Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi: 10.12980/JCLM.2.2014J25

Rhodotorula mucilaginosa BPT1 can form arthrospore in response to cold−temperature

Sahay Sanjay*, Abdul Mazid Khan, Majid Butt, Tekram Sahu, Ravinder S Rana, Deepak Chouhan, Kamlesh Ranjan, Burhan Hamid

Government Science and Commerce College, Benazir, Bhopal, India

PEER REVIEW

Peer reviewer

Dr. Gabriella Caruso, Researcher, Institute of Coastal Marine Environment (IAMC), Italian National Research Council (CNR), Messina, Italy.
Tel: +39−090−6015423
Fax: +39−090−669007
E−mail: gabriella.caruso@iamc.cnr.it

Comments

This is a valuable research work in which authors have demonstrated that the formation of arthrospore strain BPT1 of *R. mucilaginosa* is related to specific environmental conditions, suggesting some strategies to control its growth and diffusion. Arthrospore formation by this strain is a novel feature recorded for the first time for this yeast species.
Details on Page 144

ABSTRACT

Objective: To study carbon and nitrogen utilization pattern and arthrospore formation in a psychrotolerant yeast isolate *Rhodotorula mucilaginosa (R. mucilaginosa)* BPT1.
Methods: Growth of the yeast on minimal synthetic medium supplemented with various carbon and nitrogen compounds as sole carbon or nitrogen source has been studied. Various physico−chemical parameters such as pH, restricted oxygen supply, temperatures, media composition and presence of methionine were tested to examine their effect on arthrospore formation by this known opportunistic pathogen.
Results: The psychrotolerant isolate BPT1 identified on the basis of D1/D2 domain of large rDNA sequence characteristics as *R. mucilaginosa* showed some deviation in carbon and nitrogen utilization patterns from those of other strains of *R. mucilaginosa* in the CBS data base. Intriguingly, the isolate produced sub−surface hyphal rays around its colony at lower temperatures (4 °C and 20 °C) on PDA medium; the ray was found to be linearly arranged arthrospores. The arthrospore was not produced in liquid medium, or in presence of methionine or under micro−aerobic condition or at higher temperature.
Conclusions: The investigation showed a novel feature *i.e.* arthrospore was formed by this yeast isolate under specific set of conditions. The results reiterated that only physiological and morphological characteristics were not sufficient to identify a yeast. The ability of *R. mucilaginosa* to form arthrospores seems to be an adaptive feature in response to extreme environmental condition, and represents adaptive ability having something to do with its ubiquity.

KEYWORDS
Rhodotorula mucilaginosa, Psychrotolerance, Sub−surface hyphal ray, Arthrospore

1. Introduction

Arthrospore is asexual propagule produced in a wide variety of fungi, and many of them are pathogenic ones[1]. This spore plays an important role in the proliferation of fungi producing, takes part in transmission of pathogenic fungi, and is also a potentially useful model for the study of cellular differentiation[1]. Formation of arthrospore has attracted the attention of scientists because it was found to be correlated with high−yield cephalosporin C production in *Acremonium chrysogenum* (*A. chrysogenum*)[2].

The arthrospores are produced by some yeasts, molds and actinomycetes by direct fragmentation of hyphae. In many cases, the arthrosporulation occurs during prolonged cultivation under limited nutrient supply[3]. Some pathogenic fungi are found to form arthrospore under such conditions as higher CO_2 concentration or presence of antifungal drugs[4]. In case of certain yeasts, *viz.*, *Arxula* spp., *Dipodascus* spp., *Geotrichum* spp. and *Trichosporon* spp., formation of arthrospores is a taxonomically useful trait used to identify the taxa at generic level[5].

Rhodotorula mucilaginosa (*R. mucilaginosa*) is widely distributed. Yeast has also been found to be associated with disease lesions[6−11]. The yeast has been evaluated

*Corresponding author: Sahay Sanjay, Government Science and Commerce College, Benazir, Bhopal, India.
Tel: 91−9425689386
E−mail: ss000@rediffmail.com

Founding Project: supported by Madhya Pradesh Council of Science and Technology, Bhopal (MP) (Grant No. 3902/CST/R&D/2011).

Article history:
Received 4 Dec 2013
Received in revised form 7 Dec, 2nd revised form 13 Dec, 3rd revised form 20 Dec 2013
Accepted 15 Feb 2014
Available online 28 Feb 2014

for bioremediation and production of carotene and other important chemicals[12-14]. It has never been reported to form arthrospore under any circumstance.

The objective of our research is to make further studies on the strain characteristics of previously isolated and identified psychrotolerant yeast *R. mucilaginosa* BPT1, the conditions under which it forms arthrospore and make observation on the pathological and ecological implications of arthrosporogenesis in this yeast.

2. Materials and methods

2.1. Isolation of yeast

The yeast *R. mucilaginosa* BPT1 was isolated from the soil of Baramullah (Altitude-2 730 m; Latitude-34°34′ N; Longitude-74°45′ E) Jammu & Kashmir, India and maintained as described earlier[8,15].

2.2. Effect of various physic-chemical environments on arthrospore genesis

The effect of temperature on arthrosporulation was tested by incubating the potato dextrose agar (PDA) plates with the yeast at 4 °C, 20 °C, 30 °C and 37 °C for 3 d. The effect of pHs on arthrosporogenesis was examined by inoculating the isolate onto PDA with acidic (pH 3, 4, 5 and 6) or alkaline (pH 8, 9, 10 and 11) reactions and incubating at 4 °C for 15 d. The yeast was also grown in ME broth supplemented with methionine 1% (w/v) and in minimal synthetic medium or minimal Czapek Dox medium containing 0.5% (w/v) glucose at 4 °C for 30 d to see the effect of these conditions on arthrospore formation[3,15,16]. The yeast was cultured on slide in ME agar medium for 5 d at 4 °C to study the effect of micro-aerobic condition on arthrospore formation.

2.3. Light microscopy

The yeast was inoculated in malt extract broth and PDA and incubated at various temperatures (4 °C, 20 °C, 30 °C and 37 °C) and colony morphology was examined after 5-10 d. The cells were mounted in sterile distilled water and observed under microscope (make Olympus; model CH20i) and photographed with Magnus Image Projection System. A 1×4 mm cut was made over the periphery of actino-colonies (colonies showing hyphal growth at periphery) and medium along with embedded hyphal growth was picked up and observed under microscope without teasing it.

2.4. Physiological characterization

To characterize physiologically, the yeast isolate BPT1 was inoculated in minimal synthetic medium containing a carbon or nitrogen compound as sole carbon or nitrogen source respectively[15]. We have tested all the prescribed carbon sources except arbutin, D-glucono-1, 5-lactone, 2-keto-D-gluconate, 5-keto-D-gluconate, D-gluconate, D-glucuronate, D-glucarate and D-galactonate[5]. The culture was incubated at 20 °C for 5 d.

2.6. Molecular characterization

For molecular characterization, sequence of D1/D2 domain 26s rDNA was cloned and sequenced as reported earlier[15]. A representative sequence of 10 most similar neighbours was retrieved from GenBank and aligned using clustal W2 for multiple alignments with the default settings. The multiple-alignment file in mega format was then used to create a neighbor-joining phylogram in MEGA 5 software[17].

3. Results

3.1. Isolation of yeast

The yeast *R. mucilaginosa* BPT1 was one of the 23 yeasts isolated from the soil of Baramullah (Figure 1A)[15]. The cold-active isolate BPT1 forming arthrospores-like structure was selected for further characterization.

3.2. Effect of temperature on arthrosporogenesis

The isolate BPT1 was found to grow optimally in the temperature range of 20-30 °C and pH range of 3-7. The colony morphology showed variation with change in incubation temperature; at 4 °C and 20 °C, the colony showed subsurface hyphal rays at the periphery (Figure 1B and 1C respectively), at 30 °C (Figure 1D) and at a combined incubation temperatures of 20 °C for 2 d and 30 °C thereafter (Figure 1E) showed entire periphery. The sub-surface rays in this isolate were found to be linearly arranged arthrospore (arthroconidia) under microscope (Figure 1F).

3.3. Effect of other conditions on arthrosporulation

The yeast did not form arthrospores in ME broth and minimal synthetic medium containing 0.5% (w/v) glucose[15,16], supplemented with methionine 1% (w/v) and incubated at 4 °C for 30 d as against the earlier report[3]. The yeast was cultured on slide for 5 d in ME agar medium or inoculated in ME broth as stationary culture for 30 d at 4 °C. There was no arthrosporulation under these micro-aerobic and long culture conditions as shown by the isolate BPT1.

3.4. Further characterization of the yeast

To characterize physiologically, the isolate BPT1 was inoculated in synthetic dextrose medium containing various carbon compounds (except arbutin, D-glucono-1,5-lactone,

2-keto-D-gluconate, 5-keto-D-gluconate, D-gluconate, D-glucuronate, D-glucarate and D-galactonate) and nitrogen compounds as sole carbon or nitrogen sources respectively[5,15]. The culture was incubated at 20 °C for 5 d. The result showed some difference in its utilization pattern of certain carbon sources as compared to available data (www.cbs-knaw.nl). Most importantly, it could utilize α-D-glucoside, melibiose, lactose, inulin, starch, erythritol, myo-inositol, methanol, butanol and D-glutamate as carbon source, and creatin and creatinine as sole nitrogen sources unlike those of type and most CBS strains.

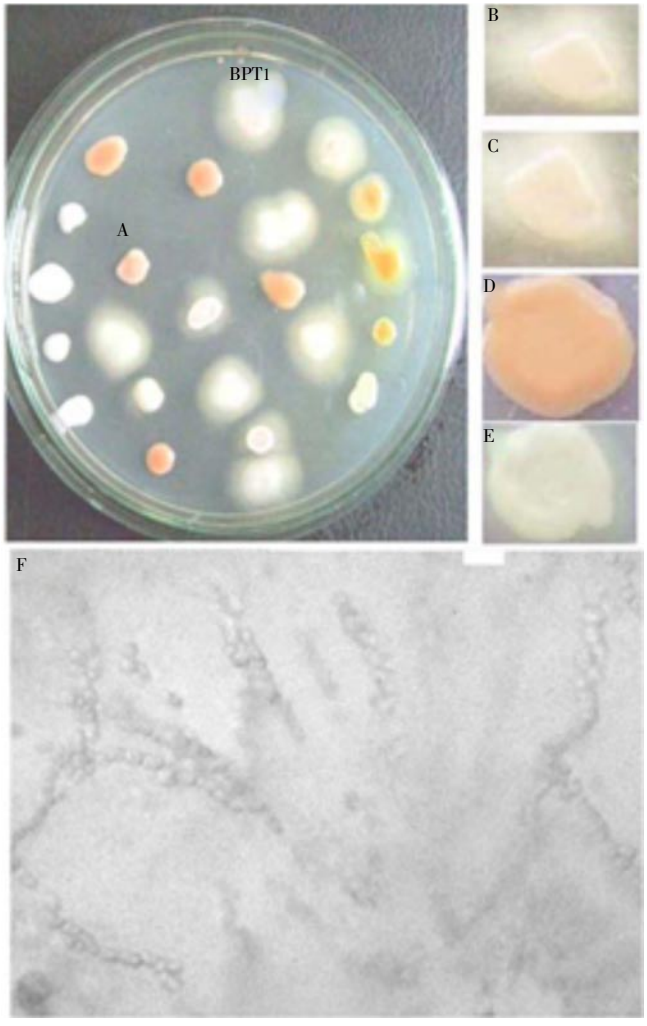

Figure 1. Twenty three yeasts isolated from the soil of Baramullah.
A: Colonies of cold-active yeast isolates; B-E: Colony morphology of BPT1 grown at 4 °C (B), 20 °C (C), 30 °C (D) and 20 °C for 2 d followed by incubation at 30 °C (E); F: Arthrospore formation by submerged pseudohyphae on PDA medium at 4 °C.

The yeast BPT1 showed pink-white colony and fluid-mucoid-butyrus texture (under different incubation temperatures). The urease and diazonium blue B positive BPT1 does not form teleutospore. The D1/D2 sequence based phylogenetic analysis showed 99% similarity to type and many other strains of *R. mucilaginosa* in the GenBank database (Figure 2), and hence the isolate was named *R. mucilaginosa* BPT1[15]. The D1/D2 sequence has been extensively used for the identification of yeasts and *R. mucilaginosa* earlier[7]. The GenBank accession number of its nucleotide sequence is JN091167[15].

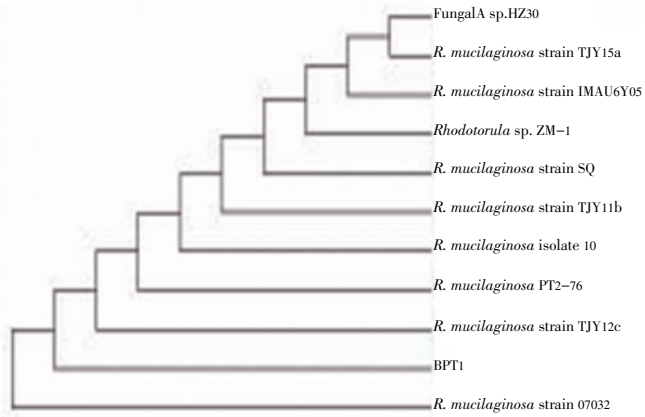

Figure 2. Phylogenetic tree based on the D1/D2 sequences of the large-subunit rDNA of *R. mucilaginosa* BPT1 and those of ten closely related yeasts retrieved from the GenBank database. The tree was constructed by using the neighbor-joining method in MEGA 5 software[18].

4. Discussion

The isolate BPT1 shows some difference in carbon and nitrogen utilization pattern as compared to the CBS strains. The carbon utilization profile indicates that BPT1 is an extremophilic yeast physiologically as well. The genotypic/phenotypic variants of this yeast have also been studied earlier[6]. These studies indicate ability of this yeast to evolve and adapt rapidly which in turn seems to be the reason for its ubiquity.

The yeast BPT1 shows arthrospore formation is not as usual means of asexual reproduction. Nor the arthrospore formation occurs under any other tested conditions except a very special set of conditions *viz.*, semi-solid medium and a temperature range of 4-20 °C. The yeast thus forms conditional arthrospores, which is not a unique phenomenon in fungi. In pathogenic yeast *Candida albicans*, exposure of cells to CO_2[5], in old-culture of *Schizosaccharomyces japonicas* var. *japonicas,* the presence of gradient of nitrogen source in semi-solid medium and in the human pathogen *Penicillium marneffei*[18], the shift in growth temperature from 25 °C to 37 °C induces the arthrosporulation[3]. In other fungal and yeast species too, arthrospore formation has been found to be regulated variously by environmental factors and different fungicides[4]. Coincidently, many of these fungi have been found to be pathogenic to normal or immunocompromised human beings[19]. Recent gene expression study shows that the expression pattern of gene in arthrosporogenesis is different from that in the development of other asexual body and that the arthrospore is more resistant to various fungicides than microconidia[20,21]. Do these evidences suggest that arthrospore can help in any way in the pathogenesis? Further study needs to evaluate the role of arthrospore in the pathogenesis.

Although the yeast *R. mucilaginosa* has been found to be associated with disease lesions[9-11], the temperature

at which this yeast forms arthrospore does not correspond with the normal body temperature of the hosts. Hence the arthrospore formation in this yeast does not seem to be associated with the pathogenesis. Nonetheless, it needs *in vivo* study in case of *R. mucilaginosa* since other stresses posed by host during the course of pathogenesis may induce arthrosporulation.

As against the previous reports, the arthrospore formation in this yeast is not stimulated by physiological changes during cultivation indicating a different mechanism/pathway existed in this yeast isolate[3,17]. Arthrosporulation has been found in the human pathogen *Penicillium marneffei* to be regulated by conserved components of signal transduction pathways ST12 or the G-protein alpha-subunit GASC[3]. In *A. chrysogenum* cephalosporin C regulator 1 (CPCR1), a member of the winged helix/regulator factor X transcription factor family regulating cephalosporin C biosynthesis, has been found to control arthrospore development as well. Interestingly, the process is independent of another regulator (the forkhead transcription factor AcFKH1) though the latter directly interacts with CPCR1 indicating a diversion of arthrospore formation pathway from this junction[3]. The downstream target of CPCR1 in this fungus seems to be the genes encoding chitinolytic or proteolytic enzymes. Cell wall chitinases are thought to be involved in sporulation in filamentous fungi, as the specific chitinase inhibitor, allosamidin retards the fragmentation of hyphae into arthrospores in *A. chrysogenum* and autolysis in *P. chrysogenum*[3]. Chitinases contribute to breakage and reforming of bonds within and between chitin polymers. This leads to remodeling of the cell wall during growth and morphogenesis[3]. In *Saccharomyces chrysogenum*, the efficient cell separation has been reported to be dependent on the expression of a chitinase encoded by the CTS1 gene. Expression of this gene is greatly reduced in DCBK1 cells, lacking a putative serine/threonine protein kinase[3]. In *R. mucilaginosa* BPT1, a chitinase dependent arthrospore formation does not seem to be possible rather some sensory pathway that can distinguish between the conditions in ME broth and PDA media seems to be responsible for induction of arthrosporogenesis since the yeast does not exhibit fragmentation of pseudohypha at lower temperatures in ME broth.

The formation of arthrospores by the disintegration of pseudohypha shown by BPT1 seems to be evolved for pushing the cells through the soil, thus helping not only in propagation but in the dispersal of cells to the neighborhood. Such dispersal in cold condition is likely to be associated with its ability to move in neighborhood to explore food when the condition of decomposition of complex food into simpler utilizable forms hardly exist. A low temperature inducing morphogenesis has been reported[22], it is yet to be known whether low temperature acts as cue for the arthrosporulation or condition inhibitory to cell-cell adhesion in pseudohypha. The adaptation reiterates the ability of yeasts to exhibit morphological variation under different ecological conditions and hence cannot be a reliable basis for their identification. On the other hand, the present report reveals a new method of asexual reproduction which may be associated with its highly prolific and ubiquitous nature and presents a new dimension to be considered for its effective management in the affected patients. The yeast may also serve as additional model system for studying arthrospore morphogenesis. Moreover, this is the first report of the ability of *R. mucilaginosa* to form arthrospore.

In conclusion, the arthrospore formation by *R. mucilaginosa* BPT1 is a novel feature recorded for the first time for this yeast species. The arthrosporulation in this yeast was not induced by the factors such as presence of methionine, higher CO_2 concentration or oxygen tension, shifting growth temperature to higher side (25 to 37 °C) or long culture period as reported for other fungi and yeast. It forms arthrospore under low temperature condition (4-20 °C) and semisolid medium. The finding indicates that the evolution of arthrosporulation in this yeast species is related to psychrotrophy. It's medical dimension needed to be investigated. The carbon and nitrogen utilization pattern of the yeast strain showed some difference from those reported earlier.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The research was supported by Madhya Pradesh Council of Science and Technology, Bhopal (MP) in the form of a major project grant number 3902/CST/R&D/2011, Dated 4.1.11 to the corresponding author.

Comments

Background

R. mucilaginosa BPT1 is a psychrotolerant yeast isolated from the soil of Baramullah (India), recognized to be an opportunistic pathogen. Knowledge on the environmental conditions which stimulate or inhibit the formation of arthrospore is an interesting topic of research, in relation to its ubiquitous distribution.

Research frontiers

The present research work describes the morphological, biochemical and molecular characteristics of *R. mucilaginosa* strain BPT1, examining how these vary in response to different physico-chemical parameters such as temperature, pH, presence of methionine, various carbon and nitrogen sources, limited oxygen availability.

Related reports

Compared to previous studies, the authors show that the arthrospore formation in this yeast isolate, differently from other yeasts, is not stimulated by physiological changes during cultivation. Therefore authors suggest that

different mechanisms/pathways exist in this strain. Suitable temperature and organic substrates should be available to support its growth and dispersion in the environment. The authors also demonstrate that arthrosporulation does not occur under micro-aerobic conditions and that this process is related to psychrotrophy; this has important implications in environmental medicine.

Innovations and breakthroughs

The most interesting feature of the present study is the conclusion drawn by the authors, who explain the ubiquitous distribution of the opportunistic pathogen *R. mucilaginosa* BPT1 in relation to the ability of this strain to evolve and develop adaptations to different environmental niches, as suggested by the differences found in the carbon utilization profiles.

Applications

Although arthrospore formation has previously been documented in a wide variety of fungi and yeasts, this study is the first report on the ability of *R. mucilaginosa* BPT1 to form arthrospores, and provides information to prevent the dispersion of this specific strain through the control of environmental factors affecting its growth.

Peer review

This is a valuable research work in which authors have demonstrated that the formation of arthrospore strain BPT1 of *R. mucilaginosa* is related to specific environmental conditions, suggesting some strategies to control its growth and diffusion. The process was assessed through the application of different ranges of temperature, pH, as well as supplementation with methionine or use of different carbon and nitrogen sources. The phylogenetic analysis by D1/D2 sequence analysis showed strong similarity of BPT1 strain with other related *R. mucilaginosa* strains. Arthrospore formation by this strain is a novel feature recorded for the first time for this yeast species.

References

[1] Barrera CR. Formation and ultrastructure of *Mucor rouxii* arthrospores. *J Bacteriol* 1983; **155**(2): 886-895.

[2] Yang Y, Xia J, Li J, Chu Ju, Li L, Wang Y, et al. A novel impeller configuration to improve fungal physiology performance and energy conservation for cephalosporin C production. *J Biotechnol* 2012; **161**(3): 250-256.

[3] Hoff B, Schmitt EK, Kück U. CPCR1, but not its interacting transcription factor AcFKH1,controls fungal arthrospore formation in *Acremonium chrysogenum*. *Mol Microbiol* 2005; **56**(5): 1220-1233.

[4] Khan MSA, Ahmad I, Agil F, Owais M, Shahid M, Musarrat J. Virulence and pathogenicity of fungal pathogens with special reference to *Candida albicans*. In: Ahmad I, Owais M, Shahid M, Aqil F, editors. *Combating fungal infections*. Berlin Heidelberg: Springer; 2010, p. 21-45.

[5] Kurtzman C, Fell JW, Boekhout T. *The yeasts: a taxonomic study*. 5th ed. London, UK: Elsevier; 2011, p. 1500.

[6] Libkind D, Gadanho M, van Broock M, Sampaio JP. Studies on the heterogeneity of the carotenogenic yeast *Rhodotorula mucilaginosa* from Patagonia, Argentina. *J Basic Microbiol* 2008; **48**(2): 93-98.

[7] Gadanho M, Sampaio JP. Occurrence and diversity of yeasts in the mid-atlantic ridge hydrothermal fields near the Azores Archipelago. *Microb Ecol* 2005; **50**(3): 408-417.

[8] Hamid B, Singh P, Rana RS, Sahay S. Isolation and identification of psychrophilic yeast from the soil of northern region of India. *Inventi Rapid: Pharm Biotech Microbio* 2012; **2012**(1).

[9] Martini K, Müller H, Huemer HP, Höpfl R. Nail psoriasis masqueraded by secondary infection with *Rhodotorula mucilaginosa*. *Mycoses* 2013; **56**(6): 690-692.

[10] Kim HA, Hyun M, Ryu SY. Catheter-associated *Rhodotorula mucilaginosa* fungemia in an immunocompetent host. *Infect Chemother* 2013; **45**(3): 339-342.

[11] Nunes JM, Bizerra FC, Ferrira RC, Colombo AL. Molecular identification, antifungal susceptibility profile, and biofilm formation of clinical and environmental *Rhodotorula* species isolates. *Antimicrob Agents Chemother* 2013; **57**(1): 382-389.

[12] Jarbouj R, Magdich S, Avadi RJ, Gargouri A, Gharsallah N, Ammar E. *Aspergillus niger* P6 and *Rhodotorula mucilaginosa* CH4 used for olive mill wastewater (OMW) biological treatment in single pure and successive cultures. *Environ Technol* 2013; **34**(5-8): 629-636.

[13] Moliné M, Libkind D, van Broock M. Production of torularhodin, torulene, and β-carotene by *Rhodotorula* yeasts. *Methods Mol Biol* 2012; **898**: 275-283.

[14] Vajzovic A, Bura R, Kohlmeier K, Doty SL. Novel endophytic yeast *Rhodotorula mucilaginosa* strain PTD3 II: production of xylitol and ethanol in the presence of inhibitors. *J Ind Microbiol Biotechnol* 2012; **39**(10): 1453-1463.

[15] Sahay S, Hamid B, Singh P, Ranjan K, Chauhan D, Rana RS, et al. Evaluation of pectinolytic activities for oenological uses from psychrotrophic yeasts. *Lett Appl Microbiol* 2013; **57**(2): 115-121.

[16] Sahay S. Phenylalanine transport in *Aspergillus nidulans*: demonstration of role of phenylalanine binding protein. *Indian J Exp Biol* 1999; **37**(2): 152-156.

[17] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; **28**(10): 2731-2739.

[18] Sipiczki M, Takeo K, Yamaguchi M, Yoshida S, Miklos I. Environmentally controlled dimorphic cycle in a fission yeast. *Microbiology* 1998; **144**(Pt 5): 1319-1330.

[19] Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med* 2012; doi: 10.1126/scitranslmed.3004404.

[20] Viriyakosal S, Singhania A, Fierer J, Goldberg J, Kirkland TN, Woelk CH. Gene expression in human fungal pathogen *Coccidioides immitis* changes as arthroconidia differentiate into spherules and mature. *BMC Microbiol* 2013; **13**: 121.

[21] Coelho LM, Aquino-Ferriera R, Maffei CM, Martinez-Ross NM. *In vitro* antifungal drug susceptibilities of dermatophytes microconidia and arthroconidia. *J Antimicrob Chemother* 2008; **62**(4): 758-761.

[22] Xing YM, Zhang LC, Liang HQ, Lv J, Song C, Guo SX, et al. Sclerotial formation of *Polyporus umbellatus* by low temperature treatment under artificial conditions. *PLoS ONE* 2013; doi: 10.1371/journal.pone.0056190.