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BRIEF COMMUNICATION

Molecular targets of biofabricated silver nanoparticles in *Candida albicans*

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Abstract

We have analyzed the expressions of genes which regulate Ras-cAMP-*EFG1* and CEK1-MAPK pathways involved in yeast to hyphal form morphogenesis in *Candida albicans*. The expression profile of genes associated with serum-induced morphogenesis showed reduced expressions of genes involved in these pathways by the treatment with biofabricated silver nanoparticles. Cell elongation gene, *ECE1*, was downregulated by 5.1 fold by the treatment of silver nanoparticles. Expression of hyphal inducer gene, *TEC1* was downregulated by 6.28 fold. Negative regulators of yeast to hyphal transition, *TUP1* and *RFG1* were downregulated by 2.45 and 5.43 fold, respectively. Current study suggests that silver nanoparticles affect gene expression and may subsequently reduce virulence in *C. albicans*. Targeting genes involved in virulence may be an acceptable novel treatment strategy for pathogenic fungal infections.

Introduction

Candida albicans is considered as a major fungal pathogen of the humans causing considerable morbidity and mortality. Currently prescribed antifungal antibiotics are often failures in clinical situations, due to the development of multiple drug resistance, formation of drug resistant biofilms on biotic as well as abiotic surfaces and transformation from yeast to hyphal (Y-H) form morphology [1]. This has resulted in the use of higher dosages of antibiotics and prolonged therapy which may result in undesirable side effects to the patients [2]. There is a need to find alternative therapy which is free from side effects. There is considerable interest in using nanotechnology-based drugs [3]. The targets of silver nanoparticles in *C. albicans* include: cell wall, cell membrane, mitochondrial function, changes in membrane fluidity, changes in fatty acids, inhibition of ergosterol synthesis, formation Reactive oxygen species, release of silver ions, ultra structural changes, apoptosis, cell cycle arrest etc. [4]. Silver nanoparticles (SNPs) have been shown to inhibit the expression of genes involved drug efflux in drug resistant clinical isolates [5]. As Y-H form morphogenesis is one of the major virulence factors in *C. albicans* and necessary for penetrating the epithelial tissues and escape from immune response [6]. Y-H form transition involves two signaling pathways, Ras1-cAMP-PKA and Cek1-MAPK pathway [6]. Targeting of hyphal specific genes involved in these pathways may be a promising strategy for anticandida drug development [7].We are presenting information on the downregulation of genes involved in Y-H signal transduction in *C. albicans* after the treatment of SNPs. This is the first report on SNPs targeting signal transduction in *C. albicans*.

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Materials and methods

Synthesis of SNPs

Silver nitrate ($AgNO_3$) was procured from Himedia Chemicals Ltd, Mumbai, India. Silver nitrate solution (1 mM)

was prepared freshly for the synthesis of SNPs. The aqueous leaf extract of *Polyalthia longifolia* was used for the biogenic synthesis of the SNPs. One milliliter of the plant leaf extract was added to 19 ml of 1 mM aqueous silver nitrate solution in a 250 ml Erlenmeyer flask and incubated for 15 min at 70°C. The biogenic synthesis of SNPs was observed spectrophotometrically at different time intervals. The biogenic synthesis of SNPs was observed by the color change from colorless to brown in the colloidal solution. The synthesis of SNPs was characterized at different time intervals using a UV–Vis double beam spectrophotometer (Shimadzu UV–Visible Spectrophotometer) at the wavelength range 200–800 nm, and scanning interval was 0.5 nm [3].

Gene expression studies

The expression of genes during Y-H form conversion was studied by using real time PCR. *C.albicans* (1×10^6 cells/ml) cells were inoculated and incubated for 90 min in 20% serum containing SNPs (3 μg ml^{-1}). Cells without treatment were served as a control (Fig. 1). Total RNA was isolated by using the RNease® Mini Kit (Nucleo-pore,India) and converted to cDNA by using SuperScript® III First strand synthesis for RT-PCR (Invitrogen by Life technologies, USA) [8–10]. qRTPCR reactions were done as per Jadhav et al.(2017). Actin, a house keeping gene was used as an internal control in this study (Table 1). Transcript levels of genes were calculated using formula $2^{-\Delta\Delta CT}$ [8–10].

Results and discussion

Expressions of 12 genes were downregulated by the treatment of biofabricated SNPs. In the Ras1-cAMP-*EFG1* pathway, *PDE2* was downregulated by 2.03 fold while *BCY1* and *EFG1*were downregulated by 1.01 and 1.33 fold (Table 2). Expression of *TEC1* and *ECE1* were significantly downregulated by 6.29 and 5.10 fold. Also UME6 gene was downregulated by 3.46 fold. Cek1-MAPK pathway genes, *CST20* (4.13 fold), *HST7* (3.72 fold), and *CEK1* (2.64 fold) were downregulated on treatment with SNPs (Fig. 2). The negative regulators of Y-H transition, *TUP1, MIG1*, and *RFG1* were downregulated by 2.45, 1.06, and 5.43 fold, respectively (Fig. 2). Out of all the genes in this study promising downregulation was exhibited by *TEC1*,*ECE1*, *CST20*, and *RFG1* genes after the treatment of biofabricated SNPs.

Here, for the first time we are reporting that biosynthesized silver nanoparticles may affect signal transduction pathways in *C. albicans* by downregulating the expression of genes which are important for Y-H form transition (Table 2). Serum-induced Y-H form the signal transduction pathway involves two signaling pathways, Ras1-cAMP-PKA and Cek1-MAPK pathway (Table 2). Y-H form transition is negatively regulated by hyphal suppressor genes *NRG1, MIG1, TUP1*, and *RFG1*. Molecules like,indole, isatin, capric acid, caprylic acid, moxyfloxacin, and dicyclomine are found to modulate expression of genes involved in Y-H form the signal transduction pathway [8–10].

In current study we found that biofabricated SNPs downregulated the expression of 12 genes involved in the Ras-mediated signal transduction pathways (Fig. 2). PDE2 is upstream component of the Ras1-cAMP-PKA pathway. Phosphodiesterases (PDEs) are unique enzymes decomposing cyclic adenosine and guanosine 3′, 5′-monophoshophates (cAMP and cGMP) [11, 12]. We found that *PDE2* gene expression was downregulated by 2.03 fold after the treatment of biofabricated SNPs (Fig. 2).

In *C. albicans* PKA regulatory subunit *BCY1* plays an important role in the regulation of cell differentiation and death [12]. It also shows essential activity on viability of *C. albicans*. Deletion of *BCY1* leads to multiple cellular morphologies and promotes the development of filaments [13] (Ding et al., 2016). After exposure to SNPs the gene *BCY1* was downregulated (Fig. 2). The downstream

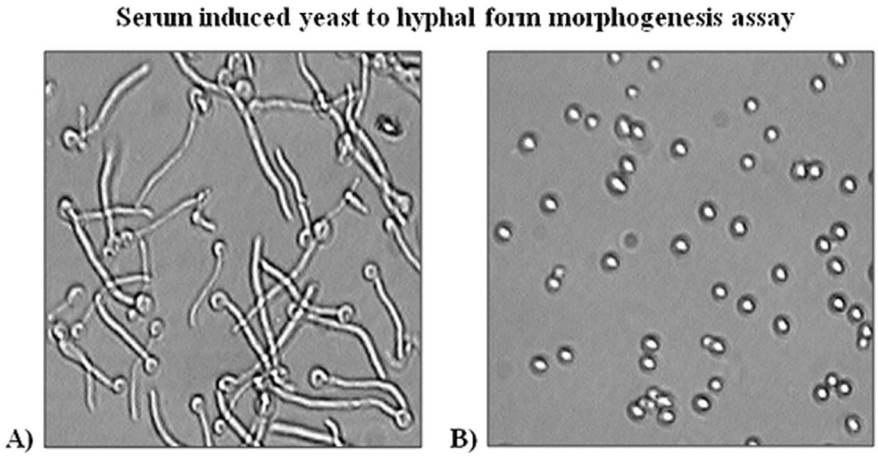

Fig. 1 Serum-induced yeast to hyphal form morphogenesis assay, **a** control **b** inhibition of serum-induced yeast to hyphal form morphogenesis in presence of silver nanoparticles (3 μg ml^{-1}) in *C. albicans* (ATCC 90028)

Table 1 List of the primers

Primers	Sequence(5′ 3′)
ACTIN-F	5′ATGGACGGTGAAGAAGTTGC 3′
ACTIN-R	5′ACCTCTTTTGGATTGGGCTTCA 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′
UME6-F	5′ TCTACTTCTAATCCAATGGTG 3′
UME6-R	5′ TATCATTACTTGATTTTTTCCGAG 3′
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′
CST20-F	5′ TTCTGACTTCAAAGACATCAT 3′
CST20-R	5′ AATGTCTATTTCTGGTGGTG 3′
MIG1	5′CTTCAACTAGCCTATATTCCGATGG 3′
MIG1	5′-CTTTCT GTAGGTACCAACAACTAC 3′
TUP1	5′ GAGGATCCCATGTATCCCCAACGCACCCAG 3′
TUP1	5′GGCGACGCGTCGTTTTTTGGTCCATTTCCAAATTCTG 3′
RFG 1-F	5′ CACACATAGGTACCCCAATACAC 3′
RFG 1-R	5′ CACTTTAAACAGATAAACTCGAGGATATG 3′

Table. 2 Effect of biofabricated silver nanoparticle on gene expression in *C. albicans* during yeast to hyphal form transition

Gene name	Fold change in gene expression on treatment of biofabricated silver nanoparticle (3 μg ml^{-1})
Ras1-cAMP-Efg1 pathway	
PDE 2	Downregulation (2.03 fold)
BCY 1	Downregulation (1.01 fold)
EFG 1	Downregulation (1.33 fold)
TEC 1	Downregulation (6.29 fold)
ECE 1	Downregulation (5.10 fold)
UME6	Downregulation (2.46 fold)
Cek1-MAPK pathway	
CST 20	Downregulation (4.13 fold)
HST 7	Downregulation (3.72 fold)
CEK 1	Downregulation (2.64 fold)
Hyphal suppressor genes	
MIG 1	Downregulation (1.06 fold)
RFG 1	Downregulation (5.43 fold)
TUP 1	Downregulation (2.45 fold)

component *ECE1* required for cell elongation during filamentation in *C. albicans* was significantly downregulated by SNPs up to 5.1 fold (Fig. 2). *ECE1* has a critical role in invasion of epithelial cells during infection [14]. The downregulation of expression of *ECE1* may reduce the filamentation and invasive property (Fig. 2).

SNPs downregulated the expression of *CST20, HST7,* and*CEK1* (Fig. 2). The downregulation of *CST20, HST7, and CEK1* may contribute to the inhibition of yeast-to-hyphal form morphogenesis in *C. albicans* [15, 16]. The hyphal suppressor genes like *NRG1, MIG1, RFG1*, and *TUP1* are inhibitors of yeast-to-hyphal transition [17–19]. Hyphal suppressor genes *NRG1, TUP1* were significantly upregulated by indole and isatin. *NRG1* and *TUP1*, negative regulators of hyphal formation, were overexpressed in the presence of capric or caprylic acid [8] but biofabricated SNPs did not upregulate the expression of the hyphal suppressor genes (Fig. 2).

Downregulation of the regulators of Y-H signal transduction pathway in *C. albicans* may result in the inhibition of Y-H form transition. Molecular targets in the Y-H signal transduction pathway will be novel targets to control the pathogenesis of *C. albicans* [20]. Biofabricated SNPs

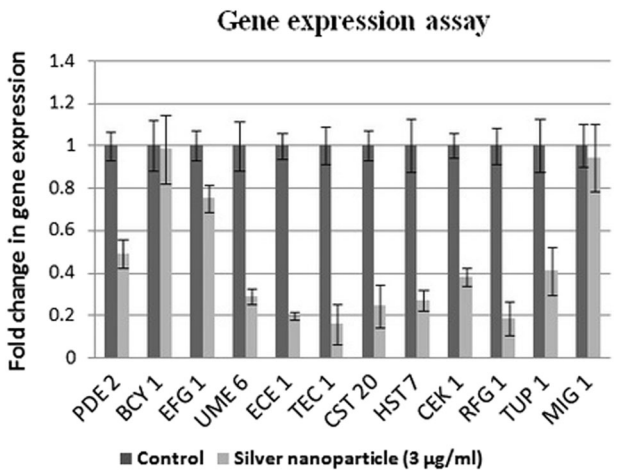

Fig. 2 Gene expression assay. Gene expression profile in presence of biofabricated silver nanoparticle during yeast to hyphal transition in *C. albicans* (ATCC 90028) as compared to control

downregulated the expression of genes involved in Ras–cAMP-*EFG1* pathway and CEK1-MAPK pathway, which were responsible for Y-H form transition in *C. albicans*. The expression of *ECE1* and *TEC1*, which are downstream regulators of Ras–cAMP-*EFG1,* was downregulated by the treatment of biofabricated SNPs. Similarly *CST20*gene expression in the CEK1-MAPK pathway was showed reduced expression in *C. albicans* cells.

This study revealed that genes involved in the Y-H transition pathway are downregulated by the treatment of biofabricated SNPs. Biofabricated SNPs targeted important components in the Y-H transition pathway which is pivotal for virulence and biofilm formation. Hence it may affect the pathogenicity of *C. albicans*. Targeting of virulence factors by SNPs may be a novel paradigm for developing drugs against *C. albicans* since it has multiple targets in *C. albicans*. As it has multiple targets in *C. albicans*, the chances of developing drug resistance are less. Multiple molecular and cellular targets may favor broad-spectrum antifungal activity and minimal host toxicity. In vivo studies need to be done to confirm the efficacy of SNPs against candidiasis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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