we combined the traditional approach of screening and searching for antifungal secondaries metabolites with modern advances in sequencing, genomics, metabolomics, transcriptomics, HPLC, LC-MS, and NMR.

Solid broth and fungi were isolated through in vitro cultivation via the CIP 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, to narrow down mammanians host cell toxicity, activity against a broad spectrum of fungal pathogens including wild-type reference strains, and established antifungal drug resistance variants and species identification of the producing strains. Continuing, lead hits were purified with structurally 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 will be performed.

Additionally, aside from discovering a novel antifungal compound, another project goal is to gauge if purified spec- troscopy can provide an early suggestion regarding the mode of action of the present antifungal agent. For this, a P-SC study was performed which showed that different antifungal drug classes provide distinct signature responses profiles by which they can be classified. As such, when active strain broths show unique signature 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 unknown or literature never described the species as a producer of a known, or variant of a known antifungal compound. Moreover, several novel species are novel based on Blommaert-cloning. Generally proving our current lead should include-bacteria: Pseudomonas, Tekkanamara, Parabehaella, and fungi: Athelia, Pichia. Within the collection, the Pseudomonas species appear to produce variants of the antifungal non-ribosomal peptidolysis class.

S.6.4

The role of NRPS in inflammation in host defense during Talaromyces marneffei infection

Liu Shu1, Wu Jinping2, Xi Liyan1, Xin Zhou1
1Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China
2Reindorp University Medical Center, Nijmegen, Netherlands

S.6.4.1 Poor oral paper session, September 21, 2022, 4:45 PM - 6:15 PM

Talaromyces (Pichia) marneffei (T. marneffei) is the only thermally dimorphic pathogen in Talaromyces. The pathogenesis of T. marneffei in mammals is not yet fully understood. Inhalation of T. marneffei conidia without normal clearance may result in conidial dissemination throughout the body and lead to disseminated infection. In TSM patients, studies have shown that at least 90% of patients present with 1-3 LCM-MS, and were constantly associated with the severity of organ and outcomes of post-treatment. This means poor outcome is likely associated with an overall severe immune response. Several studies have identified inflammation activation as an essential immune response in host defense against fungal pathogens. Among them, NRPSs are known to be involved in T. marneffei-induced immunopathology to be elucidated. Therefore, in the present study, we aimed to address the role played by the NRPSs in the T. marneffei systemic infection in mice.

We established T. marneffei infected mice pulmonary model with two groups of mice, including the NRIP-/- mice and wild-type mice. We found that infected mice displayed NRPS activation and increased production of IL-1β upon pulmonary T. marneffei infection. Further, we demonstrated that T. marneffei conidia activated the NRPSs inflammation both in vivo and macrophages. And T. marneffei conidia induced IL-1β release by infected macrophages is NRPS inflammation-dependent. In vitro study, we found that NRPS contributes to the development of pathology in the early stage of pulmonary T. marneffei infection. However, NRIP-/- mice showed a similar fungal load to the WT in the middle stage of infection and the lung tissue number per micrometer of the WT mice could not be significantly reduced. Moreover, NRPS contributes to pathogenic inflammation in pulmonary T. marneffei infection and contributes to survival prolongation and pulmonary injury.

So, in the present study, we demonstrated that the NRPSs activation is caused during T. marneffei infection. But NRPSs inflammation plays a dual role during T. marneffei infection: anti-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 inflammation in the latter stage of TSM detrimentally contributes to pathogenesis and suggests that targeting the inflammation may be a therapeutic option to treat pathogenic T. marneffei infections.

S.6.4.2 Unraveling the role of DOG genes in a novel alternative pathway of glycogen biosynthesis in Candida albici- can and its influence on virulence

Chinnaty Awards1, Tuger Van Gemert1, Lina Hermens1, Patrick Van Dijk1
1Laboratory of Molecular Cell Biology, Department of Biotechnology, Section of Molecular Biotecnology of Plants and Microorganisms, Institute of Botany and Biotechnology, KU Leuven, Leuven, Belgium
2Biomedical MIR/MOSCA, Department of Imaging and Pathobiology, KU Leuven, Leuven, Belgium

S.6.4.2 Poor oral paper session, September 21, 2022, 4:45 PM - 6:15 PM

DOG genes, encoding for 2-deoxy-D-glucose-6-phosphate phosphatase for low molecular weight phosphates, with an unknown biological function. In contrast to bacterial glucoseresinovar which has two DOG homologs, C. albicans only has one DOG gene. We hypothesized that DOG plays an important role under osmotic or toxic stress by biosynthesizing glycogen, which is known to be useful for cell survival and virulence of this pathogenic yeast, via a novel alternative pathway.

The known classical pathway of glycogen production begins when the glycolytic intermediate molecule dihydroxyacetone phosphate (DHAP) is conserved into glyceraldehyde-3-phosphate (G3-P) by a pair of glyceraldehyde-3-phosphate-dehydrogenases, Gpd1 and Gpd2. G3-P is further dephosphorylated into glyceraldehyde-3-phosphate-phosphomonoester, Gpd1 and Gpd2. However, an alternative pathway, where DHAP is dephosphorylated into DHA, which is subsequently converted into glyceraldehyde has been proposed, but the enzymes involved in this process have not yet been described. We recently showed that in Candida albicans, the DOG strain are involved in the production of DHA from DHAP, thereby allowing the synthesis of glycogen in the absence of the classical pathway. Overexpression of the DOG genes restored the osmo-tolerance of the gpd1 Δ gpd2Δ double deletion strain. Furthermore, phosphatase overexpression also conferred DHAP tolerance (Lee et al., submitted).

Since DOG has a potential role in biosynthesis glycogen 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 evident knowledge gap. We have now generated multiple deletion strains, using CRISPR-Cas9 for the C. albicans counterparts of the GPM, GPD, and DOG genes as well as multiple DOG overexpression strains in which we observed the restoration of osmotic stress tolerance phenotypically and in vitro 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 DOG gene deletion and biofilm formation in vitro as well as in vivo, the latter with our carbon-based biofilm substrates mouse model system. We also linked DOG and its role in glycogen biosynthesis to the survival of biofilm in mouse macrophages. Future, we would be setting up a high throughput small compound screening for this phasotrope as a potential antifungal target.