

## NEW RECORDS OF SEVEN FUNGAL SPECIES FOR BANGLADESH

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### Abstract

From three farming stages of *Arthrospira platensis* (Nordstedt) Gomont [*Spirulina platensis* (Gomont) Geitler], seven fungal species, namely, *Cladosporium varians* Braun, Melnik & K. Schub., *Fusarium trichothecioides* Wollenw., *Geotrichum candidum* Link, *Mucor circinelloides* Tiegh., *M. hiemalis* Wehmer, *Penicillium frequentans* Westling and *Verticillium albo-atrum* Reinke & Berthold were recorded for the first time from Bangladesh.

### Introduction

Fungi are the second largest kingdom, with about 70,000–100,000 known species (Kendrick, 2000). But knowledge on the mycodiversity is still deficient and incomplete, making inventory ponderous due to the inadequate number of researchers in this area (Mueller *et al.*, 2004). A total of 275 fungal species under 125 genera have been reported from Bangladesh (Siddiqui *et al.*, 2007). *Spirulina*, the most widely exploited food microalga, usually expose under open air during commercial production. Air of the earth contains various fungal propagules. These airborne propagules can be settled on culture, slurry and powder of *Spirulina*. Eleven fungi species were isolated and identified from culture, slurry and powder of *Spirulina* at Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Out of 11 species seven were recorded for the first time from Bangladesh. This study presents taxonomic description of these seven fungal species under five different genera.

### Materials and Methods

Fungi were isolated from culture, slurry and powder of *Arthrospira platensis* (Nordstedt) Gomont [*Spirulina platensis* (Gomont) Geitler; common name: *Spirulina*] at Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka following Agar and/or Dilution Plate techniques during the last week of December 2012.

Colony character of pure culture of each isolate was studied. They were examined microscopically after preparation of glass slides with cover slip or sticky tap by using lactophenol-cotton blue solution (0.05 gm cotton blue in 100 ml lacto-phenol). Microscopic images of each isolates were captured with the aid of Olympus-DP20 digital camera attached with Olympus CX41 compound microscope at 400x. Isolates were identified up to species level with the help of standard mycological literature (Bensch *et al.*, 2012; Booth, 1971; Crous and Groenewald, 2013; Ellis, 1971; Ellis *et al.*, 2007; Harvey, 1965; Jabnoun *et al.*, 2010; Kurtzman *et al.*, 2011; Raper and Thom, 1949; Schipper, 1973, 1976, 1978; Zare, 2003).

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Pure cultures of the described species are preserved at the Department of Botany, Jagannath University, Dhaka, Bangladesh.

### Results and Discussion

A total of 11 fungal species belonging to eight different genera were studied and identified. Among these, seven species *viz.*, *Cladosporium varians* Braun, Melnik & K. Schub., *Fusarium trichothecioides* Wollenw., *Geotrichum candidum* Link, *Mucor circinelloides* Tiegh., *M. hiemalis* Wehmer, *Penicillium frequentans* Westling and *Verticillium albo-atrum* Reinke & Berthold are found new records for Bangladesh. Four others were *Arthrinium phaeospermum* (Corda) Ellis, *Cladosporium sphaerospermum* Penz., *Cunninghamella bertholletiae* Stadel and *Nigrospora oryzae* (Berk. & Br.) Petch.

Taxonomic descriptions of the newly recorded species are as follows:

1. ***Cladosporium varians*** U. Braun, V.A. Melnik, and K. Schubert, Mikologiya I Fitopatologiya 42(3): 215 (2008). (Figs 1 & 8).

Colonies on PDA reaching 29.5 mm in diameter after 14 days, dark grey-olivaceous, olivaceous or iron-grey, reverse grey olivaceous, velvety to powdery, growth regular, flat to low curved, margin white, extensive. Mycelium mainly immersed, pale olivaceous grey; consisting of branched or unbranched, 4.1–5.0  $\mu\text{m}$  wide hyphae. Conidiophores macronematous or micronematous, solitary, straight or rising, unbranched or branched or dichotomous branched, terminally or laterally formed from hyphae, septate, 58.1–87.2  $\times$  2.8–5.5  $\mu\text{m}$ . Conidiogenous cells are cylindrical oblong, integrated, terminal, 15.6–17.5  $\times$  6.9–7.5  $\mu\text{m}$ . Conidia broadly ellipsoidal to ellipsoidal or subglobose, aseptate conidia 5.4–12.9  $\times$  3.9–5  $\mu\text{m}$ , single septate conidia 10.0–17.5  $\times$  3.8–5.6  $\mu\text{m}$ , ramoconidia 11.4  $\times$  5.0  $\mu\text{m}$ .

*Specimen examined:* Isolate no. K.S. Hossain 267, 30 December 2012, from spirulina powder, BCSIR, Dhaka, collected by Amena Kibria.

*Notes:* *Cladosporium varians* belongs to the *C. cladosporioides* complex, but differs from *C. cladosporioides* by its long, frequently branched conidiophores. Furthermore, the tips of the conidiogenous cells are often somewhat swollen or unilaterally swollen, and the ramoconidia have up to four septa, and subglobose conidia are not abundant. *C. tenuissimumis* another comparable species, which is, however, easily distinguishable by its setiform, usually unbranched conidiophores (Bensch *et al.*, 2012).

2. ***Fusarium trichothecioides*** Wollenw., J. Wash. Acad. Sci. 2:147 (1912). (Figs 2 & 9).

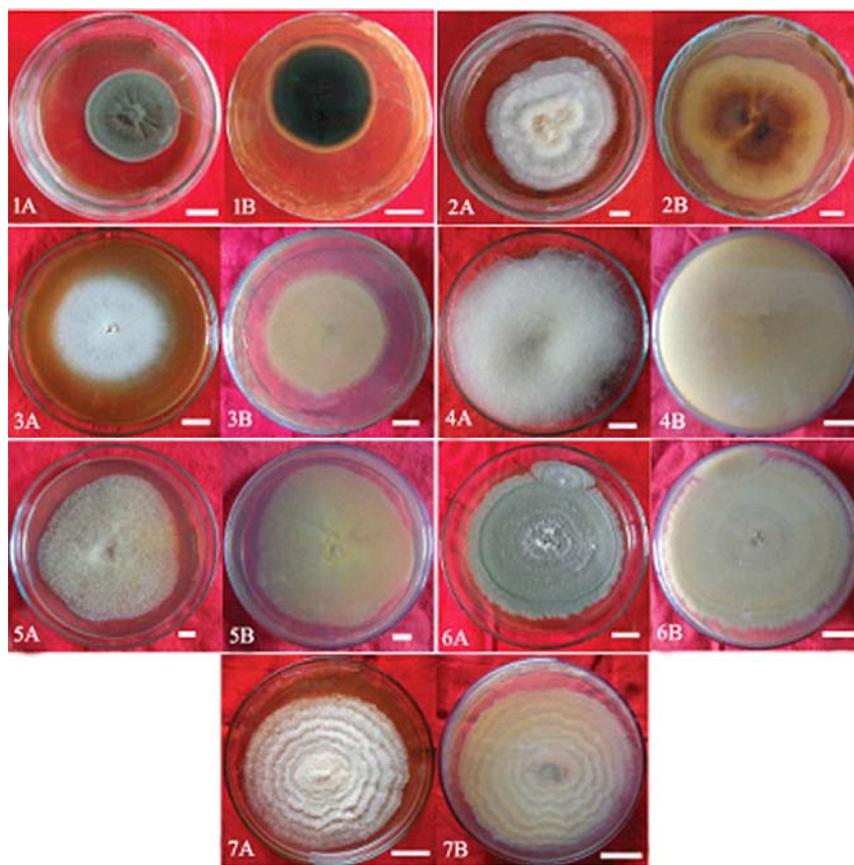
Colonies attaining 5 cm in diameter after 4 days on PDA, 6.5 cm in diameter after 6 days on SNA, pale pinkish-white, and reverse light brownish pink. It grows sparingly at 37° C, negative on urease reaction. It's able to grow without vitamin supplement. Mycelium cottony, extensive, 2.5–4.4  $\mu\text{m}$  wide. Conidiophores comprise of a basal cell bearing 2 or 3 apical phialides, 18.0  $\times$  3.8  $\mu\text{m}$ . Macroconidia 14–24  $\times$  2.5–5  $\mu\text{m}$ , 3 to 5 septate or 8–16  $\times$  2.5–5.0  $\mu\text{m}$ , 1 to 2 septate, formed frequently from lateral conidiophores. Microconidia are not present. Chlamydospores are smooth walled, terminal, intercalary, spherical, 8–15  $\mu\text{m}$  in diameter. Sporodochia, ascus and ascospore absent.

*Specimen examined:* Isolate no. K.S. Hossain 251, 24 December 2012, from spirulina slurry, BCSIR, Dhaka, collected by Amena Kibria.

3. **Geotrichum candidum** Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3(1): 3–42 (1809). (Figs 3 & 10).

Colonies on PDA reaching 4.5 cm in diameter after 6 days, grows rapidly, white to cream colored, powdery, flat with aerial mycelium. Mycelium consisting of septate, hyaline, dichotomously branched and 3–5  $\mu\text{m}$  wide hyphae. Arthroconidia hyaline, one-celled, smooth, slimy, subglobose to cylindrical, 4.7–10.1  $\times$  2.7–5.4  $\mu\text{m}$ , released by the separation of a double septum. Disjunctive cells, blastoconidia, budding and ascospore not found. It grows sparingly at 37° C, negative on urease reaction. It's able to grow without vitamin supplement.

*Specimen examined:* Isolate no. K.S. Hossain 262, 24 December 2012, from spirulina powder, BCSIR, Dhaka, collected by Amena Kibria.



Figs 1-7. Colonies of the seven fungal species on PDA medium at different incubation periods, A. Front views; B. Reverse views. 1. *Cladosporium varians*, 2. *Fusarium trichothecioides*, 3. *Geotrichum candidum*, 4. *Mucor circinelloides*, 5. *Mucor hiemalis*, 6. *Penicillium frequentans*, 7. *Verticillium albo-atrum*. [Scale bars = 10 mm]

4. **Mucor circinelloides** Tiegh., *Annales des Sciences Naturelles Botanique* 1: 94 (1875).

(Figs 4 & 11).

Colonies on PDA attaining 6.1 cm in diameter, up to 13 mm high at room temperature; 6.9 cm in diameter at 30° C and 7.8 cm in diameter and 13 mm high at room temperature on Beer Wort Agar after 4 days. Colonies pale smoke gray without any zonation, reverse off white. Sporangiohores branched, 6.2–16.2 µm in diameter. Sporangia 18.75–53.75 × 19.38–50.63 µm, globose or spherical. Columellae globose or spherical, 16.25–41.25 × 18.13–31.88 µm, collars present. Sporangiospores 5.6–13.1 × 2.25–3.13 µm, broadly ellipsoidal to ellipsoidal, few fusiform. Chlamydospores present, 7.50–16.88 µm thick.

*Specimen examined:* Isolate no. K.S. Hossain 255, 24 December 2012, from spirulina culture, BCSIR, Dhaka, collected by Amena Kibria.

5. **Mucor hiemalis** Wehmer, *Annales Mycologici* 1(1): 37 (1903).

(Figs 5 & 12).

Colonies on PDA attaining 8.3 cm in diameter at room temperature; on Beer Wort Agar 4.4 cm in diameter at 30° C and 5.2 cm in diameter and 5 mm high at room temperature after 4 days. Colonies cottony to fluffy, pale yellow-brown colour, reverse light yellowish to ochreous. Sporangiohores branched, 2.5–13.7 µm in diameter. Sporangia globose, 40.63–65 × 37.5–62.5 µm. Columellae globose to subglobose, 12.5–36.88 × 12.5–32.5 µm, collars present. Sporangiospores 4.4–12.5 × 2.8–8.1 µm, subglobose to ellipsoidal, few ovals. Chlamydospores 5.63–16.88 µm thick.

*Specimen examined:* Isolate no. K. S. Hossain 259, 24 December 2012, from spirulina culture, BCSIR, Dhaka, collected by Amena Kibria.

6. **Penicillium frequentans** Westling, *Arkiv för Botanik* 11(1): 133 (1911).

(Figs 6 & 13).

Colonies on PDA 5.2 cm in diameter after 10 days at room temperature, spreading rapidly, radiately wrinkled, broadly zonate, margin thin. Mycelium consisting of hyaline, aseptate, branched hyphae. Conidiophores arise from hyphae velvety, crowded, short, septate, heavily springing, up to 200.6 µm long and 2.6–3.6 µm wide, with smooth or finely roughed walls, and apices enlarged up to 4 µm or more in width. Sterigmata mostly 8.0–12.0 × 3.0–3.5 µm, formed in crowded clusters numbering 10 to 12 or more, produce conidial chain. Conidia mostly 2.6–3.1 µm in diameter, smooth, globose to subglobose, thin-walled.

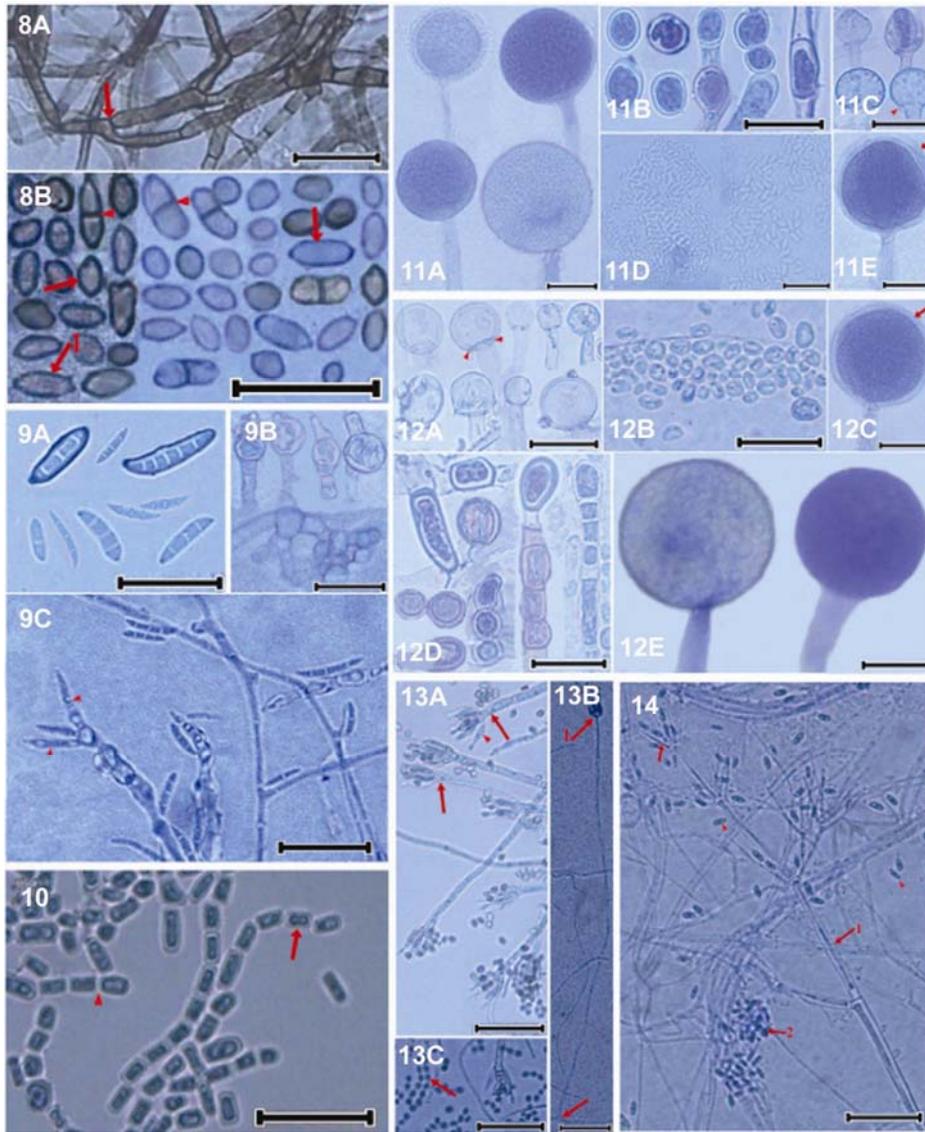
*Specimen examined:* Isolate no. K.S. Hossain 263, 27 December 2012, from spirulina slurry, BCSIR, Dhaka, collected by Amena Kibria.

7. **Verticillium albo-atrum** Reinke & Berthold, *Die Zersetzung der Kartoffel durch Pilze* 1: 75 (1879).

(Figs 7 & 14).

Colonies on PDA reaching 4.1 cm in diameter after 14 days at room temperature, white, velvety, thinning at margin, several centric weavy zone present, margin weavy. Reverse light brownish yellow. Mycelium septate, hyaline. Conidiophores branched, hyaline, more or less erect, and verticilliate, c. 4.25 µm thick. Phialides 2–4, 2 µm thick. Conidia broadly ellipsoidal to ellipsoidal, few oval, end tapered, ellipsoidal to sub cylindrical, hyaline, aseptate, 3.9–5.5 × 1.7–2.4 µm.

*Specimen examined:* Isolate no. K.S. Hossain 266, 29 December 2012, from spirulina culture, BCSIR, Dhaka, collected by Amena Kibria.



Figs 8–14. Microscopic images of the seven fungi. 8. *Cladosporium varians*, A. Dichotomous branching of conidiophores, B. Different aseptate (arrow), single septate (arrowhead) conidia and ramoconidia (arrow-1); 9. *Fusarium trichothecioides*, A. Macroconidia, B. Chlamydospores, C. Conidiophores bearing conidia on apical phialides (arrowhead); 10. Double septation (arrowhead) and arthroconidial chain of *Geotrichum candidum* (arrow); 11. *Mucor circinelloides*, A. Sporangia, B. Chlamydospores, C. Columellae with collarette (arrowheads), D. Sporangiospores, E. Sporangia with incrustated wall (arrow); 12. *Mucor hiemalis*, A. Columellae with collarette (arrowheads), B. Sporangiospores, C. Sporangia with incrustated wall (arrow), D. Chlamydospores, E. Sporangia; 13. *Penicillium frequentans*, A. Sterigmata (arrowhead) on width apex of conidiophore (arrow), 13B. Long monovercillate conidiophore arise from mycelium (arrow to arrow-1), C. Chain of conidia; 14. Conidiophores (arrow-1), whorl of phialides (arrow), conidia (arrowhead) and a cluster of conidia (arrow-2) of *Verticillium albo-atrum*. [Scale bars = 25  $\mu$ m]

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