Scientific Research Publishing

Advances in Microbiology, 2017, 7, 707-718
http://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Phenazine Methosulphate Modulating the Expression of Genes Involved in Yeast to Hyphal Form Signal Transduction in *Candida albicans*

Ashwini Khanderao Jadhav[1], Priyanka Jangid[1], Rajendra Patil[2], Wasudev Gade[2], Kiran Kharat[3], Sankunny Mohan Karuppayil[1]*

[1]School of Life Sciences, SRTM University, Nanded, India
[2]Department of Biotechnology, Savitribai Phule Pune Univesity, Pune, India
[3]Department of Biotechnology, Deogiri College, Aurangabad, India
Email: *prof.karuppayil@gmail.com

How to cite this paper: Jadhav, A.K., Jangid, P., Patil, R., Gade, W., Kharat, K. and Karuppayil, S.M. (2017) Phenazine Methosulphate Modulating the Expression of Genes Involved in Yeast to Hyphal Form Signal Transduction in *Candida albicans*. *Advances in Microbiology*, **7**, 707-718.
https://doi.org/10.4236/aim.2017.711056

Received: October 2, 2017
Accepted: November 7, 2017
Published: November 10, 2017

Abstract

Candida albicans has ability to switch from yeast to hyphal form which is an important virulence factor. The objective of the research is to study the effect of Phenazine Methosulphate (PMS) on virulence factors and to study expression profile in yeast to hyphal form transition in *C. albicans*. Phenazine Methosulphate (PMS) acted as an inhibitor of yeast to hyphal form transition, adhesion and biofilm formation in *C. albicans*. RTPCR study demonstrated that PMS Modulate the expression of genes involved in Ras1-cAMP-Efg1 and Cek1-MAPK signal transduction pathways. Cell cycle of *C. albicans* was arrested at S phase on treatment of PMS. Hyphal suppressor genes like Tup1, Mig1 and Nrg1 were upregulated by PMS. Based on our data on expression of genes during yeast to hyphal form transition in presence and absence of PMS, we hypothesize that inhibition of hyphal formation may be due to the overexpression of negative regulators of hyphal growth. Targeting of hyphal specific genes involved in these pathways may be a promising strategy for anti-candida drug development.

Keywords

Phenazine Methosulphate, *Candida albicans* Y-H Form Transition, Biofilm, Tup1, Mig1 and Nrg1

1. Introduction

Yeast to Hyphal (Y-H) form morphogenesis is one of the major virulence factors in *C. albicans* and this may facilitate penetration of epithelial tissues and escape from host immune response [1] [2]. Serum induced Ras mediated signaling pathways

involve Ras1-cAMP-PKA and Cek1-MAPK pathway responsible for Y-H form transition [3]. Y-H transition is negatively regulated by the hyphal suppressor genes, *Nrg*1, *Mig*1, *Tup*l and *Rfg*1 [4]. Upregulation of hyphal specific genes are reported during yeast to hyphal form transformation [5]. Targeting of hyphal specific genes involved in these pathways may be a promising strategy for anti-*Candida* drug development [6]. In this study the effect of Phenazine methosulphate (PMS) on the expression of genes involved in Yeast to hyphal transition is explored.

Phenazine methosulphate (PMS), an analog of 5-methylphenazine-1-carboxylic acid (5 MPCA) is reported to exhibit antifungal activity against hyphal growth and biofilm formation of *C. albicans* in *Candida albicans* [7] [8]. PMS generate reactive oxygen species (ROS) which is responsible for killing or inhibition of growth in *C. albicans* [7]. PMS and 5MPCA generally bind with cellular amines covalently and accumulate in the cell leads to killing of *C. albicans* [7] [9].

In this study we have identified the potential targets of PMS in *C. albicans.* The repositioning of PMS as an antifungal agent could be a promising strategy for the treatment of fungal infections.

2. Materials and Methods

2.1. Medium and Culture Conditions

The standard strain of *Candida albicans* ATCC 90028 provided by Institute of Microbial Technology, Chandigarh, India. Yeast extract Peptone Dextrose (YPD) agar medium was used to maintain culture and the culture was stored at 4˚C. PMS and all the media components purchased from HiMedia, India. A single isolated colony of *C. albicans* from YPD agar plate was used to inoculate 50 ml YPD broth in a 250 ml conical flask. The flasks were incubated for 24 h in an orbital shaking incubator (100 rpm) at 30˚C [10].

2.2. Minimum Inhibitory Concentration

The effect of PMS on the growth of *C. albicans* in planktonic form was studied by using the standard broth microdilution methodology based on the Clinical Laboratory Standards Institute guidelines (Pfaller *et al.*, 1994). Various concentrations of PMS ranging from 0.062 to 2 mg/ml were prepared in RPMI-1640 medium in 96 well plates (Costar, Corning Inc. USA). Cell suspension (1×10^3 cells ml^{-1}) was added to 100 µl of RPMI-1640 medium in each well. The plates were incubated at 35˚C for 48 h and growth was analyzed by measuring absorbance at 620 nm using a microplate reader (Multiskan EX, Thermo Electron Corp. USA). The lowest concentration of PMS which caused a 50% reduction in the absorbance compared to the control was considered as the minimum inhibitory concentration (MIC) for the growth of *C. albicans* [10].

2.3. Minimum Fungicidal Concentration (MFC)

To determine the MFC, *Candida* cells from the wells containing MIC and above the MIC were used. Aliquots of 10 µl from these wells were spread on YPD agar.

These plates were incubated for 48 h at 30˚C and observed for the presence of colonies. No appearance of colonies on the agar plates was noted as fungicidal effect. The lowest concentration of the test molecule in the microplate well from which an aliquot showing no growth was considered as the MFC [11].

2.4. Adhesion Assay

The effect of Capric and Caprylic acid on the adherence of *C. albicans* to a solid surface (*i.e.* polystyrene) was studied using a microplate-based assay. Different series of concentrations were prepared in Phosphate buffered saline (PBS) and of 50 µl of aliquots were added in to wells of microtiter plates. The final volume of the assay system in each well was kept at 100 µl. The plate is incubated at 37˚C for 90 min at 100 rpm in an orbital shaking incubator to allow adherence of cells. The adherence of cells were observed in each well by relative metabolic activity (RMA) using the XTT-assay [10].

2.5. Biofilm Development Assay

Biofilm formation was done on tissue culture treated 96-well polystyrene plates. 100 µl of Cell suspensions of 1×10^7 cells ml^{-1} were added into each well and allowed to adhere on solid surface at 37˚C for 90 min at 100 rpm. After adhesion wells were washed with PBS two times. RPMI-1640 medium (200 µl) along with various concentrations of PMS were incubated for 48 h at 37˚C. Developed biofilms were observed under an inverted light microscope (Metzer, India). XTT metabolic assay was used Quantitation of Biofilm growth. Photographs were taken with a Labomed microphotography system (Labomed, India) at 200 X magnification [12].

2.6. Mature Biofilm Assay

To determine the activity of the PMS against mature *C. albicans* biofilms, 24 h old biofilm was used. Wells were washed with PBS to remove nonadhered planktonic cells. Various concentrations of PMS prepared in RPMI 1640 were added to wells. Plates were incubated for 48 h and then washed with PBS. Wells were observed for the presence or absence of biofilm using an inverted light microscope (Metzer, India). XTT metabolic assay was used to analyze the biofilm growth [12].

2.7. XTT Metabolic Assay

Biofilm growth was quantified using XTT metabolic assay. XTT solution was prepared by mixing 1 mg/ml of XTT salt in PBS and stored at 20˚C. Menadione (Sigma Aldrich, India) solution was prepared in acetone (4 µM) and added to the XTT salt solution. The wells containing biofilms were washed with PBS to remove non-adhered cells and incubated with 100 µl of XTT-menadione solution in dark, at 37˚C for 5 h. Color formation by the water soluble formazan product was measured at 450 nm using a microplate reader (Multiskan EX,

Thermo Electron Corp. USA) [12].

2.8. Yeast to Hyphal (Y-H) Form Morphogenesis Assay

Serum-induced Y-H transition in *C. albicans* was studied in a microplate-based assay. A series of concentrations of PMS was prepared in deionized distilled water with 20% serum. 1×10^6 cells ml^{-1} Cells were inoculated in test and control wells. The final volume of the assay was 200 µl. After incubation for 2 h at 37˚C in an orbital shaker at 200 rpm, formation of germ tubes were observed microscopically. Numbers of yeast cells and hyphae were counted by using an inverted microscope (Metzer, India). Photographs were taken with a Labomed microphotography system at 200 X magnification. Inhibition of 50% hyphae formation was compared with control and considered as MIC for morphogenesis. [10].

2.9. Scanning Electron Microscopy

Adhesion of *Candida albicans* cells on polystyrene discs (1×10^7 cells/ml) were carried out in 12-well plates at 37˚C at 50 rpm for 90 min. Samples were fixed in 2.5% of glutaraldehyde in 0.1 mol phosphate buffer (pH 7.2) for 24 h at 4˚C. Samples were post fixed in 2% aqueous solution of osmium tetraoxide for 4 h, and then dehydrated in a series of graded alcohols. The samples were mounted over stubs and gold coating was performed using an automated gold coater. Images were obtained by JOEL 6360 (Japan) scanning electron microscope [12].

2.10. Hemolytic Activity

Hemolytic activity was determined by using human red blood cells [13]. Suspension of RBC was diluted 1:10 in PBS. Aliquotes of 100 µL from this suspension was added in to 100 µL of a different concentration of test molecules in the same buffer in Eppendorff tubes. 1% Triton X 100 was used for total hemolysis. After incubation of 1 h at 37˚C it was centrifuged for 10 min at 2000 rpm at 20˚C. 150 µL from supernatant was transferred to a flat-bottomed microtiter plate, and O.D. is taken at 450 nm. All the experiments were done in triplicates.

The hemolysis percentage was calculated by following formula:

% of hemolysis = $[(A_{450}$ of test compound treated Sample − A_{450} of buffer treated sample)/(A_{450} of 1% Triton X 100 treated sample − A_{450} of buffer treated sample)] × 100

2.11. Cell Cycle Studies

C. albicans yeast cells from the log phase were harvested, washed and starved for nutrients for 1 h. cells (10^3 cfu/ml) were inoculated to each flask containing 50 ml YPD broth and MIC of PMS. Flask without treatment of PMS was considered as Control. All the flasks were incubated at 30˚C for 3 h. Cells were centrifuged at 1000 rpm for 5 min. washed and fixed in 1 ml of 70% ethanol by incubating at room temperature on a rotary shaker (REMI, India) for 30 min. Cells were har-

vested by centrifugation (Genei, Bangalore, India) at 1000 rpm for 5 min, washed twice with 1 ml of 50 mM Tris (pH 7.8). Washed cells were resuspended in 500 µl of 50 mM Tris containing 10 µg RNase A and incubated for 2 h at 37˚C. After incubation samples were subjected to protease treatment (5 mg/ml pepsin in 0.05 M HCl) for 1 h at 25˚C to reduce cellular clumps. Cells were harvested by centrifugation and resuspended in 500 µl FACS-buffer (200 mM Tris/HCl pH 7.5; 200 mM NaCl; 78 mM $MgCl_2$). 15 µl of propidium iodide (1 mg/ml) was added to each tube and incubated for 30 min and stored at 4˚C. Before analysis, cells were sonicated for 10 s and analyzed using a FACS Calibur cytometer (Becton-Dickinson, San Jose, Calif.) and analyzed with Cell Quest 3.0 software [14].

2.12. Gene Expression Assay

The expression of hyphal genes during serum induced morphogenesis was measured by using Real Time PCR. *C. albicans* yeast phase cells (1×10^6 cells/ml) were inoculated and incubated for 90 min in 20% serum containing PMS (0.062 mg/ml). Total RNA was isolated by using RNeasy® Mini Kit (QIAGEN, USA) and converted to cDNA by using SuperScript® III for first strand synthesis (Invitrogen, Life technologies, USA). PCR reactions were conducted by using KAPA SYBR® Fast qPCR Kit Master mix (2x) (BIOSYSTEMS, South Africa) in 96 well PCR plates with preliminary denaturation for 3 min at 95˚C. This was followed by 32 amplifications cycles of denaturation at 95˚C for 30 s, annealing at 60˚C for 20 s and primer extension at 72˚C for 30 s (CFX 96 Real time System, Bio-Rad, USA). Primers were purchased from Eurofins Genomics India Pvt. Ltd. (**Table 1**). Actin was used as an internal control and the transcript levels of selected genes were calculated using the formula $2^{-\Delta\Delta CT}$ [15].

2.13. Statistical Analysis

Values mentioned are the mean with standard deviations, obtained from three different observations. Values in the control and treatment groups were compared using Student's *t*-test. A value of $p < 0.05$ was considered statistically significant [10].

3. Results

3.1. The Anti-*Candida* Efficacy of Phenazine Methosulphate (PMS)

PMS inhibited planktonic growth of *Candida albicans* ATCC 90028 in a concentration dependent fashion (**Figure 1**). More than 90% ($p < 0.05$) of the growth was inhibited at 0.062 mg/ml. 99% of planktonic growth was inhibited by PMS after 48 hrs of exposure at 0.25 mg/ml (**Table 1**). PMS caused significant inhibition of developing as well as mature biofilm formation (**Figure 1**, **Figure 2**). MIC values for developing biofilm and mature biofilm were 0.25 mg/ml and 0.5 mg/ml respectively. >70% of 48 h old mature biofilm growth was eradicated at 0.5 mg/ml (**Figure 1**). MIC values were established for Adhesion for PMS (**Table**

2). Adhesion of *C. albicans* ATCC 90,028 to polystyrene plate surface was inhibited significantly ($p < 0.05$) by PMS at >16 µg /ml (Figure 1). MIC50 for inhibition of adhesion was 16 µg /ml (Table 2). After treatment of PMS (0.031 mg/ml) on *C. albicans* caused cell cycle arrest in S phase (Figure 3).

Table 1. Gene specific primers used in qRT-PCR.

Primers	Sequence (5' → 3')
ACTIN-F	5'ATGGACGGTGAAGAAGTTGC 3'
ACTIN-R	5'ACCTCTTTTGGATTGGGCTTCA 3'
RAS1-F	5'GGCCATGAGAGAACAATATA 3'
RAS1-R	5'GTCTTTCCATTTCTAAATCAC 3'
PDE 2-F	5' ACCACCACCACTACTACTAC 3'
PDE 2-R	5' AAAATGAGTTGTTCCTGTCC 3'
BCY 1-F	5' CCC AAGCTTATGTCTAATCCTCAACAGCA 3'
BCY 1-R	5' GGG CTGCAGTTAATGACCAGCAGTTGGGT 3'
EFG 1-F	5' TATGCCCCAGCAAACAACTG 3'
EFG 1-R	5' TTGTTGTCCTGCTGTCTGTC3'
TEC 1-F	5' AGGTTCCCTGGTTTAAGTG 3'
TEC 1-R	5' ACTGGTATGTGTGGGTGAT 3'
ECE 1-F	5'-CCCTCAACTTGCTCCTTCACC-3'
ECE 1-R	5'-GATCACTTGTGGGATGTTGGTAA-3'
CEK 1-F	5' AGCTATACAACGACCAATTAA 3'
CEK 1-R	5' CATTAGCTGA ATGCATAGCT 3'
HST 7-F	5' ACTCCAACATCCAATATAACA 3'
HST 7-R	5' TTGATTGACGTTCAATGAAGA 3'
CPH1-F	5'ATGCAACACTATTTATACCTC 3'
CPH2-R	5'CGGATATTGTTGATGATGATA 3'
CDC35-F	5'TTCATCAGGGGTTATTTCAC 3'
CDC35-R	5'CTCTATCAACCCGCCATTTC 3'
HWP1-F	5'TGGTGCTATTACTATTCCGG
HWP1-R	5'CAATAATAGCAGCACCGAAG
MIG1	5'CTTCAACTAGCCTATATTCCGATGG 3'
MIG1	5'-CTTTCT GTAGGTACCAACAACTAC 3'
NRG1-F	5'CACCTCACTTGCAACCCC
NRG1-R	5'GCCCTGGAGATGGTCTGA
TUP1	5' GAGGATCCCATGTATCCCCAACGCACCCAG 3'
TUP1	5'GGCGACGCGTCGTTTTTTGGTCCATTTCCAAATTCTG 3'

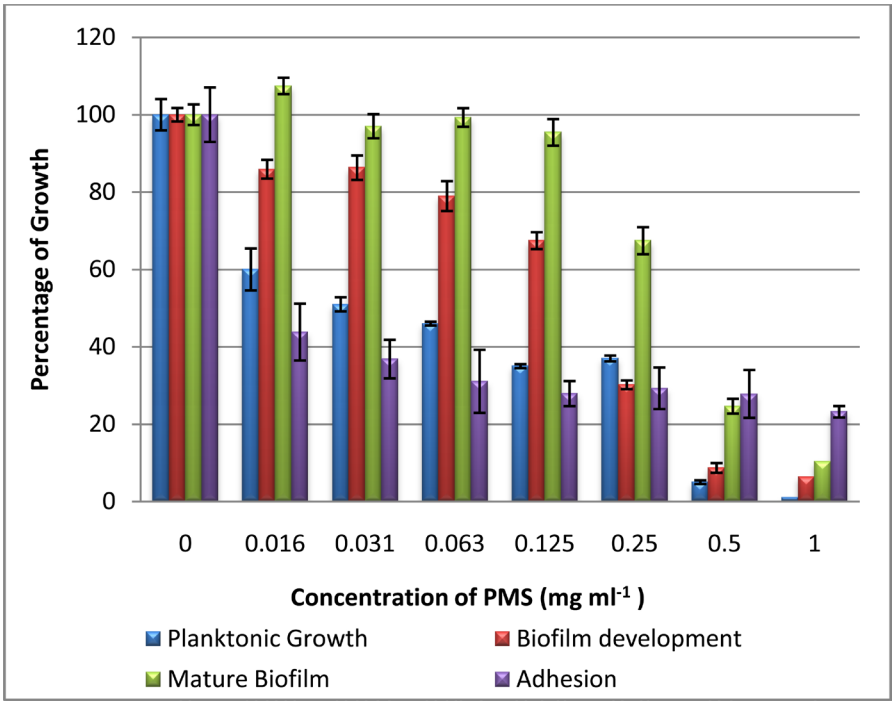
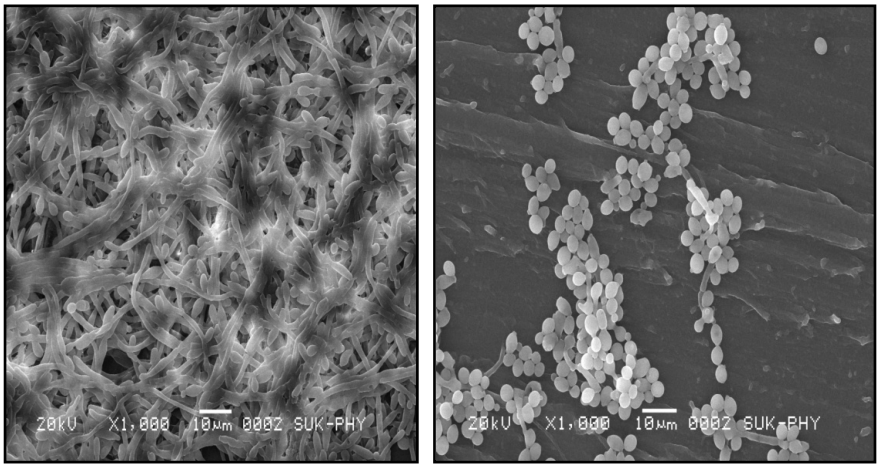


Figure 1. Effect of PMS on *C. albicans* (ATCC 90,028) Planktonic growth, Developing biofilm, Mature biofilm and Adhesion.



(a) (b)

Figure 2. Scanning electron micrographs (a) Inhibition of Biofilm formation in *C. albicans* (ATCC 90,028) in presence of PMS (0.25 mg/ml) (b) Biofilm without treatment of PMS (Control).

Table 2. The efficacies of PMS against *C. albicans* ATCC 90,028, showing the MICs for growth, adhesion, morphogenesis, and biofilm formation and MFCs for planktonic growth.

Test Molecule	MIC (mg/ml)					MFC (mg/ml)
	Planktonic Growth	Developing Biofilm	Mature Biofilm	Adhesion	Yeast to Hyphae Morphogenesis	
PMS	0.031	0.25	0.5	0.016	0.062	0.25

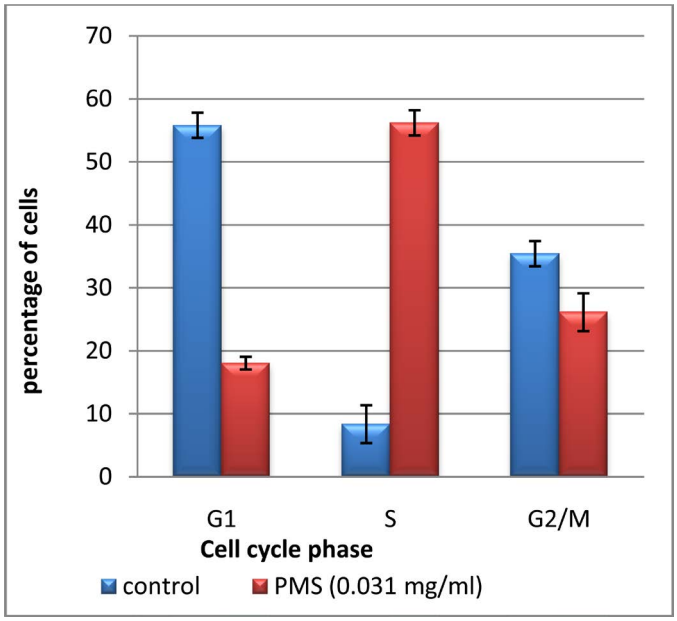
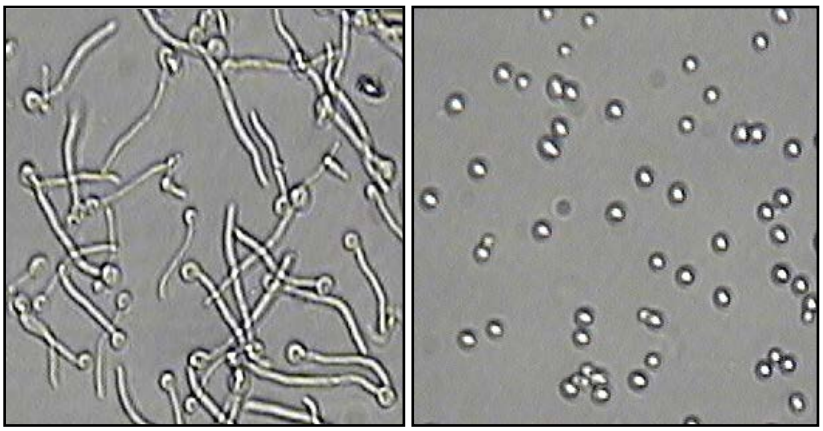


Figure 3. Effect of PMS on Cell cycle of *C. albicans.*



(a) (b)

Figure 4. (a) Serum induced yeast to hyphal form morphogenesis in *C. albicans* (ATCC 90,028) (Control). (b) Inhibition of serum induced yeast to hyphal form morphogenesis in presence of PMS (0.062 mg/ml) on *C. albicans* (ATCC 90,028).

3.2. PMS Inhibited Serum Induced Yeast to Hyphal Form Morphogenesis

Significant ($p < 0.05$) inhibition of serum induced yeast to hyphal form transition was observed in presence of PMS in a concentration dependent manner. More than fifty percentage inhibition of germ tube induction was found at 0.062 mg/ml (**Figure 4**).

3.3. PMS Altered the Expression of Genes Involved in Yeast to Hyphal Form transition Pathways

Since PMS has shown inhibition of serum induced hyphal induction in *C. albicans*, we analyzed changes in expression levels of the genes involved in Ras1-

cAMP-Efg1, Cek1-MAPK pathways with quantitative real-time PCR. It was found that the expression of Ras1 which is a master regulator was downregulated up to 1.14 fold ($p < 0.05$) by treatment with PMS. *Cdc*35 *and Pde2,* the upstream components of cAMP dependent PKA pathway were upregulated by 1.09 and 1.46 fold respectively. Similarly expression of its downstream components *Bcy*1*, Efg*1 and *Tec*1 were upregulated by 2.75, 1.12, 1.65 fold. *Ece*1 and *Hwp*1 downstream of Efg1 were significantly downregulated by 1.56 and 11.16 fold respectively (Figure 5). Expression of *Hst7, Cek*1 and *Cph*1 involved in Cek1-MAPK pathways were upregulated by 1.44, 1.43 and 2.57 fold in presence of PMS respectively (Figure 5). Negative regulators of hyphal induction, Nrg1, Mig1 and Tup1 were upregulated by 1.53, 2.96 fold and 17.95 fold respectively (Figure 5).

4. Discussion

PMS, Phenazine derivative showed inhibition of *Candida* growth and killing of *Candida* biofilm [7] [8] [16]. PMS inhibited planktonic growth, morphogenesis, biofilm formation and adhesion of *Candida albicans* ATCC 90,028 (Figure 1). Serum induced yeast to hyphal transition was also inhibited by PMS at 0.062 mg/ml (Figure 4). After 3 hr of PMS treatment, it was found that cell cycle of *C. albicans* was arrested at the S phase (Figure 3).

Adherence of *Candida* cells to surface of polystyrene Microtitre plates was analyzed by microscopically and XTT assay. It was found that adhesion was

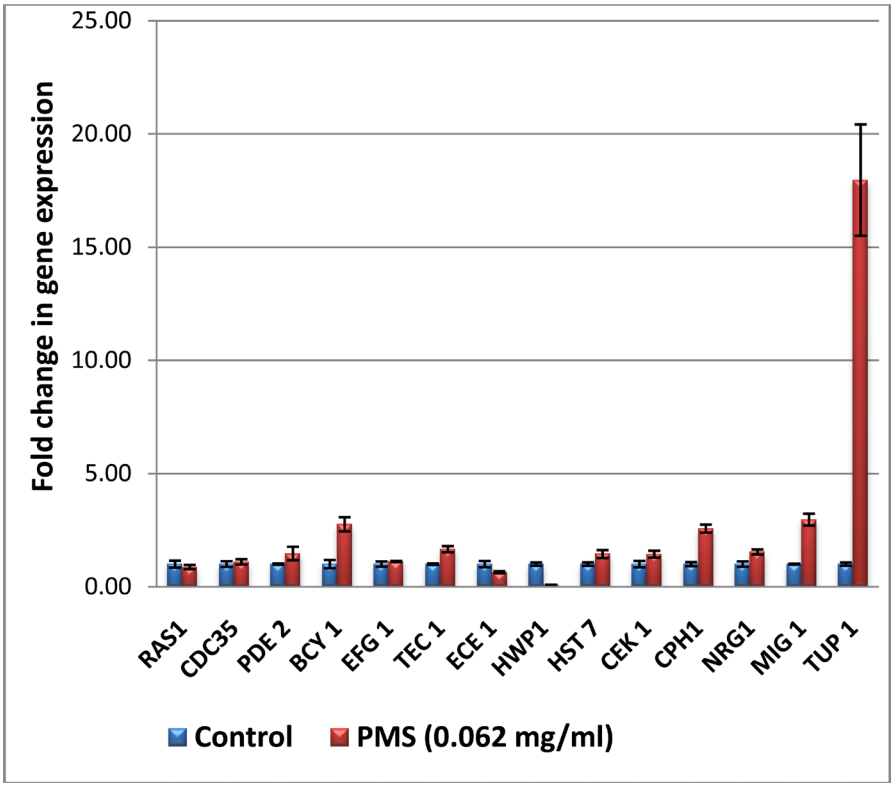


Figure 5. Gene expression profile of *Candida albicans* during serum induced yeast to hyphal form transition in presence of PMS.

significantly inhibited by PMS treatment. Adhesion may be inhibited by PMS due to the downregulation of *Hwp*1 expression by 11.16 fold (**Figure 5**). *Hwp*1 encodes a glycosylphosphatidylinositol-modified cell wall protein that can serve as a target for mammalian transglutaminases to form covalent attachments between *C. albicans* and host epithelial cells [17].

To know the effect of PMS at the transcription levels during yeast to hypha switching we performed qPCR. *Ras*1 gene which is a master regulator of both the pathways [18] was downreguated up to 1.14 fold by treatment with PMS. Expression its downstream components like Efg1 [4] which is a strong inducer of hyphal development was unaffected by the treatment of PMS. The expression of *Tec*1 was upregulated by 1.65 fold. Ece1 and Hwp1 are downstream of Efg1 were significantly downregulated by 1.56 and 11.16 fold respectively (**Figure 5**). Ece1 is required for cell elongation in *C. albicans* and *Hwp*1 is associated with adhesion and biofilm formation [17] [19]. In addition Cek1-MAPK pathway was affected by change in expression of *Hst7* followed by *Cek*1 and *Cph*1. Their expression was slightly upregulated by 1.44, 1.43 and 2.57 fold respectively. Cph1 in *C. albicans* is regulated by a mitogen-activated protein MAP kinase cascade that includes Cst20, Hst7, and Cek1 [20] [21] [22].

Hyphal suppressor gene like *Nrg*1, *Mig*1 and *Rfg*1 suppresses hyphal induction [4] [23] [24] and *Tup*1 repressor which is constitutively expressed forms a complex with MIG1, RFG1 and NRG1 negative regulators and thereby inhibit the activity of hyphal specific gene [4]. *Tup*1 encodes a transcriptional repressor that negatively controls filamentous growth in *Candida albicans* [4]. Expression of Nrg1, a negative regulator of hyphal induction was upregulated by 1.53 fold by treatment of PMS. Similarly *Mig*1 and *Tup*1 over expressed by 2.96 fold and 17.95 fold (**Figure 5**).

5. Conclusion

Our *in vitro* study revealed that PMS inhibited major virulence factors in *C. albicans* like morphogenesis and adhesion. qPCR studies showed that PMS affects the signal transduction pathways in yeast to hyphal transition in *C. albicans*. Most of the genes involved in serum induced Ras1-cAMP-Efg1 and Cek1-MAPK pathway were affected differentially by the treatment with PMS. Upregulation of Hyphal suppressor gene like Tup1 may result in inhibition of yeast to hyphal induction. PMS arrested the cell cycle of *C. albicans* at S phase. PMS did not cause hemolysis of RBCs at the effective concentrations. PMS like molecules could be good molecules to treat candidiasis. *In vivo* studies need to be done to confirm the anti-*Candida* efficacy of PMS.

Acknowledgements

AKJ and SMK are thankful to Prof. Pandit Vidyasagar, Vice Chancellor, SRTM University, Nanded for his kind support. SMK is also thankful to UGC, New Delhi for SAP DRS II Program for infrastructural support. WNG and RP are

thankful to the Department of Biotechnology Research and Development grant, Savitribai Phule Pune University, Pune, India.

Conflict of Interest

Authors declare that there is no Conflict of Interest.

References

[1] Calderone, R.A. and Fonzi, W.A. (2001) Virulence Factors of *Candida albicans*. *Current Trends in Microbiology*, **9**, 327-335. https://doi.org/10.1016/S0966-842X(01)02094-7

[2] Kumamoto, C.A. and Vinces, M.D. (2005) Contributions of Hyphae and Hypha-Co-Regulated Genes to *Candida albicans* Virulence. *Cellular Microbiology*, **7**, 1546-1554. https://doi.org/10.1111/j.1462-5822.2005.00616.x

[3] Biswas S., Van Dijck P. and Datta, A. (2007) Environmental Sensing and Signal Transduction Pathways Regulating Morphopathogenic Determinants of *Candida albicans*. *Microbiology and Molecular Biology Reviews*, **71**, 348-376. https://doi.org/10.1128/MMBR.00009-06

[4] Braun, B.R., Kadosh, D. and Johnson, A.D. (2001) NRG1, a Repressor of Filamentous Growth in *C. albicans*, is Down-Regulated during Filament Induction. *EMBO Journal*, **20**, 4753-4761. https://doi.org/10.1093/emboj/20.17.4753

[5] Carlisle, P.L., Banerjee, M., Lazzell, A., Monteagudo, C., López-Ribot, J.L. and Kadosh, D (2009) Expression Levels of a Filament-Specific Transcriptional Regulator Are Sufficient to Determine *Candida albicans* Morphology and Virulence. *Proceedings of the National Academy of Sciences,* **106**, 599-604. https://doi.org/10.1073/pnas.0804061106

[6] Jacobsen, I.D., Wilson, D., Wächtler, B., Brunke, S., Naglik, J.R. and Hube, B. (2012) *Candida albicans* Dimorphism as a Therapeutic Target. *Expert Review of Anti-Infective Therapy*, **10**, 85-93. https://doi.org/10.1586/eri.11.152

[7] Morales, D.K. and Hogan, D.A. (2010) *Candida albicans* Interactions with Bacteria in the Context of Human Health and Disease. *PLOS Pathogens*, **6**, 1-4. https://doi.org/10.1371/journal.ppat.1000886

[8] Morales, D.K., Jacobs, N.J., Rajamani, S., Krishnamurthy, M., Cubillos-Ruiz, J.R. and Hogan, D.A. (2010) Antifungal Mechanisms by Which a Novel *Pseudomonas aeruginosa* Phenazine Toxin Kills *Candida albicans* in Biofilms. *Molecular Microbiology*, **78**, 1379-1392. https://doi.org/10.1111/j.1365-2958.2010.07414.x

[9] Gibson, J., Sood, A. and Hogan, D.A. (2009) *Pseudomonas aeruginosa*-Candida *albicans* Interactions: Localization and Fungal Toxicity of a Phenazine Derivative. *Applied and Environmental Microbiology*, **75**, 504-513. https://doi.org/10.1128/AEM.01037-08

[10] Raut, J.S., Shinde, R.B., Chauhan, N.M. and Mohan Karuppayil, S. (2013) Terpenoids of Plant Origin Inhibit Morphogenesis, Adhesion, and Biofilm Formation by *Candida albicans*. *Biofouling*, **29**, 87-96. https://doi.org/10.1080/08927014.2012.749398

[11] Routh, M.M., Raut, J.S. and Karuppayil, S.M. (2011) Dual Properties of Anticancer Agents: An Exploratory Study on the *in Vitro* Anti-Candida Properties of Thirty Drugs. *Chemother*, **57**, 372-380. https://doi.org/10.1159/000330454

[12] Shinde, R.B., Raut, J.S. and Karuppayil, S.M. (2012) Biofilm Formation by *Candida albicans* on Various Prosthetic Materials and Its Fluconazole Sensitivity: A Kinetic Study. *Mycoscience*, **53**, 220-226. https://doi.org/10.1007/S10267-011-0155-Y

[13] Rajput, S.B. and Karuppayil, S.M. (2013) β-Asarone, an Active Principle of *Acorus calamus* Rhizome, Inhibits Morphogenesis, Biofilm Formation and Ergosterol Biosynthesis in *Candida albicans*. *Phytomedicine*, **20**, 139-142.

[14] Zore, G.B., Thakre, A.D., Jadhav, S. and Karuppayil, S.M. (2011) Terpenoids Inhibit *Candida albicans* Growth by Affecting Membrane Integrity and Arrest of Cell Cycle. *Phytomedicine*, **18**, 1181-1190.

[15] Chang, W., Li, Y., Zhang, L., Cheng, A. and Lou, H. (2012) Retigeric Acid B Attenuates the Virulence of *Candida albicans* via Inhibiting Adenylyl Cyclase Activity Targeted by Enhanced Farnesol Production. *PLoS ONE*, **7**, 1-10.

[16] Tupe, S.G., Kulkarni, R.R., Shirazi, F., Sant, D., Joshi, S.P. and Deshpande, M.V. (2015) Possible Mechanism of Antifungal Phenazine-1-Carboxamide from *Pseudomonas* sp. against Dimorphic Fungi *Benjaminiella poitrasii* and Human Pathogen *Candida albicans*. *Journal of Applied Microbiology*, **118**, 39-48. https://doi.org/10.1111/jam.12675

[17] Staab, J.F., Bradway, S.D., Fidel, P.L. and Sundstrom, P. (1999) Adhesive and Mammalian Transglutaminase Substrate Properties of *Candida albicans* Hwp1. *Science*, **283**, 1535-1538. https://doi.org/10.1126/science.283.5407.1535

[18] Feng, Q., Summers, E., Guo, B. and Fink, G. (1999) Ras Signaling Is Required for Serum-Induced Hyphal Differentiation in *Candida albicans*. *Journal of Bacteriology*, **181**, 6339-6346.

[19] Nobile, C.J., Andes, D.R., Nett, J.E., Smith, F.J., Yue, F., Phan, Q.T., Edwards, J.E., Filler, S.G. and Mitchell, A.P. (2006) Critical Role of Bcr1-Dependent Adhesins in *C. albicans* Biofilm Formation *in Vitro* and *in Vivo*. *PLOS Pathogens*, **2**, 636-649. https://doi.org/10.1371/journal.ppat.0020063

[20] Csank, C., Schröppel, K., Leberer, E., Harcus, D., Mohamed, O., Meloche, S., Thomas, D.Y. and Whiteway, M. (1998) Roles of the *Candida albicans* Mitogen-Activated Protein Kinase Homolog, Cek1p, in Hyphal Development and Systemic Candidiasis. *Infection and Immunity*, **66**, 2713-2721.

[21] Köhler, J.R. and Fink, G.R. (1996) *Candida albicans* Strains Heterozygous and Homozygous for Mutations in Mitogen-Activated Protein Kinase Signaling Components Have Defects in Hyphal Development. *Proceedings of the National Academy of Sciences*, **93**, 13223-13228. https://doi.org/10.1073/pnas.93.23.13223

[22] Leberer, E., Harcus, D., Broadbent, I.D., Clark, K.L., Dignard, D., Ziegelbauer, K., Schmidt, A., Gow, N.A., Brown, A.J. and Thomas, D.Y. (1996) Signal Transduction through Homologs of the Ste20p and Ste7p Protein Kinases Can Trigger Hyphal Formation in the Pathogenic Fungus *Candida albicans*. *Proceedings of the National Academy of Sciences*, **93**, 13217-13222. https://doi.org/10.1073/pnas.93.23.13217

[23] Kadosh, D. and Johnson, A.D. (2005) Induction of the *Candida albicans* Filamentous Growth Program by Relief of Transcriptional Repression: A Genome-Wide Analysis. *Molecular Biology of the Cell*, **16**, 2903-2912. https://doi.org/10.1091/mbc.E05-01-0073

[24] Murad, A.M., Leng, P., Straffon, M., Wishart, J., Macaskill, S., MacCallum, D., Schnell, N., Talibi, D., Marechal, D., Tekaia, F. and d'Enfert, C. (2001) *NRG*1 Represses Yeast-Hypha Morphogenesis and Hypha-Specific Gene Expression in *Candida albicans*. *The EMBO Journal*, **20**, 4742-4752. https://doi.org/10.1093/emboj/20.17.4742