Objectives: Our study demonstrates that PIT has a better synergic lethal effect with FLC by inhibiting ergosterol synthase and higher safety and suggests that PIT may be suppressed as a promising adjuvant to make FLC fungicidal and enhance the efficacy of FLC in treating invasive fungal infections caused by pathogenic fungi with high FLC tolerance.

Conclusions: LF demonstrated antifungal activity against yeast species Cryptococcus, Candida, and Saccharomyces and was much less effective against molds. Good synergy was achieved with AMB but not nystatin or echinocandin drugs. While the iron-chelating capacity of LF was important for the antifungal activity it was not involved in synergy. SEM revealed cell damage suggesting an interaction between AMB, LF, and the fungal membrane or cell wall. A 10 residue peptide from the C-lob of LF was synthesized and tested for activity and synergy. This peptide, dubbed lactofungin (LFG) was inactive alone but was potently synergistic with AMB, indicating a direct role in augmenting AMB activity. Synthetic membranes loaded with ergosterol but not cholesterol were depleted by AMB + LFG, demonstrating that activity was fungal-specific and was mediated through ergosterol binding.

Conclusions: LF is a complex molecule that causes fungal inhibition via iron binding and when cleaved by pepsin can produce active peptides. An AMB is a highly toxic treatment, the use of LFG as a synergist could help increase activity while lowering the effective dose, thereby reducing undesirable side effects. The action of AMB + LFG appears dependent on ergosterol, suggesting inhibition will be highly fungal-specific.

S3.4c A pipeline toward the identification of novel antifungal compounds derived from the microbial dark matter

Griet Vannoppen1, Jeroen Wagenaars2, Alannah Holdendorv3, Alexander Peys2, Hainbo Hu2, Dries De Ruysscher1,2, Jolen Masschelein3, Walter Luyster1, Paul Vandecruys1, Patrick Van Dijck1
1 Laboratory of molecular cell biology, Leuven, Belgium
2 Animal physiology and neurobiology, Leuven, Belgium
3 Laboratory for biomolecular discovery and engineering, Leuven, Belgium

Background: The current armamentarium of antifungal drugs and the contracted variety in antifungal drug classes combined with the ever-rising threat of resistant fungal pathogens highlight the urgent need for novel antifungal compounds. Natural

S3.4b Lactoferrin, a natural source of peptides that potentiate the antifungal activity of amphoterin B

Kenya Fernandes1,2, Evelyne Desplagnes1, Sarmeeer Kuljan1,2, Richard Payne1,2, Dee Carter1,2
1 University of Sydney, Sydney, Australia
2 Sydney Institute for Infectious Diseases, Sydney, Australia

Background: Lactoferrin (LF) is a broad-spectrum antimicrobial host defense molecule with the ability to carry a variety of effector molecules, including peptides that target fungal cell membranes.

Methods: Lactoferrin (LF) was obtained from a commercial supplier and two dairy companies. LF was tested on a panel of pathogenic yeast and mold species for inhibition using CLSM microdilution methods. Synthetic peptides were synthesized with antifungal drugs amphoterin B (AMB), tyrocidine (NYL), flucytosine (FLC), azole-resistant (ITC), rycomycin (VRC), and 5-fluorocytosine (5FC). The effect of LF on fungal cells was analyzed using scanning electron microscopy (SEM). The active peptides within LF were then predicted from putative and in silico digested, synthesized, and tested for synergy with amphotericin B (AMB).

Results: LF demonstrated antifungal activity against yeast species Cryptococcus, Candida, and Saccharomyces and was much less effective against molds. Good synergy was achieved with AMB but not nystatin or echinocandin drugs. While the iron-chelating capacity of LF was important for the antifungal activity it was not involved in synergy. SEM revealed cell damage suggesting an interaction between AMB, LF, and the fungal membrane or cell wall. A 10 residue peptide from the C-lob of LF was synthesized and tested for activity and synergy. This peptide, dubbed lactofungin (LFG) was inactive alone but was potently synergistic with AMB, indicating a direct role in augmenting AMB activity. Synthetic membranes loaded with ergosterol but not cholesterol were depleted by AMB + LFG, demonstrating that activity was fungal-specific and was mediated through ergosterol binding.

Conclusions: LF is a complex molecule that causes fungal inhibition via iron binding and when cleaved by pepsin can produce active peptides. An AMB is a highly toxic treatment, the use of LFG as a synergist could help increase activity while lowering the effective dose, thereby reducing undesirable side effects. The action of AMB + LFG appears dependent on ergosterol, suggesting inhibition will be highly fungal-specific.

S3.4b Lactoferrin, a natural source of peptides that potentiate the antifungal activity of amphoterin B

Kenya Fernandes1,2, Evelyne Desplagnes1, Sarmeeer Kuljan1,2, Richard Payne1,2, Dee Carter1,2
1 University of Sydney, Sydney, Australia
2 Sydney Institute for Infectious Diseases, Sydney, Australia

Background: Lactoferrin (LF) is a broad-spectrum antimicrobial host defense molecule with the ability to carry a variety of effector molecules, including peptides that target fungal cell membranes.

Methods: Lactoferrin (LF) was obtained from a commercial supplier and two dairy companies. LF was tested on a panel of pathogenic yeast and mold species for inhibition using CLSM microdilution methods. Synthetic peptides were synthesized with antifungal drugs amphoterin B (AMB), tyrocidine (NYL), flucytosine (FLC), azole-resistant (ITC), rycomycin (VRC), and 5-fluorocytosine (5FC). The effect of LF on fungal cells was analyzed using scanning electron microscopy (SEM). The active peptides within LF were then predicted from putative and in silico digested, synthesized, and tested for synergy with amphotericin B (AMB).

Results: LF demonstrated antifungal activity against yeast species Cryptococcus, Candida, and Saccharomyces and was much less effective against molds. Good synergy was achieved with AMB but not nystatin or echinocandin drugs. While the iron-chelating capacity of LF was important for the antifungal activity it was not involved in synergy. SEM revealed cell damage suggesting an interaction between AMB, LF, and the fungal membrane or cell wall. A 10 residue peptide from the C-lob of LF was synthesized and tested for activity and synergy. This peptide, dubbed lactofungin (LFG) was inactive alone but was potently synergistic with AMB, indicating a direct role in augmenting AMB activity. Synthetic membranes loaded with ergosterol but not cholesterol were depleted by AMB + LFG, demonstrating that activity was fungal-specific and was mediated through ergosterol binding.

Conclusions: LF is a complex molecule that causes fungal inhibition via iron binding and when cleaved by pepsin can produce active peptides. An AMB is a highly toxic treatment, the use of LFG as a synergist could help increase activity while lowering the effective dose, thereby reducing undesirable side effects. The action of AMB + LFG appears dependent on ergosterol, suggesting inhibition will be highly fungal-specific.
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 rediscovety and its prorated economic cost.

Study: In an effort to recover the original success stories, we combined the traditional approach of screening for active secondary metabolites with modern advances in sequencing, genomics mining, sequencing mass spectrometry, NMR, LC-MS, and NMR.

Soil bacteria and fungi were isolated through in situ cultivation via the Chip method. After application of the OSMAC approach, >800 broth were identified with activity against Candida albicans. To prioritize active strains, several criteria were set up: how to best maximize host cell toxicity, activity against a broad spectrum of fungal pathogens including wild-type resistant strains, and established antifungal drug-resistant variants and species identification of the producing strain. Continuing, lead hits were purified striking bioactivity-based semi-preparative HPLC. The resulting pure fractions were analyzed by tandem LCMS-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 impure spec- troscopy can provide an early indication regarding the mode of action of the present antifungal agent. For this, a PSC 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 impure 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 new or literature never described the species as a producer of a known, or variant of a known antifungal compound. Moreover, several novel species are named on Illumina sequencing. Generally producing our current lead list include: Fusarium, Thanatephorus, Parabasidiella, and fungi: Attaec, Penicillium. Within the collection, the Penicillium species appear to produce variants of the antistreptococcal non-ribosomal peptide class.

S3.4d The role of NRPS inosamminose in host defense during Talosmoyacea marneffii infection

Liu Sha1, Wu Jinping1, Xi Liyan1, Xin Zhou1

1Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China
2Radboud University Medical Center, Nijmegen, Netherlands

S3.4e Free oral paper session, Wednesday, 21 June 2022, 4:41 PM - 6:15 PM

Talosmoyacea (Penicillium) marneffii (T. marneffii) is the only thermally dimorphic pathogen in Talosmyace. The patho- genesis of T. marneffii in mammals is not yet fully understood. Inhibition of T. marneffii conidia without normal clearance may result in conidia dissemination throughout the body and lead to disseminated infection. In TSM patients, study shows have shown that 7-18% of patients develop disseminated TSM, and 10-20% compared with the severity of sepsis and outcome of post-treatment. This means poor outcome is likely associated with an overall strong immune response. Several studies have identified inosamminose as an essential immune response in host defense against fungal pathogens. Among them, NRPSI pharmacological study on NRPS I in T. marneffii-induced immunopathology remains to be elucidated. Therefore, in the present study, aim to address the role played by the NRPSI pharmacological study on NRPS I in T. marneffii-induced immunopathology in vivo.

We established T. marneffii infected mice pulmonary model with two groups of mice, including the NRPSI transgenic and wild-type mice. We found that infected mice displayed NRPSI inosamminose activation and increased production of IL-18 upon pul- monary T. marneffii infection. Further, we demonstrated that T. marneffii conidia antagonized the NRPSI inosamminose both in mice and human macrophages. And T. marneffii conidia induced IL-18 released by infected macrophages is NRPSI-dependent in vitro. In vivo study, we found the NRPSI contributes to the development of pathology in the early stage of pulmonary T. marneffii infection. However, NRPSI transgenic mice showed a similar fungal load to the WT in the middle stage of infection. This suggests that the possibility number of WT mice could not be seen in the NRPSI transgenic mice. Moreover, NRPSI contributes to pathology in pulmonary T. marneffii infection and contributes to insufficient recovery and pulmonary pathology.

So, in the present study, we demonstrated that the NRPSI pharmacological study on NRPS I in T. marneffii-induced immunopathology plays a dual role during pathogenic T. marneffii early inflammatory response inducing a protective environment, and a subsequent excessive damaging inflammatory response that contributes to pathology and mortality. This study identifies for the first time that activation of the inosamminose in the latter stage of TSM detrimentally contributes to pathological inosamminose activation and suggests that targeting the inosamminose may be a therapeutic option to target pathological T. marneffii-induced immunopathology.

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

Chinnmaya Awasthi1, Wouter van Genderen1, Luca Himmler2, Patrick Van Dijk3

1Laboratory of Molecular Cell Biology, Department of Biology, Section of Molecular Biotechnology of Plants and Microorganisms, Institute of Botany and Microbiology, KU Leuven, Leuven, Belgium
2Biomedical MRI/MOSAIC, Department of Imaging and Pathology, KU Leuven, Leuven, Belgium

S3.4e Free oral paper session, Wednesday, 21 June 2022, 4:41 PM - 6:15 PM

DOG genes, encoding for 2-deoxylyxose-6-phosphate phosphatase for low molecular weight phosphates, with an an- lewis biochemical function. In contrast to Saccharomyces cerevisiae which have two DOG homologs, C. albicans only have 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 virulence formation and virulence of this pathogen, via a novel alternative pathway.

The known classical pathway of glycerol production begins when the glycerol intermediate molecule dihydroxyacetone phosphate (DHAP) is converted into glycerol-3-phosphate (G-3-P) by a pair of glycerol-3-phosphate-dehydrogenases, Gap1 and Gap2. However, an alternative pathway, where DHAP is dephosphorylated into DHIA, which is subsequently converted into glycerol has been proposed, but the enzymes involved in this process have not yet been described. We recently showed that in Saccharomyces cerevisiae, the DOG enzymes are involved in the production of DHIA from DHAP, thereby allowing the synthesis of glycerol in the absence of the classical pathway. Overexpression of the DOG genes restored the osmo-tolerance of the gap1Δgap2Δ and gap1Δ gap2ΔΔΔΔΔΔΔ mutant strains. Further analysis, purifying DHAP in yeast, we showed that DHAP is not a clear inhibition zone, but not thiaminose (25 ng/ml) (Fig. 1a). Second, we showed that DOG activity varied with the broth microassays assay (Fig. 2a, b), which are key virulence factors of pathogenic fungi. Besides, DOG (25 mg/l) significantly prolonged the median survival time from 5 days (control group) to 15 days, and improved the survival rate from 0% (control group) to 62.5% (Fig. 2c, d). Furthermore, we found that DOG activity varied with the broth microassays assay (Fig. 2a, b), which are key virulence factors of pathogenic fungi. Besides, DOG (25 mg/l) significantly prolonged the median survival time from 5 days (control group) to 15 days, and improved the survival rate from 0% (control group) to 62.5% (Fig. 2c, d). Furthermore, we found that DOG (25 mg/l) significantly prolonged the median survival time from 5 days (control group) to 15 days, and improved the survival rate from 0% (control group) to 62.5% (Fig. 2c, d). Furthermore, we found that DOG activity varied with the broth microassays assay (Fig. 2a, b), which are key virulence factors of pathogenic fungi. Besides, DOG (25 mg/l) significantly prolonged the median survival time from 5 days (control group) to 15 days, and improved the survival rate from 0% (control group) to 62.5% (Fig. 2c, d).

DOG activity demonstrated to be a potential antifungal agent. Hence, we were setting up a high throughput small scale screening for this phasor as a potential antifungal drug target.