Novel antimicrobials from *Streptomyces coelicolor* when challenged by *Aspergilli* species under nutrient-deplete conditions

Ruth Nair, Rajeev Shrivastava, Cher-Pheng Ooi
Department of Biology,
Edge Hill University

1. Introduction

Background: There is an ever-increasing need to identify novel antimicrobials due to an increase in antimicrobial resistant (AMR) strains. The WHO recognises AMR as one of the top 10 global public health threats facing humanity. *Actinomycetes* are producers of 80 % of known antibiotics. *Streptomyces*, the largest genus in this order, is a major contributor to the antimicrobials produced. Environmental challenges like nutrient-depletion, competing pathogens, temperature and pH are known to enhance the production of its chemical arsenal.
Objective: To identify and isolate novel antimicrobials by co-culturing *Streptomyces* strains with pathogenic *Aspergillus* fungal strains under nutrient-depleted conditions

2. Strain Definition

Fig 1. *S. coelicolor* *(jic.ac.uk)*

Fig 2. *A. flavus* *(aspergillus.org.uk)*

Streptomyces coelicolor

- M145 (WT) (*Δact, Δred, Δcpk, Δcdc*)
- M1146 (*Δact, Δred, Δcpk, Δcdc*) rpoB[C1298T]
- M1152 (*Δact, Δred, Δcpk, Δcdc*) rpoB[C1298T] rpsL[A262G]
- M1154

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Streptomyces venezuelae

(Gomez-Escribano & Bibb, 2011)

Aspergillus spp.

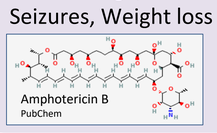
A. flavus | *A. parasiticus*

Opportunistic Filamentous Fungi
Main cause of Aspergillosis disease

Main producers of Aflatoxins

Current Treatment: Amphotericin B

Side Effects of Amphotericin B include Liver & Kidney Damage, Haemorrhage, Nausea Seizures, Weight loss



3. Minimal media enhances Antifungal activity

Differences in *Aspergillus* morphology when co-cultured with *Streptomyces* was only evident in modified NMMP (WS NMMP). There was a minor reduction in colony radius and significant discolouration (Fig 3.)

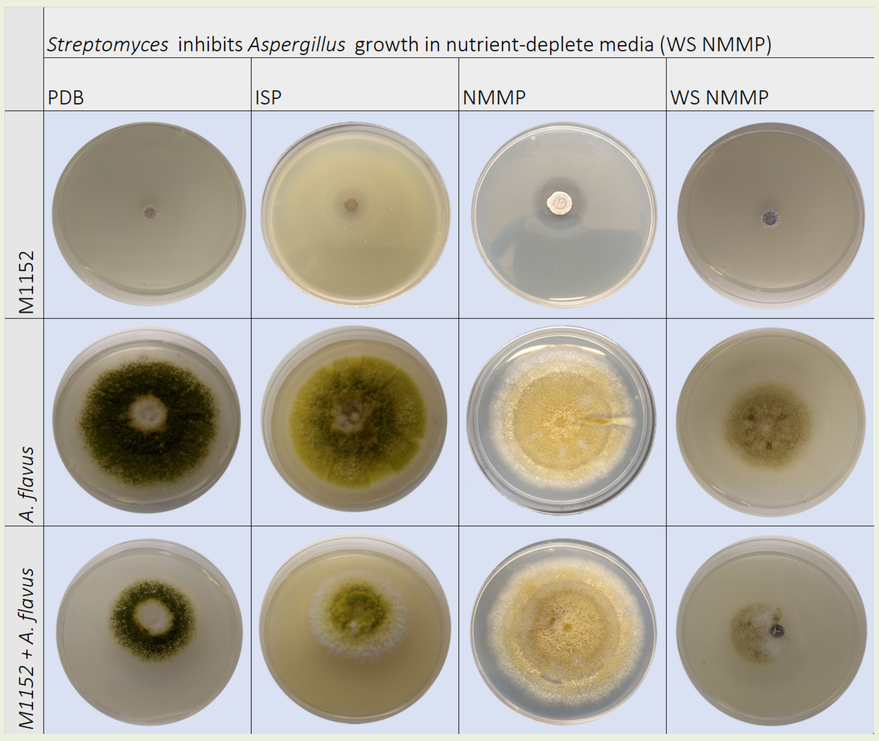


Fig 3. Plated *A. flavus* and *S. coelicolor* (M1152) on nutrient rich media (Potato Dextrose Broth, ISP 2) and minimal media (NMMP and WS NMMP)

4. *Streptomyces* reduces structural integrity

Reduced incorporation of fluorescent dye indicates defects to the chitin wall of *Aspergilli* when co-cultured with mutant *S. coelicolor* and *S. venezuelae*.

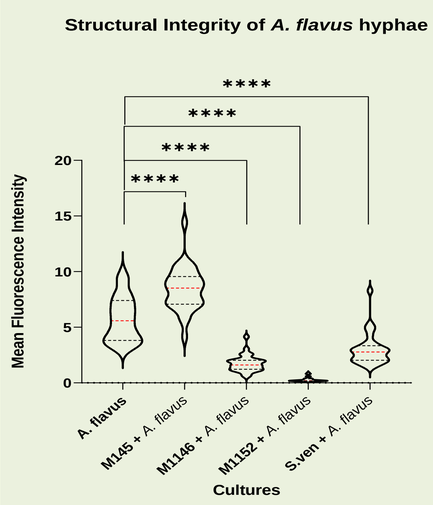


Fig 4. Fluorescent dye incorporation in *Aspergillus* hyphae is significantly reduced in mutant *S. coelicolor* and *S. venezuelae* co-cultures

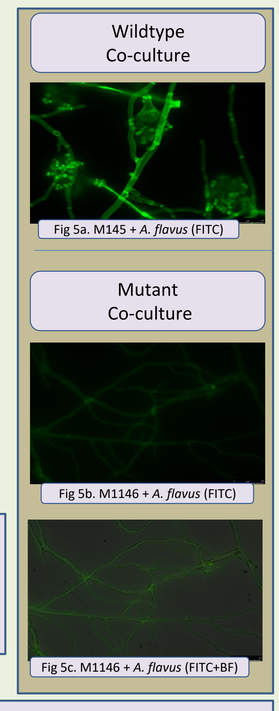


Fig 5a. M145 + *A. flavus* (FITC)

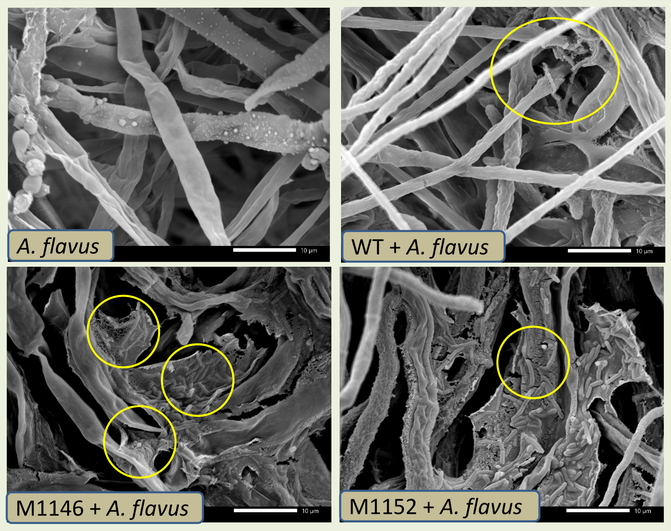
Fig 5b. M1146 + *A. flavus* (FITC)

Fig 5c. M1146 + *A. flavus* (FITC+BF)

Fig 5. Fluorescence microscopy images of *Aspergilli* hyphae when co-cultured with *S. coelicolor* WT versus the M1146 mutant

5. Damage to fungal hyphae & biofilm

The integrity of *A. flavus* hyphae, biofilm and sclerotia are compromised when co-cultured with *S. coelicolor.* (Fig 6.).



6. Inhibition of fungal sporulation

Fungal spores (green pigmentation) is reduced when co-cultured with *Streptomyces* mutants.

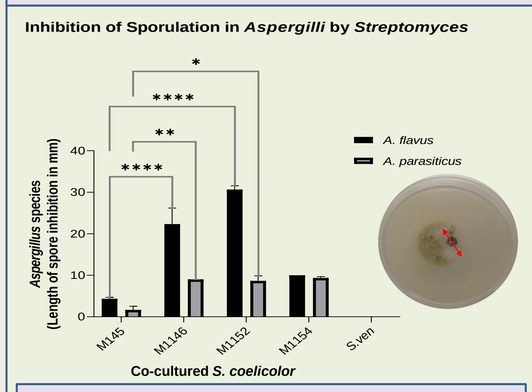


Fig 7. Reduction in *Aspergillus* spore formation is significant in mutant *Streptomyces* co-cultures

7. Chemical Extraction and Analysis

Secreted metabolites from co-cultured agar plates was extracted using ethyl acetate. After distillation using the rotary evaporator, samples were analysed in Vanquish UHPLC system with QExactive mass spectrometer. LCMS data was processed using Compound Discoverer.

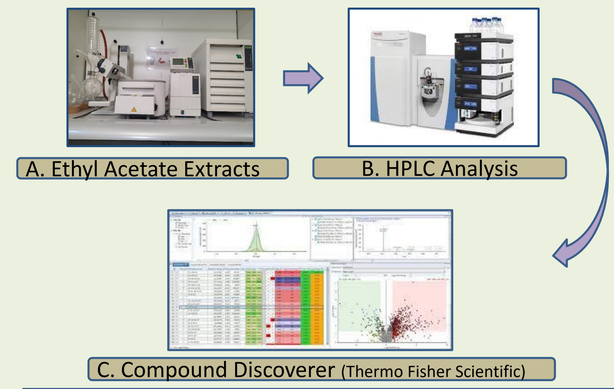


Fig 8. Workflow for analysis of novel compounds

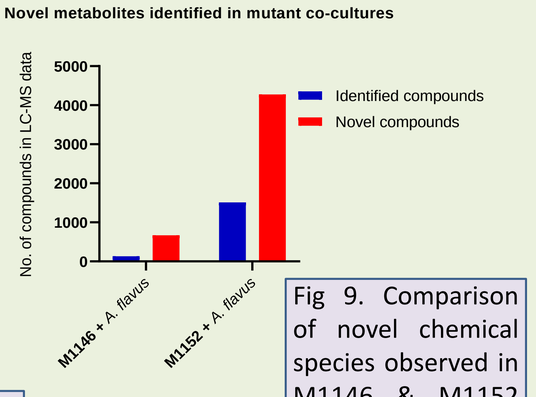


Fig 9. Comparison of novel chemical species observed in M1146 & M1152 co-cultures

8. Mutant co-cultures exhibit Antibiotic activity against ESKAPE pathogens

Zone of Inhibition observed with Ethyl Acetate Extracts

Cultures	Indicators		ESKAPE Pathogens					
	E. coli	*M. lut*	*E. fae*	*S. aur*	*K. pne*	*A. bau*	*P. aer*	*E. fae*
M145	✗	✗	✗	✗	✗	✗	✗	✗
M1146	✗	✗	✗	✗	✗	✗	✗	✗
M1152	✗	✗	✗	✗	✗	✗	✗	✗
A. flavus	✓	✗	✓	✗	✗	✗	✗	✗
M145 +*Af*	✗	✓	✓	✗	✗	✓	✓	✓
M1146+*Af*	✗	✗	✗	✗	✗	✗	✗	✗
M1152+*Af*	✓	✓	✓	✓	✗	✓	✓	✓

Antimicrobial Assay against ESKAPE pathogens reveal antibiotic activity in co-cultured *Streptomyces* species in comparison to mono-cultured *S. coelicolor* WT and mutants.

Fig 10. M1152+ *A. flavus* co-culture inhibits majority of ESKAPE strains except for *Klebsiella pneumoniae*

9. Conclusions and Future Work

1. *S. coelicolor* mutant strains and *S. venezuelae* WT exhibit anti-fungal properties under nutrient depletion.
2. Non-polar extracts from mutant *S. coelicolor + A. flavus* co-cultures inhibit ESKAPE pathogen growth.
3. > 4000 novel compounds have been identified from mutant *S. coelicolor* M1152 + *A. flavus* co-cultures.
4. RNAseq will correlate identified novel compounds with activated gene clusters in mutant *Streptomyces* (M1152) in conjunction with *in silico* analyses (AntiSMASH).
5. Reverse-Phase HPLC and X-Ray Crystallography will help isolate and identify the molecular structure of compounds of interest