Effect of low shear modeled microgravity on phenotypic and central chitin metabolism in the filamentous fungi Aspergillus niger and Penicillium chrysogenum

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ORIGINAL PAPER

Effect of low shear modeled microgravity on phenotypic and central chitin metabolism in the filamentous fungi *Aspergillus niger* and *Penicillium chrysogenum*

Yesupatham Sathishkumar · Natarajan Velmurugan · Hyun Mi Lee · Kalyanaraman Rajagopal · Chan Ki Im · Yang Soo Lee

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Abstract Phenotypic and genotypic changes in *Aspergillus niger* and *Penicillium chrysogenum*, spore forming filamentous fungi, with respect to central chitin metabolism were studied under low shear modeled microgravity, normal gravity and static conditions. Low shear modeled microgravity (LSMMG) response showed a similar spore germination rate with normal gravity and static conditions. Interestingly, high ratio of multiple germ tube formation of *A. niger* in LSMMG condition was observed. Confocal laser scanning microscopy images of calcofluor flurophore stained *A. niger* and *P. chrysogenum* showed no significant variations between different conditions tested. Transmission electron microscopy images revealed number of mitochondria increased in *P. chrysogenum* in low shear modeled microgravity condition but no stress related-woronin bodies in fungal hyphae were observed. To gain additional insight into the cell wall integrity under different conditions, transcription level of a key gene involved in cell wall integrity *gfaA*, encoding the glutamine: fructose-6-phosphate amidotransferase enzyme, was evaluated using qRT-PCR. The transcription level showed no variation among different conditions. Overall, the results collectively indicate that the LSMMG has shown no significant stress on spore germination, mycelial growth, cell wall integrity of potentially pathogenic fungi, *A. niger* and *P. chrysogenum*.

Keywords *Aspergillus niger* · *Penicillium chrysogenum* · Low shear modeled microgravity (LSMMG) · Phenotypic characteristics · Central chitin metabolism · *gfaA* gene

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Introduction

The effect of space and space flights on the survival of microorganisms was first recorded in 1935 aboard the high altitude balloon Explorer II (Francis and Cockell 2010). Detailed studies on microbial survival in space and simulated extraterrestrial environments can help for space exploration (Francis and Cockell 2010). Nowadays, the simulated microgravity condition can be achieved using high aspect ratio vessel (HARV) in

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the laboratory. The biomass can be suspended in the media inside the HARV and the vessel rotated along its horizontal axis which provides the free fall simulating the microgravity. One side of the HARV has special oxygenator membrane which facilitates the gas exchange (Nickerson et al. 2003). Effect of ground-based simulated microgravity on microorganisms and different cell lines studies suggested several physiological changes that mimic those seen in space flight (Kumari et al. 2009). Several microorganisms, *Escherichia coli* K12 MG1655 (Arunasri et al. 2013), *Salmonella enterica* (Nickerson et al. 2000), *Saccharomyces cerevisiae* (Purevdorj-Gage et al. 2006; Van Mulders et al. 2011), and different cell lines, embryonic stem cells (Wang et al. 2011), human lymphocytes (Kumari et al. 2009), have been reported for their physiological and metabolic changes under ground-based simulated microgravity conditions. Phenotypic changes of the yeast *Candida albicans* has been studied and reported that morphology was altered from round, budding yeast form to elongated, filamentous form under LSMMG conditions (Altenburg et al. 2008). Recently, phenotypic changes of *Ulocladium chartarum* colonies grown on international space station (ISS) has been reported (Gomoiu et al. 2013). It has been reported a decade ago that fungi are sensitive to changes in the gravity vector (Moore et al. 1996). However, there is limited information available on effect of LSMMG condition on filamentous, pathogenic fungal species cellular and metabolic responses.

Potential toxin or allergen producers like *Penicillium chrysogenum* and *P. brevicompactum* and potential opportunistic pathogens like *Aspergillus niger* and *A. flavus* have been isolated from HEPA filters in the US Laboratory module of the International Space Station (Vesper et al. 2008). These toxin producers can cause inflammatory and cytotoxic responses (Vesper et al. 2008). The colonized microbial consortia can result in biodegradation, and also a direct threat for crew health (Leys et al. 2009). It has been reported that the phagocytic capability of the neutrophils and monocytes from the astronauts after mission to be decreased (Kaur et al. 2004, 2005). This condition may lead to these particular opportunistic pathogens emerging as a serious threat for the health of astronauts. In addition, fungal mold growth also can act on the organic substrates of the space flight and can damage the integrity and affect its operation (Vesper et al. 2008). Thus, prime importance has to be given to phenotypic characterization of these filamentous fungi under microgravity condition. Scanning electron microscopy is considered to be a promising advanced tool to observe the trajectory of fungal hyphae since it can be altered by the growth condition and a potential indicator to monitor the effect of microgravity condition on fungal physiology. Transmission electron microscopy enables the analysis of internal subcellular organelles, vacuolation pattern, abnormal distribution of polysaccharides, autolysis and degradation of cytoplasmic contents (Ahmad and Khan 2012). Confocal microscopy, when combined with a suitable fluorescent dye, can distinguish the subcellular visualization by "optical sectioning" using a monochromatic laser light in a scanning raster across the hyphae (Czymmek et al. 1994).

A study by Johanson et al. (2002) compared the cells stress response pathway of *S. cerevisiae* at different time points under LSMMG condition. The stress response pathway was shown to be shifted from normal gyrorotatory growth to rotational growth through binding of stress responsive elements (STRE) sites in several types of genes. These genes involved in stress response (HLJ1 (HSP40 protein), and SSA4 (HSP70 protein)) and metabolism/glucose utilization. Those involved in metabolism and glucose utilization genes encode phosphoglucomutase (PGM2), phosphoglycerate mutase (GPM2), and cytochrome-c oxidase chain Vb (COX5B). The genotype and phenotype changes by *S. cerevisiae* in response to LSMMG sensed as mechanical deformation or perturbation of the cell surface and subsequently transmitted into a molecular response (Nickerson et al. 2004). Although cellular response mechanism have been studied in *S. cerevisiae* in response to microgravity condition (Nickerson et al. 2004) analogous research in filamentous, pathogenic fungal species has yet to be undertaken. Chitin is a polymer composed of β-1,4-linked *N*-acetylglucosamine (GlcNAc) and contributes 10–30 % of the dry weight of the cell wall of filamentous fungi and is considered to be a major component of the cell wall providing physical strength (Nobel et al. 2000). Fungal species have been reported to contain multiple chitin synthase-encoding genes (Nobel et al. 2000; Ram et al. 2004). Different chitin synthases are highly regulated during the different stages of fungal growth development (Ram et al. 2004). Thus, it is necessary to conduct a comparative characterization of the spore germination, hyphae development and differential expression of chitin-encoding genes under LSMMG of *P. chrysogenum* and *A. niger* fungal species because the

majority of earlier reports have been heavily centered on the investigation of the effect of microgravity on bacteria, yeast and human cell lines (Arunasri et al. 2013; Kumari et al. 2009; Nickerson et al. 2000; Purevdorj-Gage et al. 2006; Van Mulders et al. 2011; Wang et al. 2011). The key gene involved in chitin synthesis coding for glutamine: fructose-6-phosphate amidotransferase enzyme is shown to be up-regulated under stress conditions (Ram et al. 2004). The fluorescent dye calcofluor white (CFW) has been most commonly used to detect chitin distribution in the fungal cell wall. The chitin distribution and effect of different stress conditions on expression of chitin-encoding gene can be analyzed using CFW and transcription level analysis and; possible direct correlation can be made between CFW staining and transcription levels.

In this study, a complete characterization of filamentous fungi, *P. chrysogenum* and *A. niger* with respect to their phenotypic and central chitin metabolism under LSMMG condition is presented. Specifically, the relationship between spore germination, hyphae growth and development and chitin distribution was examined. Furthermore, the transmission electron microscopic study revealed the intracellular changes in *P. chrysogenum* and *A. niger* under microgravity condition. A possible correlation was made between chitin distribution and transcription level in *P. chrysogenum* and *A. niger* under microgravity condition.

Materials and methods

Fungal strains

Aspergilus niger (KACC 42589) and *P. chrysogenum* (KACC 425892) were purchased from Korean Agricultural Culture Collection (Suwon, South Korea). Fungal cultures were maintained on potato dextrose agar (BD Difco, Sparks, MD) supplemented with streptomycin (100 mg l^{-1}) (Sigma-Aldrich, St. Louis, MO). Spores from 7 days old plate were used for the experiment.

Microscopic findings of phenotypic changes

Light microscopy

Spores from 7 days old culture were used for the experiment. Spore number was adjusted to the initial concentration of 1×10^6 ml^{-1} with fresh potato dextrose broth (PDB) (BD Difco, Sparks, MD) (pH 5.2) and used as seed culture, immediately. High Aspect Ratio Vessel (HARV) (Synthecon Inc.) was filled with 48 ml of PDB supplemented with streptomycin and 2 ml of seed culture was added and rotated at 25 rpm in horizontal axis and vertical axis to provide LSMMG and normal gravity conditions respectively, inside a chamber with 90 % humidity and 25 °C. The rotation was adjusted to 25 rpm as the spore aggregates formed a fluid orbit within the vessel, exhibiting continual fall without contacting the vessel wall was attained according to the manufacturers instruction (Operation Manual, The Rotary Cell Culture System, Synthecon Inc.). To rule out the effect of excessive oxygenation by the HARV on the spore germination a static condition was included. For static condition 48 ml of PDB and 2 ml of seed culture was added to HARV and incubate in vertical axis without any rotation inside a chamber with 90 % humidity at 25 °C. In all the conditions, germination rates of *P. chrysogenum* and *A. niger* were observed for 24 h in 4 h interval using light microscope (Olymbus BX41TF, Tokyo, Japan). Triplicates were maintained throughout the experiments.

Electron microscopic studies

Transmission electron microscopy

For intracellular analysis, 48 h cultures of *A. niger* and *P. chrysogenum* grown under the conditions mentioned as above were used. Primary fixation was done with 2.5 % glutaraldehyde (Daejung, Gyonggi-do, Korea) and 2 % paraformaldehyde (EMS, Hatfield, PA) buffered with 0.01 M phosphate buffered saline for 4 h at 4 °C. Post fixation was done with 1 % osmium tetroxide (Tedpella, Redding, CA) in 0.05 M PBS pH- 7.2 for 2 h. Samples briefly washed with distilled water at room temperature and kept at 4 °C with 0.5 % uranyl acetate (EMS, Hatfield, USA). Samples were dehydrated with graded ethanol series 30, 40, 50, 60, 70, 80, 90 % and three changes of 100 % ethanol (Daejung, Gyonggi-do, Korea) 10 min for each alcohol dilution. Transition was done with 100 % propylene oxide (EMS, Hatfield, USA). Infiltration was done with Embed812 resin overnight at 70 rpm using Peneteron (Sunkay Laboratories, Japan) and allowed to polymerize at 70 °C for 48 h. Sections of 80 nm thickness were taken using Leica UCT

ultramicrotome (Leica Mikrosysteme GmbH, Austria) and collected on copper grid and stained with Reynold's lead citrate (EMS, Hatfield, PA) and examined on a transmission electron microscope (Hitachi H7650, Tokyo, Japan).

Scanning electron microscopy

For SEM studies, 48 h cultures of *A. niger* and *P. chrysogenum* grown under the conditions mentioned as above were used. Samples were fixed and dehydrated with ethanol as explained in the transmission electron microscopy section. Samples were kept in desiccator until they were completely dried. Samples were placed on a stub coated with carbon electron conductive tape. Sputter coated with gold (Polaron SC7640) at 20 mA for 80 s and examined under scanning electron microscope (Hitachi S-3000N, Japan).

Confocal microscopy

For chitin distribution studies, 48 h cultures of *A. niger* and *P. chrysogenum* grown under the conditions mentioned as above were used. Samples were washed briefly by 0.01 M PBS. Mycelia were stained with 1 mg/ml solution of calcofluor white M2R (Fluka, Buchs, Switzerland) for 5 min and visualized using Zeiss Meta 510 LSM confocal Microscope (Zeiss, Jena, Germany) with the excitation/emission set at 488/405 nm.

Central chitin metabolism

RNA isolation and qRT-PCR

For RNA isolation, 48 h cultures of *A. niger* and *P. chrysogenum* grown under the conditions mentioned as above were used. RNA was isolated according to the manufacturer's protocol provided by RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). RNA was assessed by OD 260/280 ratio and it ranged from 1.8-2.0. Quantitation was done using Epoch Microplate Spectrophotometer (Biotek, Winooski, VT).

gfaA transcription level was analyzed using real time PCR was performed using the Applied Biosystems 7500 Fast system (Foster city, CA). One step QuantiTect SYBR Green RT-PCR (Qiagen, Hilden, Germany) was used to perform the assay. Total reaction volume was 25 µl containing 2X Master Mix (provides final concentration of 2.5 mM $MgCl_2$), primers (0.5 µM) (Macrogen, Daejeon, Korea), QuantiTect RT mix, and Template (500 ng). For reverse transcription, template was pre-incubated for 30 min at 50 °C and PCR initial activation step for 15 min at 95 °C then subjected to 40 cycles under the following thermal conditions: 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s. The values of the relative quantification (Rq) were averaged and fold changes were calculated by normalizing expression levels to internal standards. All values reported represent the mean of at least three independent experiments. Statistical differences were determined by the t test for mean comparison using SigmaPlot 10.0 software (Systat Software Inc., Chicago, IL).

Results and discussion

Effect of microgravity on spore germination of *A. niger* and *P. chrysogenum*

To obtain healthy germination of *A. niger* and *P. chrysogenum*, as mentioned in materials and methods, fresh seed spores of *A. niger* and *P. chrysogenum* were obtained and inoculated into working cultures. The germination rates of *A. niger* and *P. chrysogenum* in the microgravity, normal and static conditions are shown in Fig. 1. Both fungal strains had a similar spore germination rate until 8 h (Fig. 1). A spore was considered germinated when the length of its germinal tube reached one half of the diameter of the spore (Zhao et al. 2010). The *A. niger* and *P. chrysogenum* had no spore germination at any conditions before 4 h in all three conditions and 8 h germination rate was almost similar for both strains in all three conditions (Fig. 1). The *A. niger* spore germination rate was found to be increased under LSMMG condition at 12 h than the normal and static conditions (Fig. 1a). However, *A. niger* spore germination rate had no significant difference at 24 h in LSMMG and static conditions (Fig. 1a). *P. chrysogenum* showed rapid spore germination rate at 12 h under LSMMG and difference in the germination rate between LSMMG and other two conditions decreased gradually at the following readings at 16, 20, 24 h reading (Fig. 1b). However, *P. chrysogenum* spore germination rate in

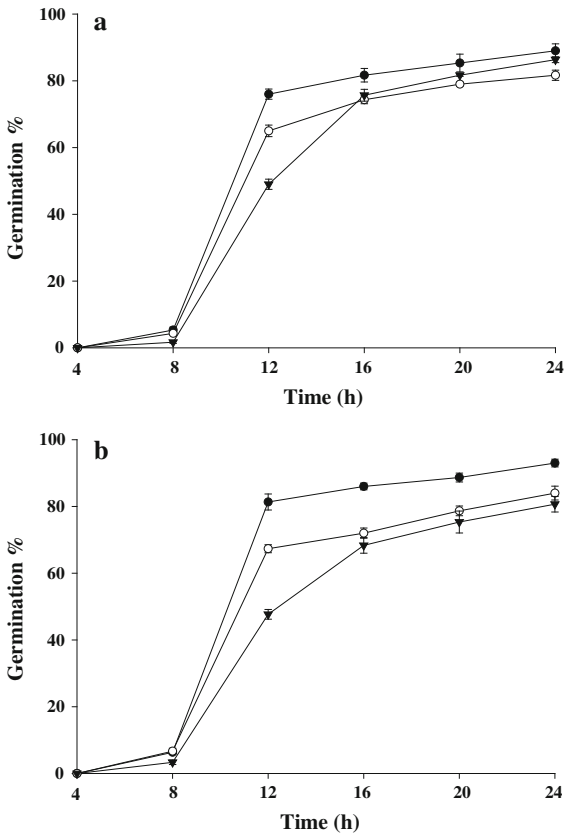

Fig. 1 *Aspergillus niger* (**a**) and *P. chrysogenum* (**b**) spore germination growth curve, spore germination was observed at 12 h under microgravity (*black circle*), normal (*open circle*), and static (*black down pointing triangle*) conditions

LSMMG condition was found to be comparatively higher than the normal and static conditions (Fig. 1b). It was interesting to observe that *P. chrysogenum* spore germination was better in LSMMG condition than the normal and static conditions (Fig. 1b). Light microscopic images of spore germination of *A. niger* and *P. chrysogenum* are shown in Fig. 2. After 12 h of incubation, multiple germ tube formation was observed in *A. niger* spores under LSMMG condition whereas one germ tube was formed in normal and static conditions (Fig. 2a–c). While *P. chrysogenum* spores had no significant multiple germ tube formation in all three conditions (Fig. 2d–f). It is theoretically possible that some stress conditions are not lethal but results in increased emergence of germ tubes and branches and selects new growth sites (Hernandez-Rodriguez et al. 2012).

Morphological features of *A. niger* and *P. chrysogenum* under microgravity condition

One of the aims of this study was to compare the effectiveness of microgravity condition on morphological features of filamentous fungi, *A. niger* and *P. chrysogenum*. The fungal hyphae morphology can be used as a potential selective indicator to study the effect of microgravity condition on fungal physiology. The scanning electron microscopic images of *A. niger* and *P. chrysogenum* under microgravity condition are shown in Fig. 3. The *A. niger* mycelium grown LSMMG condition showed homogenous hyphae with smooth cell wall (Fig. 3a, b) and showed no differences when compared with normal and static conditions. While results regarding effect of microgravity on *P. chrysogenum* mycelium were consistent with the results of *A. niger* (Fig. 3g–l). Some studies were focused on short time effect of laboratory scale LSMMG on microbial growth. Significantly, a report by Purevdorji-Gage et al. (2006) suggested that yeast cells *S. cerevisiae* (1,400 min old culture) grown under LSMMG conditions did not differ in growth rate, size, shape or viability from the controls. The results highlighted here are somewhat consistent with the previous report on short term effect of LSMMG on phenotype of *Saccharomyces cerevisiae* (Purevdorj-Gage et al. 2006). Overall, the scanning electron microscopy results suggest that the LSMMG was not stressful on *A. niger* and *P. chrysogenum* mycelia.

Effects of LSMMG condition on cell-wall chitin contents of *A. niger* and *P. chrysogenum* were examined using laser scanning confocal microscopy in combination with fluorescent dye calcofluor. Confocal images of calcoflour stained *A. niger* and *P. chrysogenum* are shown in Fig. 4. Chitin microfibrils found to be structurally important component of fungal cell wall and significantly contribute to the overall integrity of the filamentous fungal cell wall (Zhao et al. 2010). No significant difference in the fluorescent intensity was observed along with the *A. niger* and *P. chrysogenum* cell wall among LSMMG, normal and static conditions (Fig. 4). The diameter of the hyphae was analyzed using LSM image browser in five individual regions of interests (ROI) for each condition and no significant difference was observed for *A. niger* and *P. chrysogenum* (data not shown). The results collectively indicated that chitin distribution in

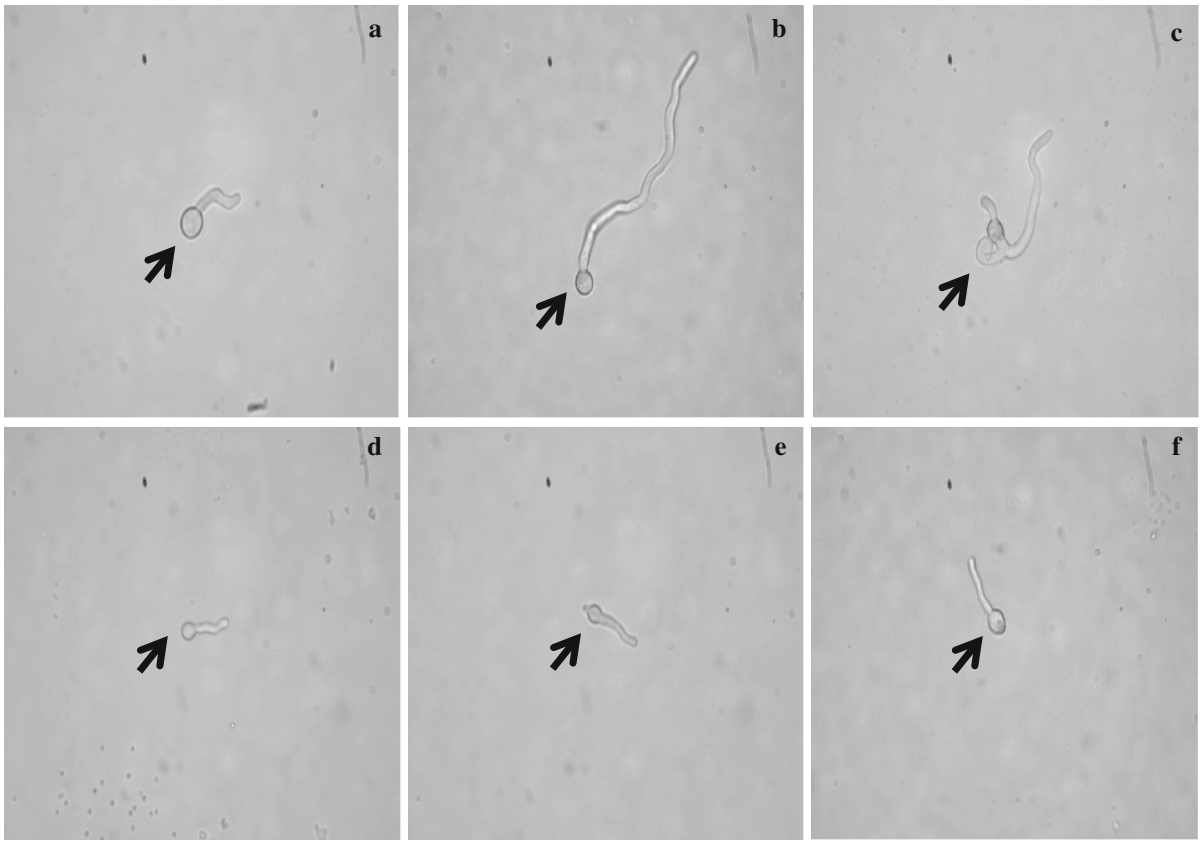

Fig. 2 *A. niger* (**a**, **b**, **c**) and *P. chrysogenum* (**d**, **e**, **f**) spore germination at 12 h. **a**, **d** static condition, **b**, **e** normal condition, **c**, **f** LSMMG condition. *Arrows* are indicating the spores

A. niger and *P. chrysogenum* cell wall was not affected by LSMMG condition.

Intracellular changes in *A. niger* and *P. chrysogenum* under microgravity condition

Pellets of live cells of *A. niger* and *P. chrysogenum* were collected, fixed, dehydrated, transited and sectioned using ultamicrotome. The sections were analyzed for intracellular changes, using transmission electron microscopy. Figures 5 and 6 shows the transmission electron microscopy images of *A. niger* and *P. chrysogenum* grown under microgravity, normal and static conditions. The intracellular observations of *A. niger* hyphae grown under LSMMG showed no difference in the sub cellular distribution compared to normal and static conditions (Fig. 5) except for the increased distribution of extracellular melanosome-like structure along the cell wall (data not shown). Additionally, no woronin bodies were observed near the septa suggesting that microgravity had not pose stress on *A. niger*. *A. niger* grown under normal gravity showed normal granulation of the cytoplasm and slight increase in number and size of vacuoles were observed compared to microgravity and static conditions and electron dense gamma particles were observed in the cytoplasm (Fig. 5d–f). In comparison, scanning electron microscopy images had shown slightly distorted cell organelles under static condition (Fig. 3e, f). Figure 6a–c shows the ultra-structure of *P. chrysogenum* hyphae grown under LSMMG revealed regular submicroscopic and clearly visible organelles. Very interestingly, increased number of cellular energy generator mitochondria with membranous cristae was observed all along the cytoplasm and not only near the apex of the

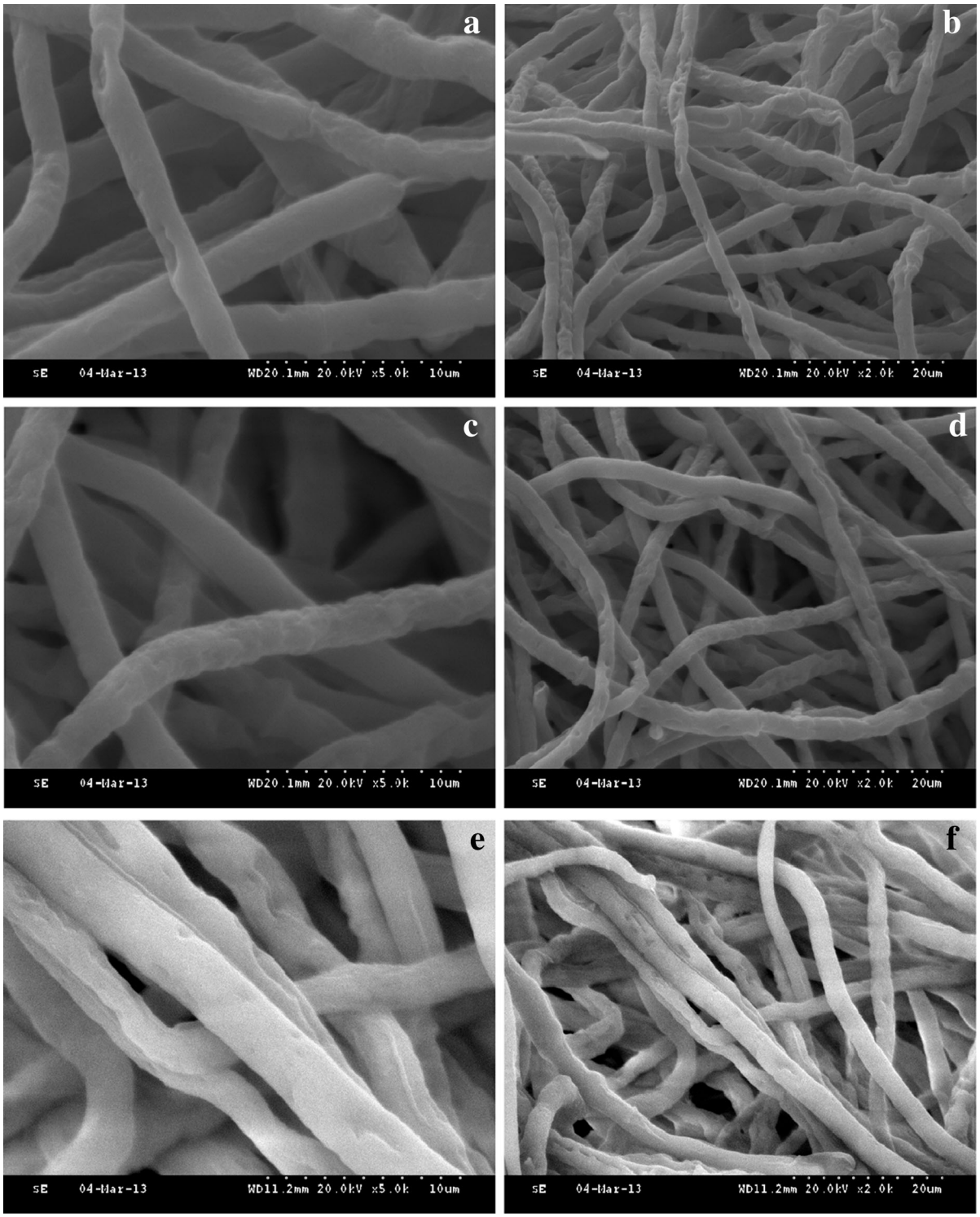

Fig. 3 Scanning electron microscopy images of *A. niger* (**a**–**f**) and *P. chrysogenum* (**g**–**l**) mycelia. **a**, **b**, **g**, and **h** LSMMG condition, **c**, **d**, **i**, and **j** normal condition, **e**, **f**, **k**, and **l** static condition

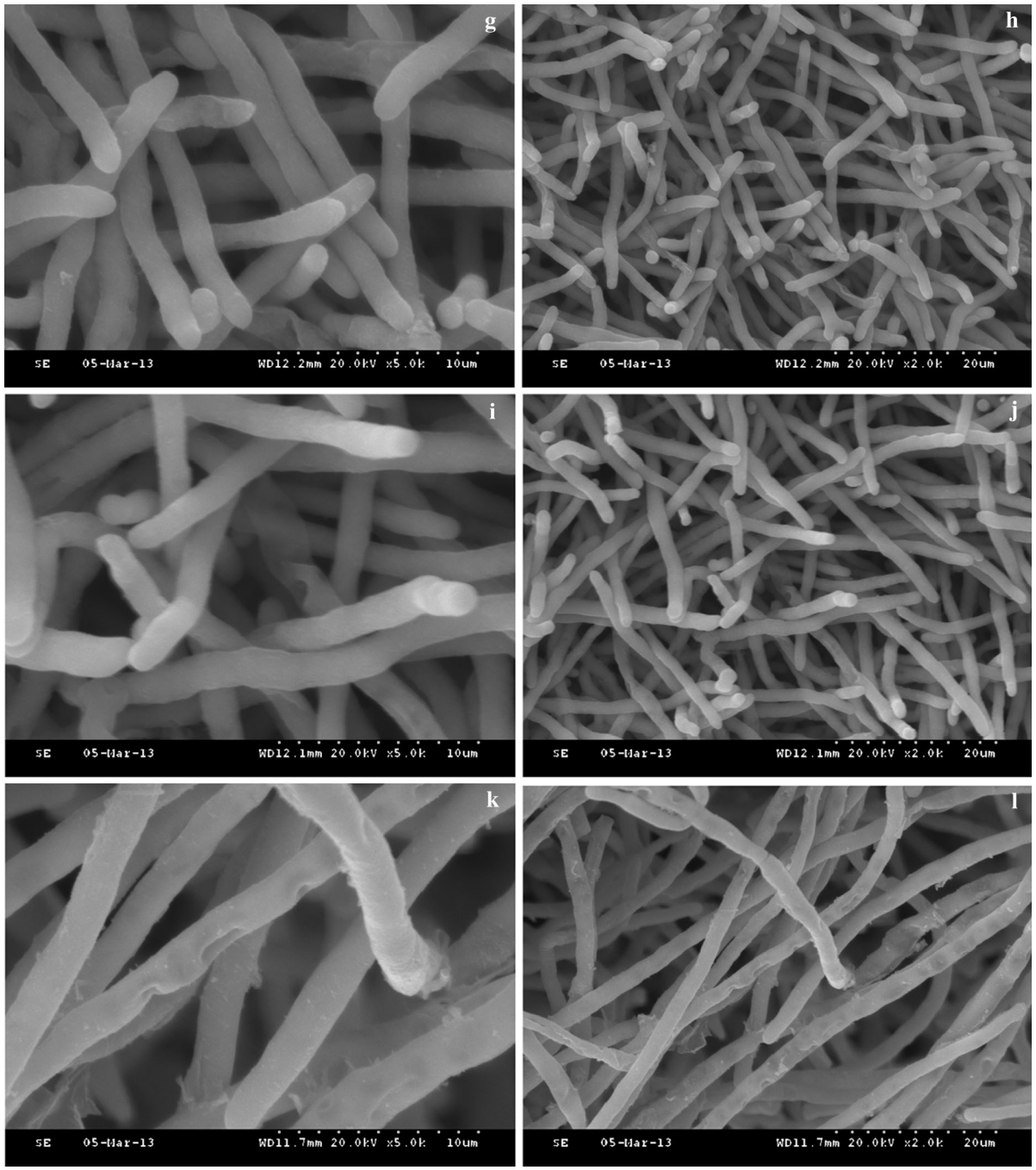

Fig. 3 continued

hyphae. Visualization of increase number of mitochondria in *P. chrysogenum* under microgravity condition is a new observation. *P. chrysogenum* cell wall appeared to be healthy and intact suggesting normal chitin distribution. *P. chrysogenum* hyphae grown under normal gravity showed healthy structures same as other conditions except for increase in vacuole size and number of electron dense gamma particles (Fig. 6d–f). Conidiophore formation was observed in the *P. chrysogenum* hyphae grown under

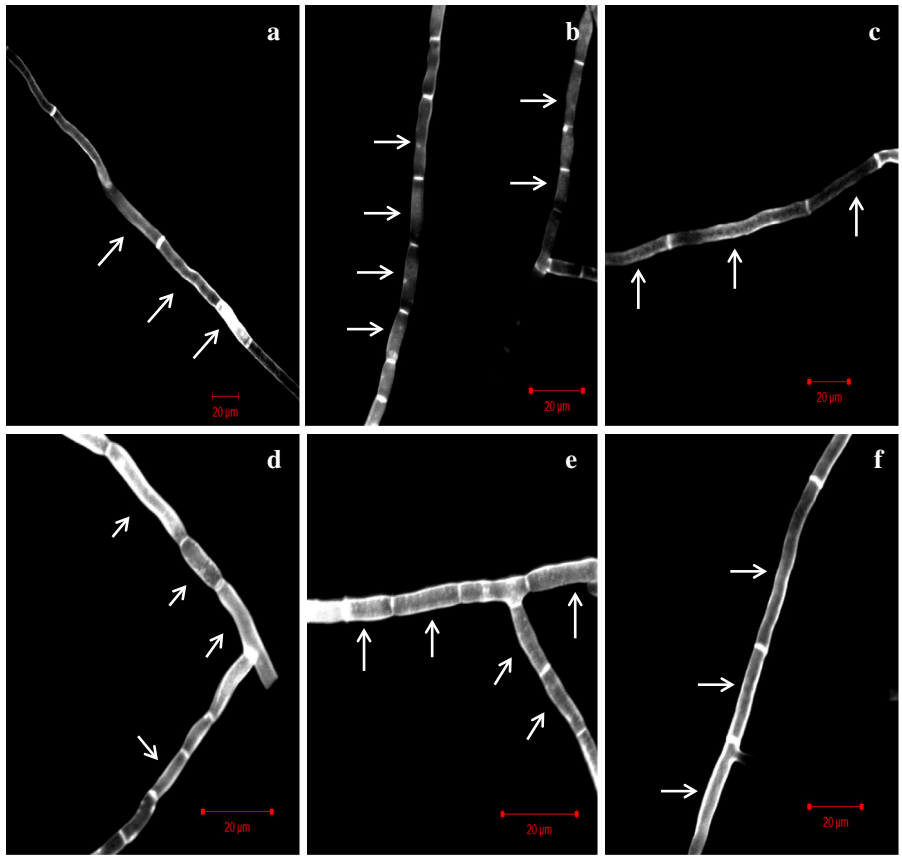

Fig. 4 Confocal laser scanning microscopy images of calcoflour stained *A. niger* (**a**, **b** and **c**) and *P. chrysogenum* (**d**, **e** and **f**) mycelia. **a**, **d** LSMMG condition, **b**, **e** normal condition, **c**, **f** static condition. *Arrows* are indicating some of randomly selected regions for the measurement of hyphae diameter

static condition (data not shown). We also scanned *P. chrysogenum* for the presence of stress responsive woronin bodies and none were observed, which suggests that *P. chrysogenum* can grow well under microgravity.

Effect of simulated microgravity in central chitin metabolism in *A. niger* and *P. chrysogenum*

The chitin biosynthetic pathway is generally activated when fungi undergo environmental stress. Earlier studies in filamentous fungi *A. niger*, *P. chrysogenum*, *Fusarium oxysporum* and the yeast *S. cerevisiae* have been indicated that increase in transcript levels of the *gfaA* gene resulted in increased accumulation of chitin in the cell wall (Ram et al. 2004). *gfaA* encodes the enzyme glutamine: fructose-6-phosphate amidotransferase. This enzyme is involved in the formation of glucosamine-6-phosphate from glutamine and fructose-6-phosphate. This is the first and also the rate limiting step for the metabolic pathway of UDP-*N*-acetylglucosamine, a sugar donor for the synthesis of chitin. This makes *gfaA* an ideal gene for expression studies to check whether LSMMG impose any stress to the cell wall of *A. niger* and *P. chrysogenum*. To evaluate the *gfaA* transcription level, RNA was isolated from 48 h cultures of *A. niger* and *P. chrysogenum* grown under microgravity, static and normal conditions. Primers were designed based on previous reports (Table 1; Ram et al. 2004). Appropriate housekeeping genes, *act* and *benA* genes respectively coding for actin and β-tubulin, were maintained as indigenous controls to monitor the transcription alterations (Bohle et al. 2007; Murray and Hynes 2010). High specificity and sensitivity in qRT-PCR was achieved using HotStarTaq DNA polymerase together with specialized buffer containing ROX dye for fluorescence normalization. Figure 7 showed the transcription level of *gfaA* gene of *A. niger* and *P. chrysogenum* under LSMMG, normal

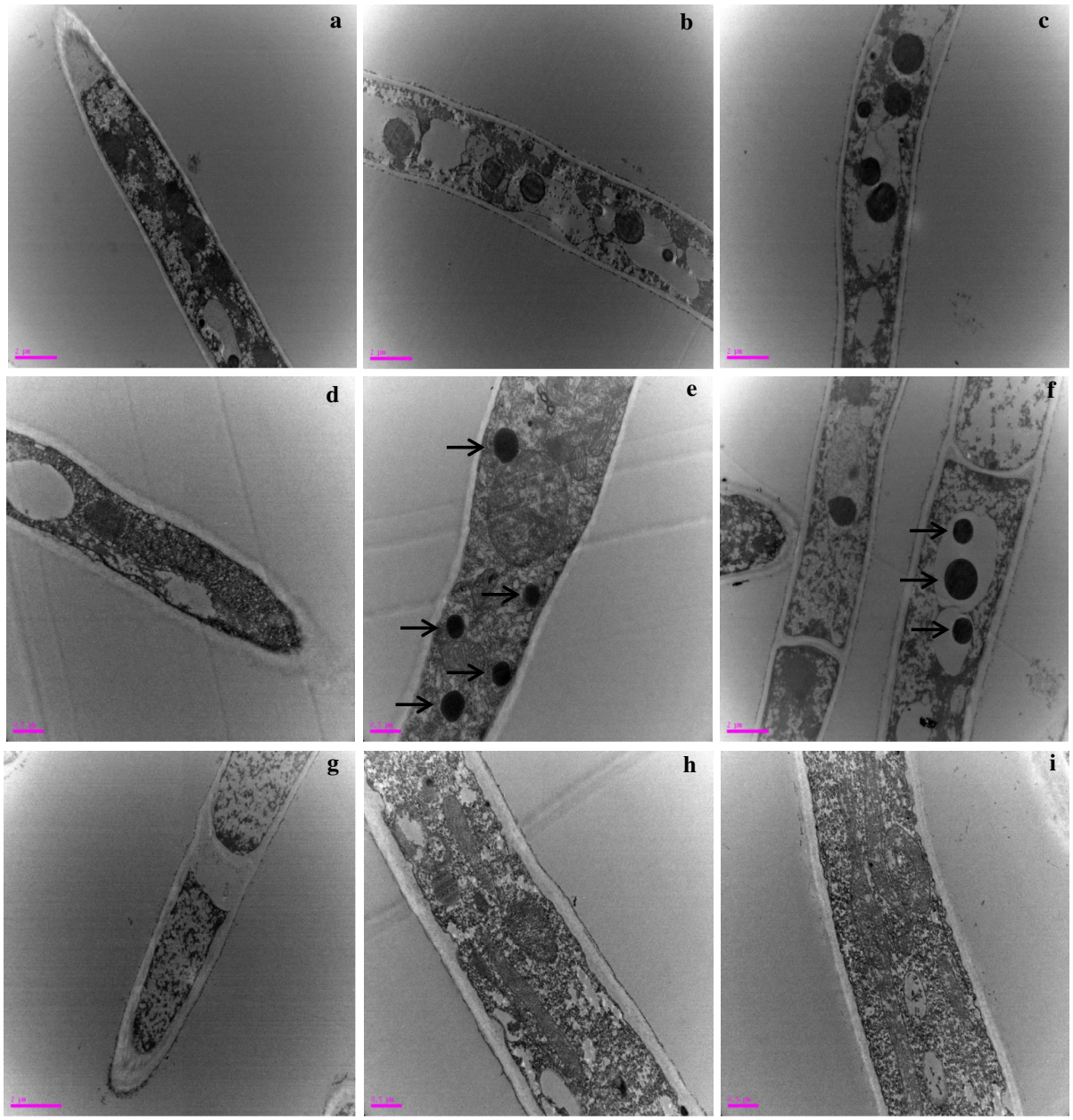

Fig. 5 Transmission electron microscopy images of *A. niger* mycelia. **a–c** LSMMG condition, **d–f** normal condition, **g–i** static condition. *Arrows* are indicating electron dense particles

and static conditions. Of the *A. niger* and *P. chrysogenum* grown under LSMMG condition, the transcription level of *gfaA* gene of *P. chrysogenum* showed highest values than *A. niger* [please refer Rq values of M in Fig. 7, Rq was 2.81 for *P. chrysogenum* (Fig. 7b) and Rq was 1.26 for *A. niger* (Fig. 7a)]. The transcription level of *gfaA* gene of *P. chrysogenum* showed 1.8 fold increases in LSMMG condition compared with controls (Fig. 7b). However, it has been suggested that less than 3–5-fold difference may not be considered as significant changes in transcription level. Very interestingly, no

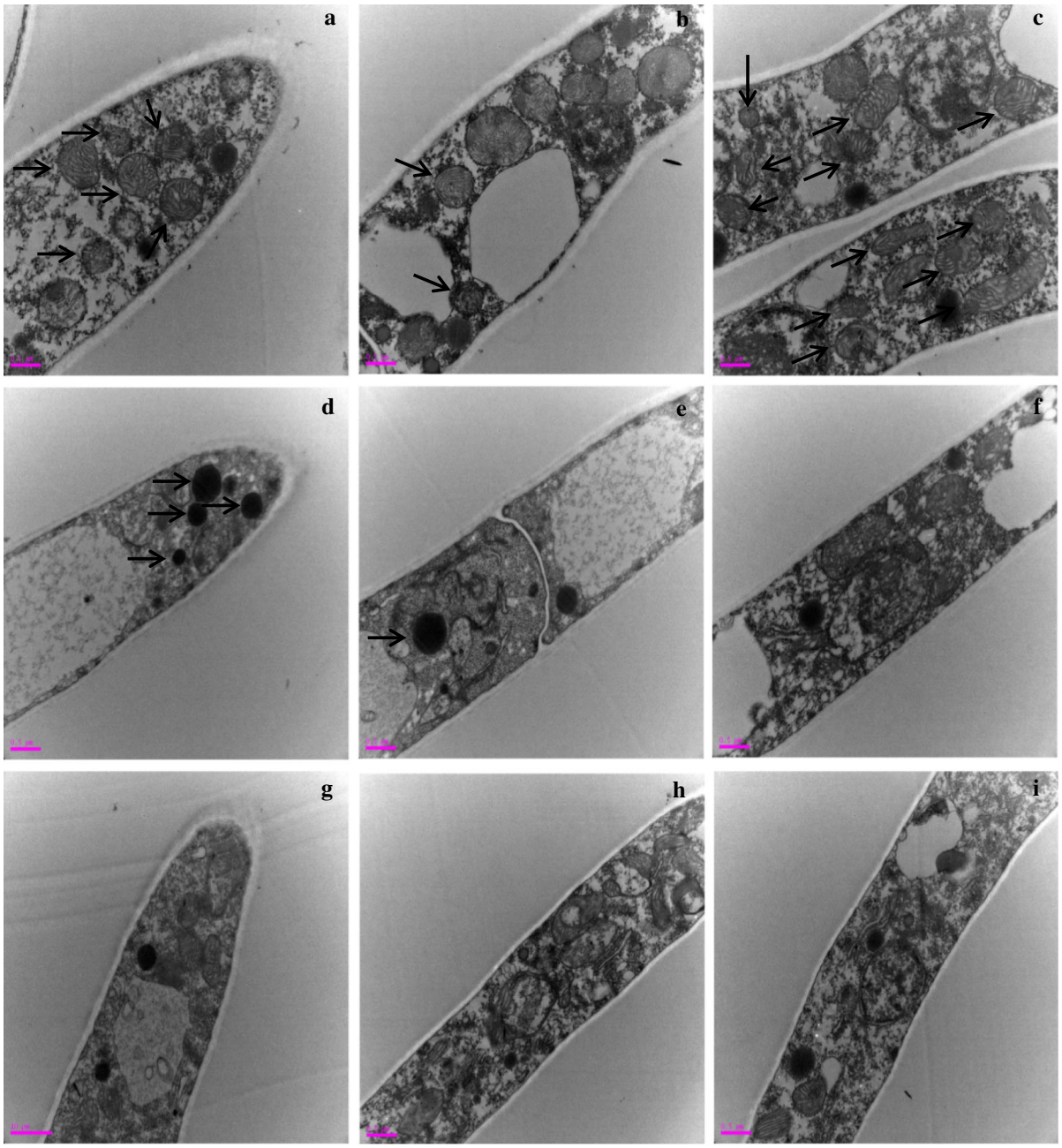

Fig. 6 Transmission electron microscopy images of *P. chrysogenum* mycelia. **a–c** LSMMG condition (*Arrows* are indicating mitochondria), **d–f** normal condition (*Arrows* are indicating electron dense particles), **g–i** static condition

significant differences were observed in the transcription level of *gfaA* gene of *A. niger* grown under LSMMG, normal and static conditions. Negative controls showed no amplifications. The *p* values of *A. niger* and *P. chrysogenum* also indicated no significant variations (Fig. 7). The results of alterations in *gfaA* transcription level suggests that there are no potential consequences in *A. niger* and *P. chrysogenum* chitin biosynthetic pathway due to microgravity condition. The results supporting the phenotypic findings and collectively suggesting that LSMMG condition was not harmful to cell-wall physiology

Table 1 List of primers used in the qRT-PCR experiments

Gene	Primer name	Sequence (5′–3′)	Reference
Penicillium chrysogenum (*gfaA*)	PFP	CGARTAYMGNGGNTAYGA	Ram et al. (2004)
Penicillium chrysogenum (*gfaA*)	PRP	CGTGNGTNGCCAANCKNGT	Ram et al. (2004)
Aspergillus niger (*gfaA*)	AFP	CCAYATHAAYGCNGGNCC	Ram et al. (2004)
Aspergillus niger (*gfaA*)	ARP	CGCCYTGNARRCARTCNAC	Ram et al. (2004)

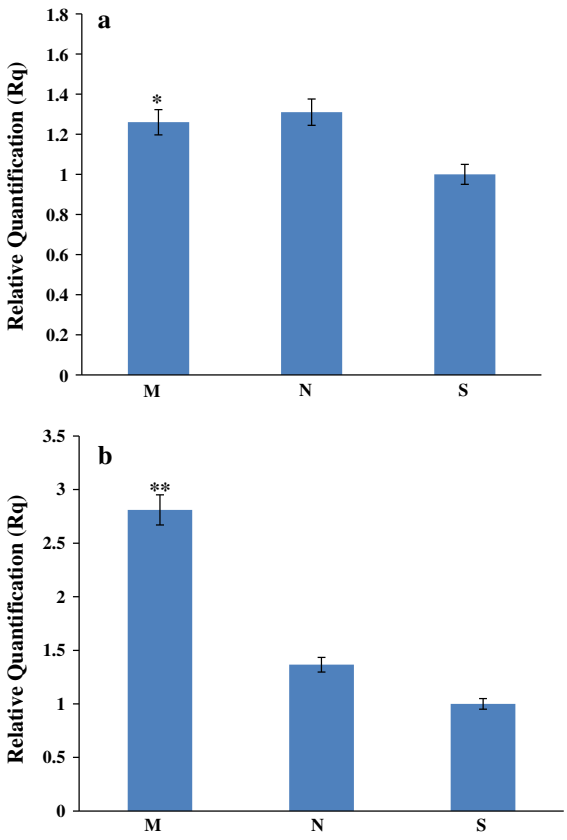

Fig. 7 Transcription levels of *gfaA* gene analysis by qRT-PCR. **a** *A. niger*, **b** *P. chrysogenum*. *M* LSMMG condition, *N* Normal condition, *S* static condition. (*$p = 0.07$, **$p = 0.0015$, p values referring comparison between LSMMG and normal gravity conditions)

and chitin biosynthetic pathways of filamentous fungi *A. niger* and *P. chrysogenum*.

Conclusion

Potential pathogenic fungi, *A. niger* and *P. chrysogenum* have been subjected to LSMMG condition to understand the phenotypic changes in combination with central chitin metabolism. The extensive phenotypic characterization of *A. niger* and *P. chrysogenum* revealed that LSMMG condition was not potentially lethal to the growth of *A. niger* and *P. chrysogenum*. Importantly, spore germination and electron microscopic physiological examination results correlates well. The cell wall integrity examination using confocal microscopy in combination with calcofluor results correlates well with transcription levels of *gfaA* gene. More detailed ground based experiments should conduct necessarily to evaluate the pathogenicity of *A. niger* and *P. chrysogenum* under microgravity at the molecular level.

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