WHAT DOES THE NAME *STACHYBOTRYS CHARTARUM* MEAN?

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ABSTRACT

The fungus *Stachybotrys chartarum* is the type species of the genus *Stachybotrys*. Certain strains of the species are known to produce trichothecene mycotoxins. It is a cellulolytic saprophyte with a worldwide distribution and frequently recovered in water-damaged buildings. Evidences of the detrimental effects on human health due to respiratory exposure to this fungus have been reported. *Stachybotrys chartarum* isolated from the lung of a child diagnosed with pulmonary hemosiderosis was reported in Texas for the first time in 1999. However, morphological and mycotoxin profile studies showed that this species is not well delineated. This paper reviews and summarizes data and evidences from studies of isolates of four *Stachybotrys* species regarding the taxonomic status of the epithet *S. chartarum*.

KEY WORDS

Conidia, Morphology, Mycotoxin profile, *Stachybotrys chartarum*, Taxonomic status.

INTRODUCTION

Stachybotrys chartarum (Ehrenberg ex Link) Hughes (=*Stachybotrys atra* Corda), is a cellulolytic saprophyte with worldwide distribution. *Stachybotrys chartarum* is frequently isolated from paper, wallpapers and gypsum boards in the buildings or residences, which experienced water damage. The fungus produces several mycotoxins, highly toxic macrocyclic trichothecenes and related trichoverroids as well as immunosuppresants and endothelin receptor antagonists (Jarvis and Hinkley, 1999). Its negative effects on animal and human beings have been studied since the 1930's (Haugland and Heckman, 1998; Kendrick, 2000). It was demonstrated to be associated with "sick building syndrome" in wet buildings. It has increasingly attracted public attention to its effect on human health following the reports concerning its association with idiopathic pulmonary hemorrhage in infants from Cleveland, Ohio (Dearborn et al. 1999). Subsequently, *Stachybotrys chartarum* was reportedly isolated for the first time from the lung of a child diagnosed with pulmonary hemosiderosis in Texas (Elidemir *et al*., 1999). Another case of infant pulmonary hemorrhage associated with the presence of *Stachybotrys atra* (=*S. chartarum*) was reported and mycotoxin analysis demonstrated that the isolate was highly toxigenic (Flappan *et al.* 1999). Vesper and Vesper (2002) studied and hypothesized that stachylysin, a hemolysin, produced by *S. chartarum* could be a contributing factor to infant pulmonary hemorrhage and hemosiderosis. The health issue related to the presence of *Stachybotrys chartarum* in buildings or residences has attracted the attention of news media. However, the species concept of *S. chartarum* is not well delineated. It has been subject to controversy since it was proposed as the type species of genus *Stachybotrys* under the name of *Stachybotrys atra* by Corda in 1837. In last several years, studies (Kong 1997; Cruse et al, 2002; Andersen et al., 2003) showed that *S. chartarum sensu lato* included several close related species and cryptic species, which raise the question concerning common identification and reports of *S. chartarum*. The objective of the paper is to discuss morphological studies of four *Stachybotrys* species and their isolates and the problematic aspects of *S. chartarum* identification.

METHODS

Three isolates (M 3N-5, M 21024, M100) of *Stachybotrys chartarum* and two isolates of *S. yunnanensis* (030115-063, PK401), and one isolate *S. cholorohalonata* (PK326), and *S. microspora* (PK306), respectively, recovered from indoor samples submitted to P & K Microbiology Services, Inc. were used for the study. For single spore isolation, the cultures were transferred onto MEA medium for one week at 25°C. Agar pieces of 5 × 5 mm with fungal mycelia were cut from MEA medium and placed into test tubes containing 10 ml sterilized water. The test tubes were vortexed for 30 s to dispense conidia into water. The conidium suspension was diluted 10 times by pipetting 1 ml into 9 ml of sterile water. Following the same procedure, the conidium suspension was further diluted to 100 x dilutions. Test tubes with conidia suspensions diluted at 1, 10, and 100 times were vortexed for 10 s to evenly suspend conidia in water. Ten µl conidia suspension was pipetted from each dilution onto MEA. The conidia suspensions were evenly spread on the media with a sterile triangle metal bar. The petri dishes were incubated for 24 hours at 25 °C. Petri dishes were then examined for under a dissecting microscope at 60 x and single spore isolation was made by removing and transferring single germinating spore onto an MEA plate for morphological comparisons. Real time

PCR detection analysis was conducted using an ABI Prism, 7000 Sequence Detection System for the isolates used in this study with the primer for *S. chartarum sensu lato* (Haugland et al, 1999).

The data were analyzed with COSTAT, statistical software.

RESULTS AND DISCUSSIONS

Taxonomic history and current status of *S. chartarum* and related species

The genus *Stachybotrys* and its type species have been subject to controversy since they were proposed. *Stachybotrys chartarum* (Ehrenberg ex Link) Hughes was first described by Corda in 1837 under the name *Stachybotrys atra* Corda as the type species of a new mitosporic genus *Stachybotrys*. In Corda's description conidia of this species are two-celled (Corda, 1837). The description of two-celled conidia is one of the controversial aspects. Up to present all accepted members of *Stachybotrys* are unicellular conidia without exception (Jong and Davis, 1976). Later Hughes' reexamination of type material and recombination showed that Corda' description was inaccurate. This inaccuracy led to the revision of Corda' description by Bisby (1943). Prior to critical revision of the description of the species by Bisby in 1943 over 20 species had been described. According to his extensive studies of cultures and herbarium materials Bisby (1943) revised the species and generic descriptions from two-celled conidia to one-celled conidia and kept the name *Stachybotrys atra*. Since Bisby did not reexamine the type material, he speculated that the guttate in the conidia misled Corda to believe the conidia were two-celled. Our observation showed that some isolates developed biguttulate (having two oil drops) conidia. He also reduced the number of species from over 20 to two based on his belief that a great variability existed in the species of *S. atra*.

After reexamining the type material of *S. atra*, Hughes (1956) identified and recombined it as *S. chartarum* (Ehrenberg) Hughes. He did not write a new description for the species based on his examination of the type material. Ellis (1971) still recognized *S. atra*. He also accepted a new variety of *S. atra* proposed by Mathur and Sankhla published in1966 according to the smaller sizes of conidia (6-8 × 4-5 µm) and the shape (elliptical or pyriform to globose) with the name *S. atra* Corda var. *microspora* Mathur and Sankhla.

Jong and Davis (1976) suggested that proper combination of the species should be *Stachybotrys chartarum* (Ehrenberg ex Link) Hughes. They also reexamined the type culture of *S. atra* var. *microspora* and found that the deposited material was mixed with *S. chartarum*. The mixed two fungal entities were re-isolated and identified. Jong and Davis proposed *S. atra* var. *microspora* as a new species and recombined it as *Stachybotrys microspora* (Mathur & Sankhla) Jong & Davis.

According to Bisby and Hughes's studies, *Stachybotrys chartarum* has three homotypic synonyms:

- *Stilbospora chartarum* Ehrenb. 1818
- *Oidium chartarum* Ehrenb. ex Link 1824
- *Oospora chartarum* (Ehrenb. ex Link) Wallr. 1833.

In addition, 16 heterotypic synonyms have been listed (Jong and Davis, 1976). *Stachybotrys atra* Corda, the type species when *Stachybotrys* was proposed as a new genus in 1837, is one of the heterotypic synonyms of *S. chartarum* (Jong and Davis, 1976).

At present the name *Stachybotrys chartarum* is well accepted and used by a majority of mycologists, but inconsistent descriptions of this species (Bisby, 1943; Ellis, 1971; Jong and Davis, 1976) has resulted in a continuation of the controversy concerning its species delineation. More studies have revealed the complexity of this species.

Kong (1997) published *Stachybotrys yunnanensis* as a new species in 1997. The key morphological character of this species is that its conidia are cylindrical to subcylindrical (9.4 ± 0.82 × 3.8 ± 0.47 µm; L/W ratio, 2.5) (Figure 3). The author indicated that this species closely resembles *Stachybotrys chartarum*. At present there has been no addition identification or report of this new species other than the 1997 publication. It is likely that isolates of *S. yunnanensis* are identified as *Stachybotrys chartarum*. Cruse *et al.* (2002), using markers for three polymorphic protein-coding loci, examined 23 isolates identified as *S. chartarum* and found two cryptic species present within the isolates. The authors claimed that the two cryptic species were indistinguishable morphologically. Andersen *et al.* (2002) studied isolates formerly identified as *S. chartarum* and found that there were two chemotypes: one producing atranones, the other macrocyclic trichothecene, as well as one undescribed taxon

identified based on the analysis of morphology, growth, and, more importantly, metabolite production. The undescribed taxon has since been described as a new species, *S. chlorohalonata* (Andersen *et al.*, 2003). Morphological characters used to differentiate *Stachybotrys chlorohalonata* from *S. chartarum* are that the former develops smooth conidia (8.5 ± 4.2 × 5.4 ± 0.39 µm; L/W ratio, 1.8), and more restricted colonies and produces a green extracellular pigment on Czapek yeast agar (CYA) medium. More significantly the two species have different tri5, chs1 and tub1 gene fragments (Andersen *et al.*, 2003).

Variations of morphological characters of *S. chartarum sensu stricto*

Stachybotry chartarum sensu stricto (excluding isolates identified as *S. chlorohalonata* and *S. yunnanensis*) is still a species with a great variation in morphology. This may explain, in part, the inconsistency in descriptions as aforementioned. The sizes of phialides observed in the present study (Table 1) were smaller in some cases than the ones reported in the major literature: 9-14 × 4-6 µm (Jong and Davis, 1976) and 10 -13 × 4-6 µm (Ellis, 1971), but generally were in agreement. The variations of the phialides sizes between M 3N-5 and the three other isolates were significant. The variation in phialide sizes was not as great as the variation in conidia sizes.

Yang (1995) pointed out that the sizes of conidia described by Ellis (1971) and Jong and Davis (1976) had significant differences, especially the width: 7-12 × 4-6 µm (Jong and Davis, 1976; Domsch et al. 1993) and 8-11 × 5-10 µm (Ellis, 1971). The width of conidia reported in the two descriptions barely overlapped. Similar inconsistency or variation was reported in the study of Bisby (1943). In his study, the sizes of phialides and conidia in cultures 1 and 3 were similar: phialide 10-15 × 5-7 µm, conidia 8-11 × 3.5-6 µm. In culture 2 conidia were 8-11 × 5-10 µm in one-month-old cultures. Our results were in general agreement with the conidia dimensions of Jong and Davis (1976) and Bisby's (1943) in cultures 1 and 3 (Table 2) (Figures 1 and 2). The variations in conidia length were significant except between isolates M 21024 and M100. Significant differences in width were found among all isolates. Why did the measurements of conidial size show such a significant discrepancy? Bisby observed that conidia from young culture were much narrower. It is recognized that younger conidia are narrower than mature ones. However, the mature conidia observed in this study were not as wide as those described by Ellis (1971) and Bisby (1943).

Such a great variation in sizes and shapes of phialides and conidia adds confusion to the identification of *S. chartarum*. It might mean that the species concept of *S. chartarum* proposed by Bisby is still too broad. One or two synonyms could be revived upon further study. The existence of undescribed species could be another explanation.

Real Time PCR analysis to differentiate *S. chartarum* from closely related species

In addition to *S. chartarum*, several species of *Stachybotrys* including *S. chlorohalonata, S. elegans, S. microspora*, *S. nephrospora*, and *S. yunnanensis* were isolated from samples collected in indoor environments (Li and Yang, in preparation; Andersen *et al.*, 2003). Real Time PCR detection using *S. chartarum* primer and probe (Haugland et al, 1999) did confirmed identities the three strains identified as *S. chartarum*. The primer and probe did not react with isolates identified as *S. elegans, S. microspora* and *S. nephrospora.* However, the primer and probe failed to differentiate *S. chlorohalonata* and *S. yunnanensis* from *S. chartarum* (Table 3). This raises the issue that the current primer and probe set is for the detection of *S. chartarum sensu lato*.

Mycotoxin profiles

According to the mycotoxin profiles of *S. chartarum*, Andersen et al. (2002) reported that two different chemo-types exist among the isolate identified as *S. chartarum* isolated from water damaged buildings. One chemotype produced atranones and the other, macrocyclic trichothecene. They also found that an undescribed *Stachybotrys* species (later on described as *S. chlorohalonata*) co-existed with the two chemotypes of *S. chartarum* in water-damaged buildings (Andersen et al. 2002). Chromatograms and LC-UV profiles of isolates of *S. chartarum* were different from those of *S. microspora* and *S. nephrospora* (Nielson, unpublished).

Practical aspects in identifying *Stachybotrys chartarum* and closely related species

Identifying *S. chartarum* seems to be an easy task due to its unique conidiophore, phialide arrangement, and ornamented conidia, but it can present problems due to the great variation in the size, and shape of the conidia, and especially the color and roughness (immature conidia hyaline, mature dark olive gray, opaque; smooth-walled to coarsely roughened with warts and ridges). In addition, other species of *Stachybotrys* have been identified indoors (Li and Yang, in preparation). Differentiation among these species and *S. chartarum* is mainly dependent upon the shape, color, size, and ornamented surface

of their conidia (Jong and Davis, 1976). The color and ornamentation of conidia change with age. After 7 days of incubation, color and ornamentation of conidia may not be fully developed in strains of *S. chartarum*. Therefore, there is a risk of misidentifying these isolates. Young isolates with cylindrical or subcylindrical conidia may be identified as *S. yunnanensis*, *S. cylindrospora* or *S. chartarum*. It can be a challenge to differentiate *S. chlorohalonata* from *S. chartarum*, *S. albipes*, and *S. elegans* when an isolate is not fully developed. Caution therefore should be taken when identifying isolates of *Stachybotrys chartarum* from indoor sources and linking them to mycotoxin production, especially at early growth stages, because the *Stachybotrys chartarum* may not be a genuine one. The types and amount of mycotoxins produced vary with *Stachybotry* taxa. Identification of *S. chartarum sensu lato* is not necessary indicating the production of macrocyclic trichothecenes (Anderson et al, 2002). An experienced mycologist should be consulted when identification of *Stachybotrys* cultures to species is necessary. Molecular DNA approaches have been suggested to offer an alternative method for detecting *S. chartarum* (Haugland et al., 1999; Vesper et al. 2000; Haugland et al., 2001). Real Time PCR testing conducted in the senior author's laboratory suggested that the primer and probe could not differentiate *S. chartarum* from *S. chlorohalonata* and *S. yunnanensis* if they are considered three valid species.

CONCLUSION AND IMPLICATIONS

Based on the present study and descriptions in major literature it appears that *S. chartarum* is at present not a well-delineated species. Questions can be raised on the identification of *Stachybotrys chartarum* and the implication and complication that are associated with the species. The answer to the question is pending future studies on type materials (including the closely related synonyms), morphology, and phylogenetic analyses. Since PCR primer and probe designed for *S. chartarum* also detected *S. yunnanensis* and *S. chorolohalonata*, further research is in need to study the phylogenetic relationship of these closely related species to determine whether *S. yunnanensis* and *S. chorolohalonata* are valid species. If they are, the primer and probe for detecting *S. chartarum* should be redesigned.

ACKNOWLEDGMENT

The authors are grateful to Mingsheng Peng and Brian Kerin for their technical assistance.

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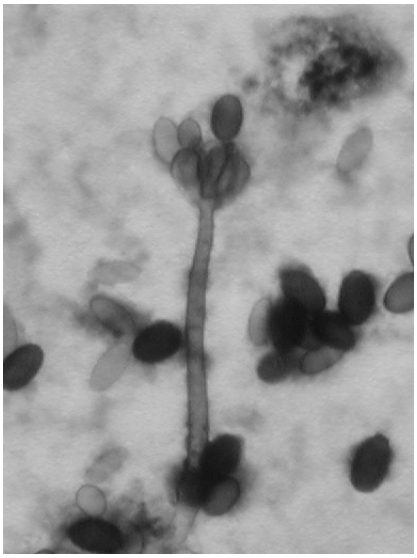

Figure 1. a. *Stachybotry chartarum* M100. Scale ___ 10 µm.

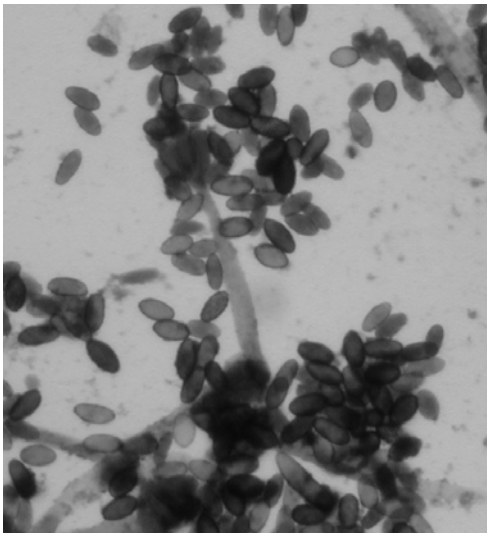

Figure 2. *Stachybotrys chartarum* M21024. Scale ___ 10 µm.

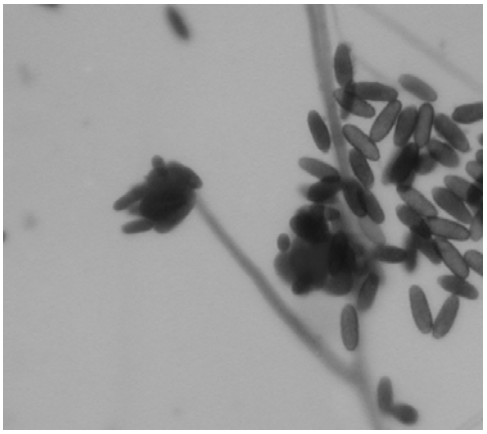

Figure 3. The conidia and conidiophore of *Stachybotrys yunnanensis*. Scale ____ 10 µm.

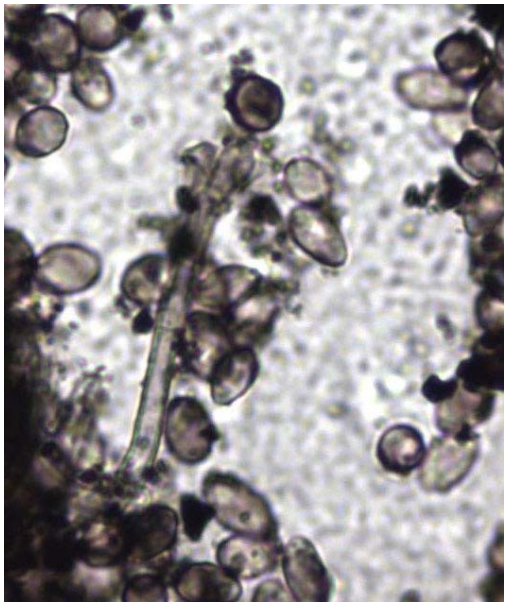

Figure 4. The conidia and conidiophore of *Stachybotrys chlorohalonata*. Scale ____ 10 µm.

Table 1. Sizes of phialides of three strains of *Stachybotrys chartarum* grown on MEA.

Strain	n	Length (µm)	Width (µm)	L/W[3] Ratio
M 3N-5	30	8.2 ± 1.33[1] a[2]	5.0 ± 1.44 a	1.7 ± 0.53 a
M 21024	30	9.8 ± 1.14 b	4.6 ± 0.47 ab	2.1 ± 0.32 b
M100	30	9.4 ± 1.47 b	4.4 ± 0.70 b	2.2 ± 0.49 b

[1] Mean ± SD. [2] Different letters in the same column indicate the significant difference (p <0.05). [3]Length/Width.

Table 2. Sizes of conidia of three strains of *Stachybotrys chartarum* grown on MEA.

Strain	n	Length (µm)	Width (µm)	L/W[3] Ratio
M 3N-5	30	8.2 ± 0.59[1] a[2]	4.9 ± 0.74 a	1.7 ± 0.26 a
M 21024	30	8.9 ± 1.13 b	4.6 ± 0.64 b	2.0 ± 0.39 b
M100	30	8.8 ± 0.97 b	5.3 ± 0.63 c	1.7 ± 0.22 a

[1] Mean ± SD. [2] Different letters in the same column indicate the significant difference (p <0.05). [3]Length/Width.

Table 3. Real Time PCR analysis of *Stachybotrys* species and isolates of *S. chartarum* using the primer and probe of *S. chartarum*.

Stachybotrys Taxon	Real time PCR Detection
S. chartarum	
M 3N-5	+
M 21024	+
M100	+
S. chlorohalonata (PK326)	+
S. elegans	–
S. microspora (PK306)	–
S. nephrospora (PK319)	–
S. yunnanensis	
030115-063	+
PK401	+