Palmitolealdehyde targeting conidial pigmentation and surface morphology in *Aspergillus fumigatus*

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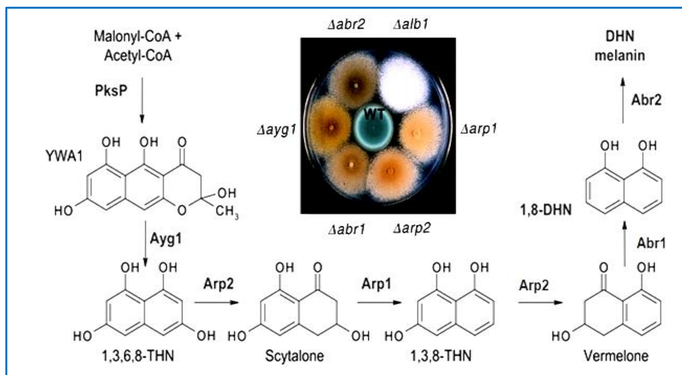
INTRODUCTION

Aspergillus fumigatus is a major fungal pathogen associated with varied invasive fungal infections all over the globe. The rapid emergence of antifungal drug resistance in *A. fumigatus* since past few years, has led to increased morbidity and mortality mainly among immunocompromised patients. The unique cell surface organisation such as the presence of DHN-melanin, hydrophobin layers, polysaccharides and glycoproteins present on *A. fumigatus* cell wall provides structural stability and also serve as topological barrier during both *in-vitro* and *in-vivo* unfavourable conditions.

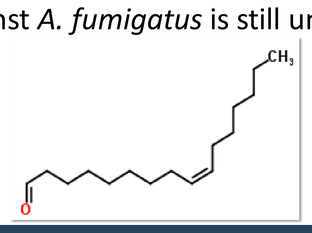
Conidial pigment DHN-melanin is present over the fungal cell wall and imparts greenish grey color to *A. fumigatus* conidia. It is a major virulence determinant that binds to the antimicrobial peptides and reduces the effectiveness of antifungal drugs. Among all color mutants, only the demelanised white *A. fumigatus* colonies are avirulent .



DHN melanin synthesis pathway in *A. fumigatus* and conidia color at different mutant stages.

Modified from Pihet et al., 2009

Palmitolealdehyde (C9H) is a naturally occurring fatty aldehydic antimicrobial compound that is present in various medicinal plants but its melanin inhibiting property against *A. fumigatus* is still unexplored.



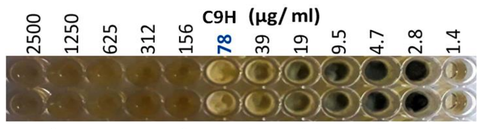
OBJECTIVE

The objective of the present study was:

- To investigate the antifungal efficacy of the phytocompound (C9H) targeting conidial pigmentation and surface morphology in *A. fumigatus* and compared with Δ*pksP* strain (negative control).
- To understand the cell cytotoxicity of the compound C9H in comparison to common antifungal drug amphotericin B.

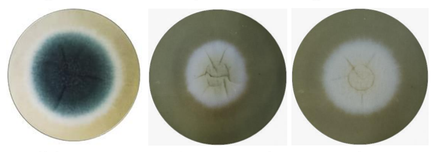
METHODOLOGY AND RESULTS

1. **Minimum effective concentration of C9H for demelanization using broth micro-dilution method**



A. fumigatus formed white demelanized colonies at 78 µg/ml C9H

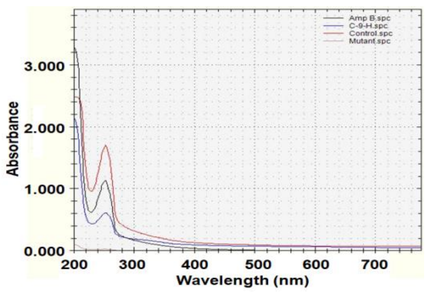
2. **Phenotypic characterization of *A. fumigatus* on czapek dox agar**



White demelanized *A. fumigatus* colonies similar to Δ*pksP were* formed after treatment with 78 µg/ml C9H whereas wild type colonies were greenish grey in color.

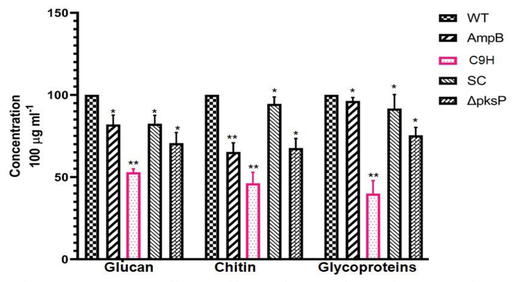
3. **Biochemical tests for evaluation of:**

A) **Melanin content**



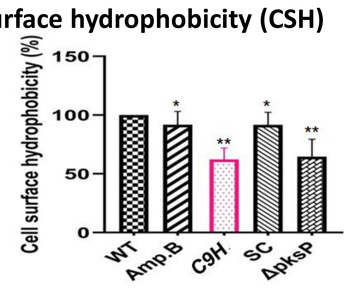
There was 96% inhibition of melanin content in C9H treated *A. fumigatus.*

B) **Polysaccharide and glycoprotein content**



Reduction in cell wall polysaccharides such as glucans (53%), chitin (46%) and glycoproteins (40%) was determined in C9H treated *A. fumigatus* ($p<0.05$)

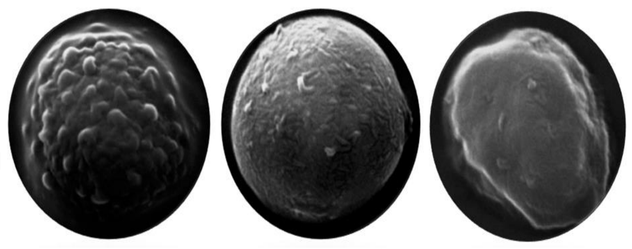
C) **Cell surface hydrophobicity (CSH)**



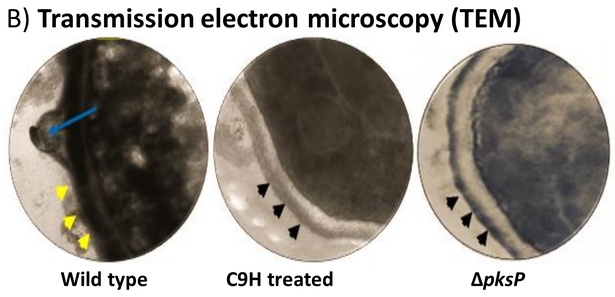
Microbial adhesion to hydrocarbons (MATHs) assay revealed up to 55% reduced surface hydrophobicity in C9H treated *A. fumigatus.* ($p< 0.0071$).

4. **Morphological study :**

A) **Scanning electron microscopy (SEM)**



B) **Transmission electron microscopy (TEM)**

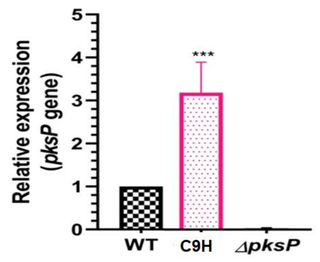


5. **Fractional inhibitory concentration indexing (FICI) using checker board assay**

Strain	Molecules	MIC_{50} values (µg/ml) Individual	Combination	FICI	Outcome
A. fumigatus	C-9-H	78	19	0.4	Addition
	AmpB	6.2	1.5		

The therapeutic efficacy of AmpB (4-fold) and C9H (2-fold) was enhanced.

6. **RT-qPCR for the transcript analysis of polyketide synthase gene *pksP/alb1* gene**



β-actin expression was used as an internal control. C9H treatment enhanced the expression of *pksP/alb1* gene (3.5 fold) in comparison to wild type *A. fumigatus.* ($p<0.008$)

7A. **Total proteome profiling using LC-MS/MS**

The total proteome analysis showed that from a total of 1809 proteins, 309 proteins were differentially expressed.

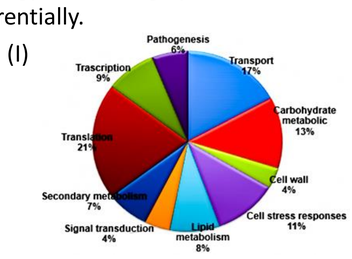
Fold change in proteins previously reported as virulence factors in *A. Fumigatus:*

Proteins	Fold change	Remarks
Integral membrane protein	2.4	Down regulated
Transport protein sec13	2	Down regulated
Thioredoxin reductase GliT	16	Down regulated
Methyltransferase GliN	12	Down regulated
Secreted antimicrobial peptide	2.4	Up regulated
Polyketide synthase	2.5	Up regulated
Catalase	2.5	Up regulated

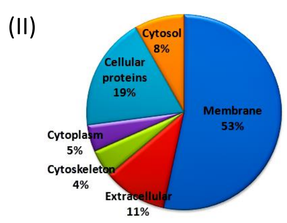
7B. **Characterization of total proteome into functional pathway via gene ontology (GO) analysis**

On the basis of cellular (I) and biological (II) gene ontology, following proteins were expressed differentially.

(I)



(II)

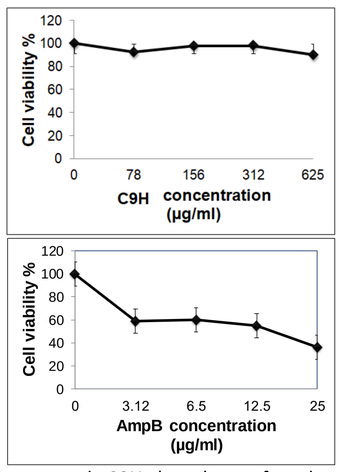


The total proteome study revealed that except PKS proteins, no other downstream proteins involved in melanin synthesis pathway were present in C9H treated *A. fumigatus* protein sample. This affirmed the inhibition of the downstream reactions in the DHN-melanin synthesis pathway, stopping the formation of the final product DHN-melanin.

8. ***In-silico* screening of compound for its therapeutic activity**

S.No.	Property	Criterion	In-silico analysis
1	Molecular weight	< 500	238.41
2	Hydrogen donor	< 6.0	0
3	Hydrogen acceptor	< 5.0	1
4	Rotational bonds	< 10.0	13
5	Polar Surface Area	< 150	17.07
6	Log P (lipophilicity)	< 5	6.29
7	Log S (Solubility)	-	-5.73
8	Oral bioavailability	-	30- 70%
9	Active transport	-	No
10	Passive transport	-	Yes
11	Drug likeness score	-	-1.27

9. **Cell cytotoxicity study on normal lung cell line L-132 using MTT assay**



The phytocompound C9H has been found to be non-cytotoxic for human lung epithelial normal cell line L-132 up to 625 µg/mL.. However, amphotericin B reduced the cell viability at 25 µg/mL.

CONCLUSION

The outcome of *in vitro* studies indicated that the phytocompound C9H has potential role in inhibition of conidial pigmentation leading to reduction in surface proteins and polysaccharides responsible for adherence and spreading infection in host body. The SEM and TEM observation also revealed protrusionless surface without melanin deposition in C9H treated *A. fumigatus* similar to *ΔpksP.* The combinatorial approach may also help in overcoming the severe side-effects due the high dosage of the available antifungal drugs. The molecular analysis suggested that upregulation of *pksP* gene and gene product may be in response to stress due to C9H treatment in *A. fumigatus* altering the expression of regulatory proteins responsible for maintaining cell membrane integrity. C9H is safe non-cytotoxic to human cell line. Hence, this study opens up the possibility of future aspect of C9H as promising therapeutic candidate especially towards *A. fumigatus* related infections.

FUTURE PROSPECTIVE

- Structural modification of the compound C9H to enhance its therapeutic potency against *A. fumigatus*.
- *In-vivo* studies for understanding the immunological effect of the compound C9H.

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