

# Characterization and Pathogenicity of *Alternaria burnsii* from Seeds of *Cucurbita maxima* (Cucurbitaceae) in Bangladesh

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**Abstract** In the course of survey of endophytic fungi from Bangladesh pumpkin seeds in 2011~2012, two strains (CNU111042 and CNU111043) with similar colony characteristics were isolated and characterized by their morphology and by molecular phylogenetic analysis of the internal transcribed spacer, glyceraldehydes-3-phosphate dehydrogenase (*gpd*), and *Alternaria* allergen a1 (Alt a1) sequences. Phylogenetic analysis of all three sequences and their combined dataset revealed that the fungus formed a subclade within the *A. alternata* clade, matching *A. burnsii* and showing differences with its other closely related *Alternaria* species, such as *A. longipes*, *A. tomato*, and *A. tomaticola*. Long ellipsoid, obclavate or ovoid beakless conidia, shorter and thinner conidial size (16~60 [90] × 6.5~14 [~16] μm) distinguish this fungus from other related species. These isolates showed more transverse septation (2~11) and less longitudinal septation (0~3) than did other related species. Moreover, the isolate did not produce any diffusible pigment on media. Therefore, our results reveal that the newly recorded fungus from a new host, *Cucurbita maxima*, is *Alternaria burnsii* Uppal, Patel & Kamat.

**Keywords** *Alternaria burnsii*, Bangladesh, *Cucurbita maxima*, Phylogeny, Seeds

Pumpkin (*Cucurbita maxima*) is commonly grown cucurbit vegetable in Bangladesh. Cucurbits are affected by a number of pathogens, which cause major and minor diseases in seeds, seedlings, crops, and post-harvest diseases. The Department of Agriculture of Bangladesh recorded 14 different diseases caused by fungi, bacteria, and nematodes in various cucurbits in 2010 [1]. Amongst these, the most commonly fungal diseases were reported as powdery mildew (*Oidium* spp.), anthracnose (*Colletotrichum* spp.), wilting (*Fusarium*

spp.) and seed rot (*Fusarium* spp.). In seeds, 4 major diseases were observed; anthracnose (*Colletotrichum* spp.), rooting (*Rhizopus* spp.), yellow mold (*Fusarium* spp.), and green mold (*Penicillium* spp.). There have not been any reports of *Alternaria* diseases in cucurbit seeds in Bangladesh.

Many *Alternaria* species cause diseases in a wide variety of plants of worldwide agricultural and economic importance during their growing stages or after harvest. The genus *Alternaria* Nees was originally described in 1816 with *A. tenuis* Nees as the type and only strains [2]. Since then, more than 1,100 names have been published in *Alternaria*, and Simmons [3] accepted nearly 300 species. While, some species are well known as destructive pathogens [3, 4], but the great majority are either saprophytic or have been described as occurring on hosts of little economic importance [3, 5]. The seed-borne pathogens *A. tagetica*, *A. zinniae*, *A. cosmosa*, and *A. patula* are commonly found during seed health test of ornamental plants in the family of Compositae [6, 7]. *A. alternata* infection has become the most important postharvest disease of stored mango fruits [8, 9]. Most *Alternaria* species are saprophytes that are commonly found in soil or decaying plant tissues [10]. Many species are isolated from unusual substrates such as sewage or jet fuel [11]. *Alternaria*, particularly the small-spored species, have

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been isolated frequently during the surveys of endophytes in the xylem and stem tissues of plants, such as *Pinus sylvestris* and *Fagus sylvatica* [12], in the leaves of some important medicinal plants [13], in the shoots and leaves of *Vitis vinifera* [14], and in the leaves, stems and roots of chili pepper at different growing stages [15]. The endophytic *Alternaria* may cause disease as a latent pathogen, or it may not.

The taxonomy of *Alternaria* has been based on morphological characteristics such as conidia dimension, color, and septa (longitudinal and transverse); wall ornamentation; beak type and size; conidiophore type, size, and septa; sporulation patterns (in chains or solitary; branched or unbranched); and cultural characteristics [3, 16-19]. The color, size, and shape of conidia of most *Alternaria* species vary considerably depending on the substrates and culture conditions (light, temperature, and humidity) [3, 11, 20]. Molecular approaches have been used to distinguish *Alternaria* species, e.g., sequence analyses of different genes (internal transcribed spacer [ITS], glyceraldehyde-3-phosphate dehydrogenase [*gpd*], *Alternaria* allergen a 1 [Alt a1], H3, EF-1 $\alpha$ ,  $\beta$ -tubulin, CHS, LSU, *endoPG*, mtSSU, ACT, CAL, and CHS) [21-27]. Therefore, the objectives of this study were (1) to isolate endophytic *Alternaria* from seeds of Bangladeshi pumpkins and (2) to identify the isolates based on their morphology and sequence analyses of multiple genes.

## MATERIALS AND METHODS

**Sampling.** Pumpkin seed samples were collected from the Bangladesh Agricultural Research Institute (BARI), Bangladesh. The variety name of the seeds is BARI-Kumra 2. Bottle gourd seeds whose variety name is BARI-lau1 were also collected from BARI. Seeds were washed in running tap water to remove attached particles and sterilized by sequential immersion in 95% ethanol for 2 min followed by 1% sodium hypochlorite (NaOCl) solution for 5 min and then washed again with 95% ethanol for 30 sec to remove the NaOCl. Seed samples were then washed with sterile water 3 times to remove surface sterilizing agents. Samples were allowed to dry on a paper towel in a laminar air flow chamber. Seeds were placed horizontally on separate petri dishes containing potato dextrose agar (PDA; Difco, Franklin Lakes, NJ, USA) and rose bengal chloramphenicol agar (Difco) supplemented with the antibiotic streptomycin sulfate (0.4 mg/mL) to stop bacterial growth. After incubation at 25°C for 5, 10, and 25 days, individual hyphal tips of the developing fungal colonies were collected and placed onto PDA media incubated for 5~10 days and checked for culture purity. *Alternaria* isolates that were assumed to be novel were screened, and one representative isolate from each *Alternaria* species was selected. Eventually, pure cultures of *Alternaria* were transferred to PDA slant tubes and 20% glycerol stock solution. All the isolates were assigned a strain number and deposited. Two *Alternaria* isolates

(CNU111042 and CNU111043) with similar characteristics were selected for subsequent analysis.

Cultures of CNU111042 and CNU111043 were deposited in the Chungnam National University (CNU) Fungal Herbarium and in the Environmental Microbiology Laboratory Herbarium (as EML111042 and EML111043), Chonnam National University, Republic of Korea.

**DNA extraction and sequence analysis.** The fungal isolates of CNU111042 and CNU111043 were grown on PDA for 7 days. Genomic DNA was extracted by the method described by Paul *et al.* [28]. Three genes were used in this study for PCR amplification; the ITS region of the ribosomal DNA (rDNA) [29]; the Alt a1 gene [22], and *gpd* [30]. The amplification reaction for each gene was performed in a 50  $\mu$ L reaction volume and carried out in a GeneAmp PCR System 2700 thermo cycler (Applied Biosystems, Foster City, CA, USA), using conditions described by Deng [27]. The Wizard PCR Prep Kit (Promega, Madison, WI, USA) was used for purification of successfully amplified PCR products. Sequencing of amplified DNA was performed with an ABI Prism 310 Genetic Analyzer (Applied Biosystems) using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) with the same primer used for PCR amplification.

Sequences obtained herein (Table 1) and other sequences retrieved from our prior study or from GenBank were initially aligned with the CLUSTAL X program [10], edited in BioEdit v 7.0.1 and were finalized by manual adjustment. For the combined analysis, sequences for each gene were concatenated in a single nucleotide alignment. Maximum parsimony analysis was conducted using MEGA ver. 5.05 [31]. The robustness of the phylogram in the maximum likelihood analyses was evaluated by 1,000 bootstrap replications. The best tree obtained from this search was edited in MEGA ver. 5.05 [31].

**Morphology.** The representative isolate CNU111042 (5 mm diameter of mycelial plug) was grown on PDA for 7 days at 25°C in dark to determine their cultural characteristics. The fungus was grown on V8 juice agar and potato carrot media (PCA: potatoes 20 g, carrots 20 g, agar 15 g, and distilled water 1 L; dice potatoes and carrots were cooked for 1/2 hr, strain through cheesecloth and added agar to filtrate, then autoclaved at 121°C for 15 min) for sporulation, according to methods described by Simmons [3]. Conidia were mounted in lactophenol for further measurement with an Olympus BX50 light microscope (Olympus, Tokyo, Japan) attached to an Artray Artcam 300MI digital camera (Artray Co. Ltd., Tokyo, Japan). The sporulation patterns of the conidia were observed, with micrographs collected simultaneously. The species description was based on the results of examination of random conidia.

**Pathogenicity test.** The CNU111042 and CNU111043 isolate was grown on PDA petriplate for 7 days at 25°C.

**Table 1.** Isolates used in this study for molecular data analysis

Species	Isolate ID	Status	Host	Origin	Other collections	Accession No.		
						ITS	Alt a 1	gpd
<b><i>Alternaria burnsii</i></b>	<b>CNU111042</b>	<b>R</b>	<b><i>Cucurbita maxima</i></b>	<b>Bangladesh</b>	- <sup>a</sup>	<b>KJ651268</b>	<b>KJ862256</b>	<b>KJ651271</b>
<b><i>A. burnsii</i></b>	<b>CNU111043</b>	<b>R</b>	<b><i>Cucurbita maxima</i></b>	<b>Bangladesh</b>	-	<b>KJ651269</b>	<b>KJ862257</b>	<b>KJ651272</b>
<i>A. burnsii</i>	CBS 107.38	T	<i>Cuminum cyminum</i>	India	C-20	KP124420	KP123967	KP123967
<i>A. alternata</i>	EGS 34-016	T	<i>Arachis hypogaea</i>	India	-	AF347031	AY563301	AY278808
<i>A. alternantherae</i>	CBS 124392	-	<i>Solanum melongena</i>	China	-	KC584179	-	KC584096
<i>A. arborescens</i>	EGS 39-128	T	<i>Solanum lycopersicum</i>	USA	-	AF347033	AY563303	AY278810
<i>A. brassicae</i>	CBS 116528	R	<i>Brassica oleracea</i>	USA	BMP 0322	KC584185	AY563309	KC584102
<i>A. brassicicola</i>	ATCC 96836	R	<i>Brassica oleracea</i>	USA	BMP 0325	JX499031	AY563311	KC584103
<i>A. carotiincultae</i>	CBS 109381	T	<i>Daucus carota</i>	USA	EGS 26.010	KC584188	AY563287	KC584106
<i>A. cinerariae</i>	CBS 116495	R	<i>Ligularia</i> sp.	USA	EGS 33-169	KC584190	AY563308	KC584109
<i>A. dauci</i>	CBS 117097	R	<i>Daucus carota</i>	USA	BMP 159	KC584192	HE796730	KC584111
<i>A. daucifolii</i>	EGS 37-050	T	<i>Daucus carota</i>	USA	CBS 118812	KC584193	-	KC584112
<i>A. dianthicola</i>	CBS 116491	R	<i>Dianthus</i> sp.	New Zealand	-	KC584194	-	KC584113
<i>A. elegans</i>	CBS 109159	T	<i>Lycopersicon esculentum</i>	Burkina Faso	EGS 45.072	KC584195	GQ180092	KC584114
<i>A. gypsophylae</i>	CBS 107.41	T	<i>Gypsophila elegans</i>	-	CBS 107.41	KC584199	JQ646387	KC584118
<i>A. helianthiinficiens</i>	CBS 208.863.1	R	<i>Helianthus annuus</i>	UK	-	NR077213	-	KC584120
<i>A. limoniasperae</i>	BMP 0316	R	<i>Citrus jambhiri</i>	USA	-	FJ266476	AY563306	AY562411
<i>A. longipes</i>	EGS 30-033	T	<i>Nicotiana tabacum</i>	USA	-	AY278835	AY563304	AY278811
<i>A. japonica</i>	CBS 118390	R	<i>Brassica chinensis</i>	USA	BMP 0332	KC584201	AY563312	KC584121
<i>A. macrospora</i>	CBS 117228	T	<i>Gossypium barbadense</i>	USA	BMP 0173	KC584204	AY563294	KC584124
<i>A. nobilis</i>	CBS 116490	R	<i>Dianthus caryophyllus</i>	New Zealand	-	KC584208	JQ646385	KC584127
<i>A. panax</i>	EGS 29-180	R	<i>Panax ginseng</i>	Korea	-	JQ693662	JQ646382	JQ646299
<b><i>A. palandui</i></b>	<b>EGS 37-005</b>	<b>T</b>	<b><i>Allium cepa</i></b>	<b>India</b>	-	<b>KJ862254</b>	<b>KJ862259</b>	<b>KJ862255</b>
<i>A. porri</i>	ATCC 58175	T	<i>Allium fistulosum</i>	USA	-	AF229470	AY563296	AY278806
<i>A. photistica</i>	CBS 212.86	T	<i>Digitalis purpurea</i>	UK	EGS 35-172	KC584212	AY563282	KC584131
<i>A. radicina</i>	CBS 245.67	T	<i>Daucus carota</i>	USA	BMP 0079	KC584213	AY563286	KC584133
<i>A. simsimi</i>	EGS 13.110	T	<i>Sesamum indicum</i>	Argentina	-	JF780937	-	KC584137
<i>A. solani</i>	CBS 116651	R	<i>Solanum tuberosum</i>	USA	BMP 0187	KC584217	AY563299	KC584139
<i>A. sonchi</i>	CBS 119675	R	<i>Sonchus asper</i>	Canada	EGS 46-051	KC584220	AY563307	KC584142
<i>A. tagetica</i>	CBS 479.81	R	<i>Tagetes erecta</i>	UK	EGS 44-044	KC584221	AY563297	KC584143
<i>A. tenuissima</i>	EGS 34-015	T	<i>Dianthus</i> sp.	UK	-	AF347032	AY563302	AY278809
<b><i>A. tomaticola</i></b>	<b>EGS 44-048</b>	<b>T</b>	<b><i>Lycopersicon esculentum</i></b>	<b>USA</b>	-	<b>KJ651270</b>	<b>KJ862258</b>	<b>KJ651273</b>
<i>A. tomato</i>	CBS 114.35	T	<i>Solanum lycopersicum</i>	USA	-	JX418359	JQ646389	JQ646306
<i>Embellisia allii</i>	CBS 339.71	R	<i>Allium sativum</i>	USA	EGS 38-073	KC584230	AY563322	KC584155
<i>E. didymospora</i>	CBS 766.79	-	Sea water	Adriatic sea	-	FJ357312	JN383506	FJ357300
<i>E. phragmospora</i>	EGS 27-098	T	Soil	The Netherlands	-	FJ357314	JN383509	FJ357302
<i>E. tellustris</i>	EGS 33-026	T	Soil	USA	-	JN383494	AY563325	JN383475
<i>Nimbya scirpicola</i>	CBS 481.90	R	<i>Scirpus</i> sp.	UK	EGS 19-016	KC584237	AY563320	KC584163
<i>Teretispora leucanthemi</i>	CBS 421.65	R	<i>Crhysanthemum maximum</i>	The Netherlands	-	KC584240	-	KC584164
<i>Ulocladium atrum</i>	CNU9054	R	Soil	Korea	-	JF417684	JX213312	JF417694
<i>U. botrytis</i>	CBS 197.67	T	Contaminant	USA	BMP 0354	KC584243	AY563317	KC584168
<i>U. cucurbitae</i>	EGS 31-021	R	<i>Cucumis sativus</i>	New Zealand	EGS 31-021	FJ266483	AY563315	AY562418
<i>Stemphylium botryosum</i>	ATCC 42170	T	<i>Medicago sativa</i>	USA	-	AF229481	AY563274	AY278820

Bold type indicates sequencing performed as part of the present study.

CNU, Chungnam National University Fungal Herbarium, Daejeon, Republic of Korea; CBS, Culture Collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; EGS, Collections of EG Simmons; ATCC, American Type Culture Collection, Manassas, VA, USA; BMP, personal collection of Barry M Pryor; R, reliable representative strain; T, ex-type strain.

<sup>a</sup>Not available data.

After 7 days of culture, mycelia were scratched off from the petriplate and kept under near ultra violet radiation at 12 hr/12 hr light-dark condition for induction of sporulation. Spores were collected and counted with a haemocytometer. Conidial suspensions were prepared to a concentration

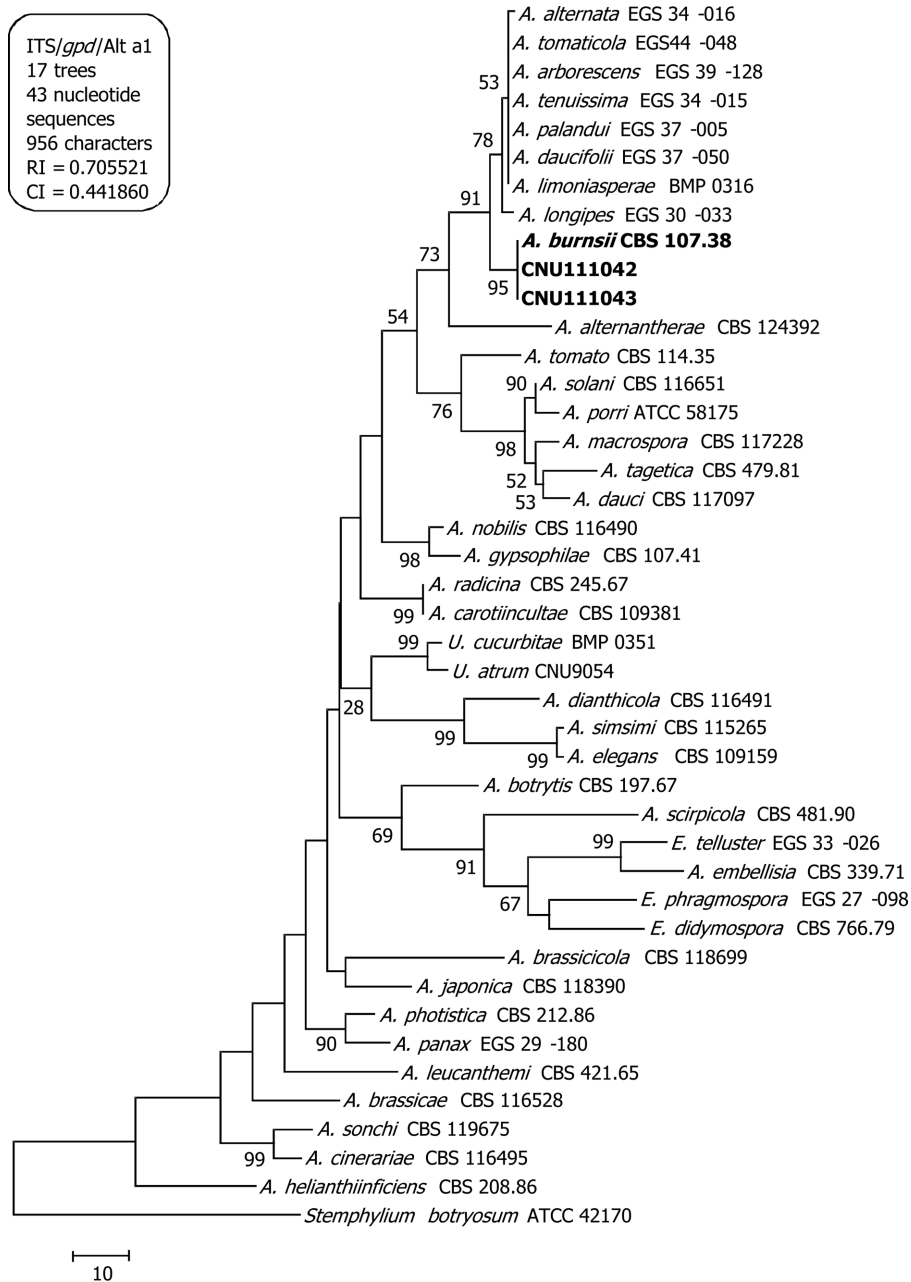
of  $1 \times 10^5$  conidia/mL. The surface sterilized seeds from pumpkin and bottle gourd (another cucurbit seeds) were sprayed with the concentrated conidial suspension. Sterilized water was used as a control. Seed infection, germination percentage, and seedling infection were checked after 5

days for the pathogenicity.

## RESULTS

**Sequence analysis.** BLAST queries of ITS, *gpd*, and Alt a1 gene sequences indicated notable relationships with GenBank sequences. The ITS sequences showed over 100% sequence similarities with *A. burnsii* and high similarity with other different *Alternaria* species including *A. longipes*,

*A. alternata*, *A. brassicicola*, *A. arborencens*, etc. Among these, one of the *A. longipes* isolates showed 100% sequence similarity; however this is not a reliable isolate. The sequence of type strain of *A. longipes* did not match well with the present *Alternaria* species. However, the ITS sequence of the isolate is typical and clearly identical to the sequences of *A. burnsii*. The *gpd* sequences showed similarities (99~100%) with *A. tomato*, *A. tomaticola*, *A. burnsii*, *A. longipes* and many other *Alternaria* species. A 100% *gpd* sequence



**Fig. 1.** Phylogenetic tree for the *Alternaria* species (isolate CNU11042) from Bangladesh pumpkin seeds and its related species generated using maximum parsimony analysis of combined data sets of internal transcribed spacer (ITS), glyceraldehydes-3-phosphate dehydrogenase (*gpd*), and *Alternaria* allergen a1 (Alt a1) gene sequences. Numbers above the nodes indicate bootstrap values (> 50%) from 1,000 replicates. The bar indicates the number of substitutions per position. *Stemphylium botryosum* ATCC42170 is the outgroup. RI, retention index; CI, consistency index.



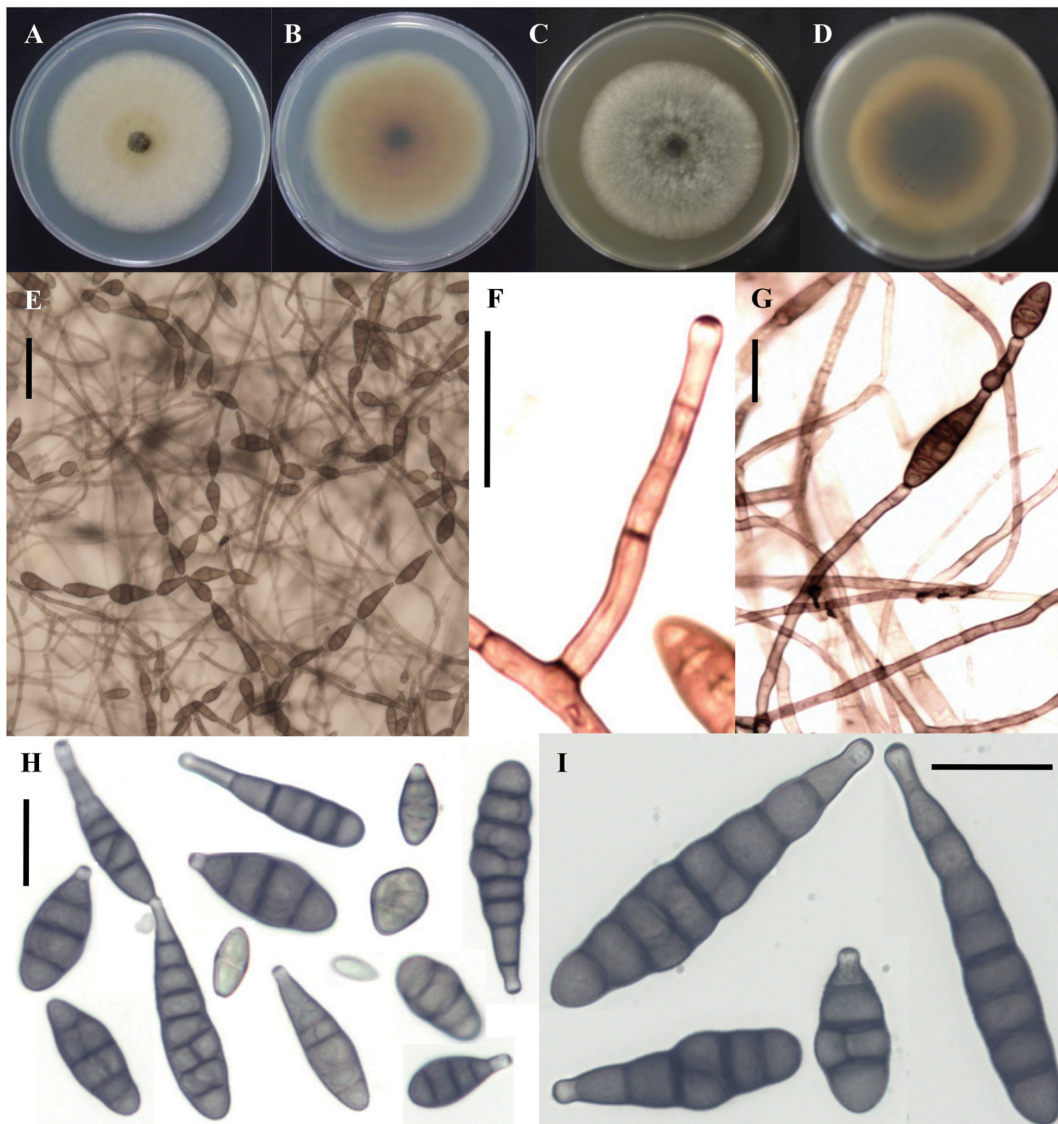
similarity was observed with the isolate *A. burnsii* CBS 107.38, but few difference was observed between the Alt a 1 gene sequence of *A. burnsii* CBS 107.38 and that of our isolates, and the overall sequence similarity was 99%. *A. tomato* CBS 114.35 showed 99% similarity in its Alt a 1 gene sequence but ITS sequence showed that the CNU111042, CNU111043, and CBS 114.35 were different fungi. In the combined dataset, isolates CNU111042, CNU111043 and *A. burnsii* (CBS 107.38), were placed in the same sub-clade (Fig. 1). It is clear that the isolates from Bangladesh pumpkin seeds are similar from those previously described as *A. burnsii*. These isolates are considered as *A. burnsii* from new host *Cucurbita maxima*.

**Morphology.** Colonies were white to buff in the obverse and buff to ochreous in the reverse, and they were 65–

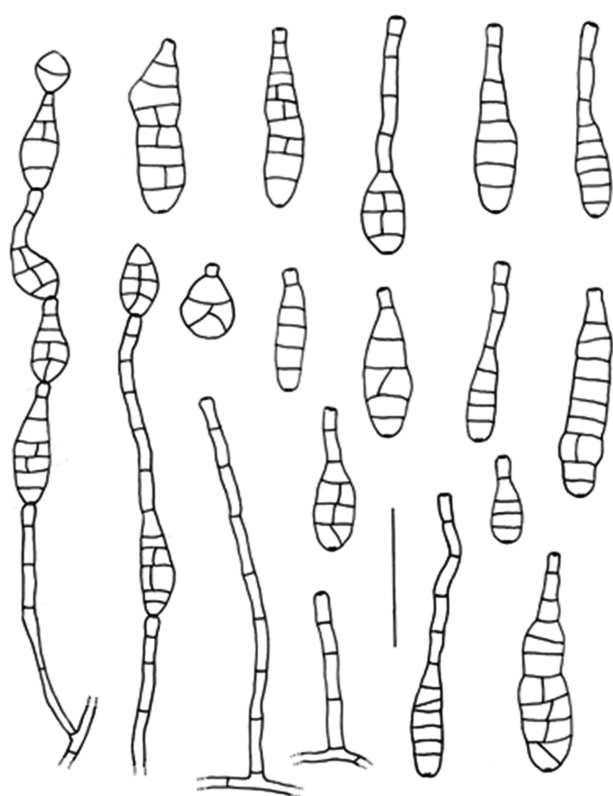
67 mm diameter when grown on PDA after 7 days at 25°C (Fig. 2A–D). Colonies on V8 juice agar were blackish-white on the obverse and in reverse, the inner part was blackish and the outer parts were brownish-white. Mycelium, superficial composed of branches; aerial hyphae observed when cultured on V8 juice agar.

Conidiophores were single, lateral from hyphae or terminal; straight or curved; smooth-walled with 1–10 septa; pale brown; usually with only one pigmented terminal conidiogenous site, sometimes with one additional lateral conidiogenous locus; sometimes slightly swollen at the apex; and 15–100 (~170) µm long, 2.5–4 (~4.5) µm wide (Figs. 2 and 3).

Conidia were in short or moderately long chains of 2–8 conidia normally, sometime more; occasionally (uncommonly) branched; normally 16–60 (~90) × 6.5–14 (~16) µm in



**Fig. 2.** Morphology of *Alternaria burnsii* CNU11042. Colonies grown on-potato dextrose agar (A, B) and V8 juice agar (C, D) (A and C, obverse; B and D, reverse) for 7 days at 25°C; sporulation pattern, conidiophores and conidia (E–I) produced on potato carrot agar (scale bars: E = 50 µm, F–I = 20 µm).



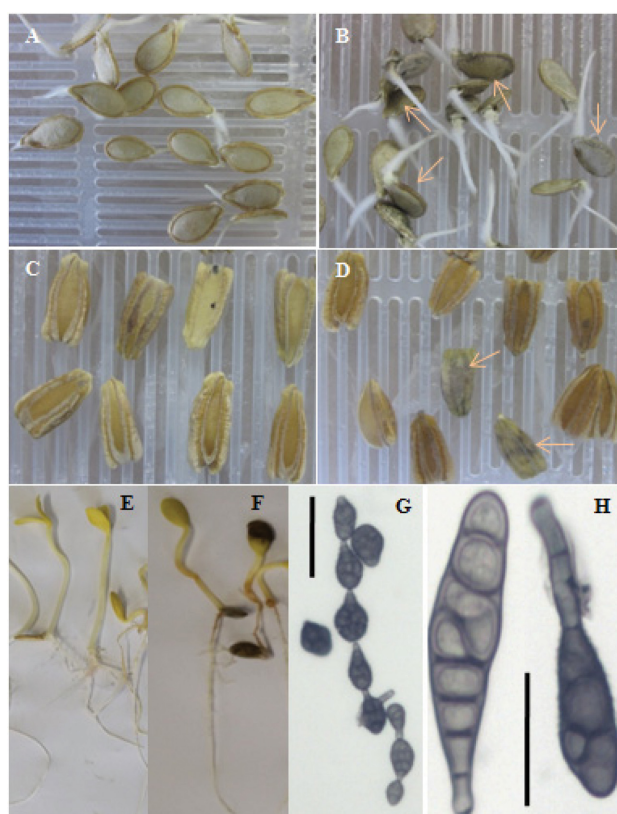
**Fig. 3.** Morphology of *Alternaria burnsii* CNU111042. Conidia, conidiophores and sporulation pattern of the culture produced on potato carrot agar (scale bar = 50  $\mu$ m).

size; ellipsoid, long ellipsoid, obclavate or ovoid with 2~11 transverse septa and 2~3 (~4) longitudinal septa; beakless or with a subcylindric or cylindric secondary conidiophores (pseudostrostrate) with 0~5 septa, being analogous to the beak with 4~60  $\mu$ m long, 3~4.5  $\mu$ m wide, which increased the conidia upto 90  $\mu$ m; dilute tan to dark brown in color with some transepts in darker contrast; mostly smooth in the conidial wall, or occasionally verruculose.

**Pathogenicity.** The isolate CNU111042 was able to cause discoloration and weak blight in pumpkin seeds. We attempted to check the pathogenicity of this isolate in seeds of other cucurbits such as bottle gourd. The isolate produced weak disease in bottle gourd seeds as well. The germination percentage of these 2 seed types was reduced, and disease was observed in seedlings (Fig. 4). The isolation procedure and the results of the pathogenicity tests indicated that the fungus might be endophytic and might act on cucurbits as a latent minor pathogen.

### DISCUSSION

Molecular data have been proven to be useful in the differentiation of many *Alternaria* species and the genetic diversity study of inter-species and intra-species fungi. Phylogenetic analysis of ITS sequences of 7 toxin-producing



**Fig. 4.** Pathogenicity of the CNU111042 isolate on seeds of *Cucurbita maxima* and *Lagenaria siceraria*. A, C, Non-treated control; B, D, Treated with *Alternaria burnsii* CNU111042 spore suspension. Seedlings of *C. maxima* (E, non-treated control; F, treated). The arrows representing infected seeds after inoculation. Reisolation of the conidia of *Alternaria* from inoculated seeds (scale bars: G = 50  $\mu$ m, H = 20  $\mu$ m).

*Alternaria* revealed that they formed a monophyletic group together with *A. alternata* isolates, which group could be clearly separated from the other morphologically distinct *Alternaria* species [24]. The morphologically similar species, *A. radicina* and *A. carotiincultae* were separated into 2 distinct lineages based on sequence analyses of genes including EF-1 $\alpha$ ,  $\beta$ -tubulin, and Alt a 1, and these results were consistent with those obtained using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat analysis [32]. Amplified fragment length polymorphism analysis previously revealed that genetic clusters among isolates of *A. tenuissima* and *A. solani* were revealed to be associated with the location of origin and the host plants, respectively [33, 34]. Molecular studies have demonstrated a clear distinction between large and small-spored *Alternaria* species which resulted in *Alternaria* species being classified into 6 genetic groups [22, 35, 36].

However, there are many species that could not be differentiated based on molecular methods, most notably, the small-spored *Alternaria* species. Among 4 morphologically distinct species-groups of *Alternaria* isolates from pistachio, *A. alternata* and *A. tenuissima* species-group isolates could

not be distinguished based on the combined data matrices of RAPD and polymerase chain reaction-restriction fragment length polymorphism analyses and the two species-groups and *A. arborescence* species-group comprised a monophyletic clade, in which the 3 species-groups could not be further resolved based on the results of ITS sequence analysis [37]. Phylogenetic analysis of several loci from small-spored *Alternaria* found no associations between phylogenetic lineages and hosts or geographic locations and strict congruence was not found between phylogenetic lineages and morphological characteristics among isolates grouped morphologically with *A. alternata* and *A. tenuissima* [38]. However, when *Alternaria* isolates were cultured and observed using defined conditions, the phenotypic plasticity could be minimized and valid taxonomic separations could be made based upon morphological characteristics [37, 39]. The standard conditions described by Simmons [3] are recommended for identification of *Alternaria*. Furthermore, use of appropriate molecular methods for species classification would permit the separation of *Alternaria* species [32].

The *Alternaria* isolate described here was seed-borne and was isolated from Bangladesh pumpkin seeds. It produced unbranched, small-spored conidia in medium-number (2~10) chains (Figs. 2 and 3). Phylogenetically, the species was closely related to *A. longipes*, as shown by the results of ITS sequencing. However, the isolate produced longer conidia and long secondary conidiophores, up to 60 µm, which was unlike what is observed in *A. longipes* [3]. Additionally, *A. longipes* produced dull-olive brown pigment on PDA media, whereas pigment was absent in CNU111042 isolate. But ITS, *Alt a 1* and *gpd* gene sequence analysis revealed similarities with related *A. burnsii* species. However, combined data analysis clearly demonstrated that the pumpkin seed isolate was previously reported as *A. burnsii*. Longitudinal septation of the new species was commonly 2 to 3 and in *A. burnsii* were 1~5 (Table 2). Transverse septation of up to 11 were seen in the new isolate, whereas in *A. tomaticola*, a maximum of 6 transverse septation were observed. Based on these properties, it was clear that the fungus is *Alternaria burnsii* Uppal, Patel & Kamat isolated from a new host.

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**Table 2.** Comparison of conidial characteristics of *Alternaria burnsii* CNU111042 and its closely related species

Species	Conidia				Pigment in PDA	References
	Size (µm)	Shape	Septa			
			Transverse	Longitudinal		
<i>A. burnsii</i>	16~60 (90) × 6.5~14 (~16)	Long ellipsoid, obclavate or ovoid	2~6 (11)	0~2 (~4)	None	This study
<i>A. tomaticola</i>	30~40 × 9~12	Ellipsoid or ovoid	6~7	1 (~2)	None	[3]
<i>A. longipes</i>	12~35 (~48) × 5~12 (~14)	Narrow-ovoid or ellipsoid	Uncommon 3~7	No or 1 (~3)	Dull-olive brown	[3]
<i>A. tomato</i>	30~50 × 10~13	Narrow-ovoid	6~9	1 (~2)	None	[3]
<i>A. burnsii</i>	25.5~105 × 8.4~20 30~50 × 9~13	Obovate Ovoid to ellipsoid	4~9 5~8	0~4 1~5	No report None	[40] [3]

PDA, potato dextrose agar.



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