Taxonomy of *Scytalidium thermophilum*, an important thermophilic fungus in mushroom compost

GERBEN STRAATSMAN and ROBERT A. SAMSON

1 Mushroom Experimental Station, Postbus 6042, 5960 AA Horst, The Netherlands and 2 Centraalbureau voor Schimmelcultures, Postbus 273, 3740 AG Baarn, The Netherlands

More than thirty isolates of the thermophilic *Torula-Humicola* complex, mainly obtained from mushroom compost, were examined and compared with the available type cultures of *Torula thermophila*, *Humicola grisea var. thermoidea* and *H. insolens*. *Scytalidium thermophilum* is considered to be the correct name for all these isolates and type cultures, but the species is very variable in macroscopical and microscopical characters. All isolates of *S. thermophilum* promoted growth of *Agaricus bisporus* mycelium.

The presence of thermophilic fungi in mushroom compost has been found to be important for the colonization of the compost by mushroom mycelium and for a good yield of fruit bodies (Olivier & Guillaumes, 1979; Ross & Harris, 1983; Straatsma et al., 1989, 1991). *Humicola grisea* Traaen var. *thermoidea* Cooney & Emerson, *H. insolens* Cooney & Emerson, and *Scytalidium thermophilum* (Cooney & Emerson) Austwick (≡ *Torula thermophila* Cooney & Emerson) have been mentioned as dominant species in mushroom compost or similar self-heating substances, although *Thermomyces lanuginosus* Tsiklinsky (≡ *Humicola lanuginosa* (Griff. & Maubl.) Bunce) also occurs (Cailleux, 1973; Bilay, 1984; Fermor, Randle & Smith, 1985).

While describing *H. grisea* var. *thermoidea*, *H. insolens* and *T. thermophila*, Cooney & Emerson (1964) expressed doubts on the position of the taxa. They indicated that *H. grisea* var. *thermoidea* might be considered as a form of *H. nigrescens* Omvik because of its lack of phialidic conidia, and a gradation might exist between isolates of *Torula* and *Humicola*. Later this intergradation in the *Torula–Humicola* complex was confirmed by Fassatićová (1967), Emerson (1968), Awao & Otsuka (1974), Ellis & Griffiths (1976) and Ellis (1982).

In the type isolate of *Torula thermophila*, ATCC 16463, both intercalary and terminal conidial chains have been recognized (Cooney & Emerson, 1964; Ellis & Griffiths, 1976). In other isolates, including the type cultures of *H. grisea* var. *thermoidea*, ATCC 16454, and of *H. insolens*, ATCC 16453, it is not possible to distinguish between elastic and thallic conidogenesis, conidiophores being undifferentiated (Ellis, 1982). The two extremes of the morphological range are isolates with spores in long chains (*T. thermophila*) and those with single spores (*H. grisea* var. *thermoidea*), with *H. insolens* having intermediate characteristics (Cooney & Emerson, 1964). Ferguson (1964) viewed these extremes as *H. insolens* and *H. grisea* var. *thermoidea*, a concept followed in ecological surveys by Eggins & Malik (1969), Evans (1971) and Fermor et al. (1979) in which *T. thermophila* was not mentioned. In other surveys only *T. thermophila* and *H. grisea* var. *thermoidea* were mentioned (Cailleux, 1973; Olivier & Guillaumes, 1976), or only two types of *H. insolens* (Chang & Hudson, 1967; Hedger, 1975), or only two strains of an unidentified *Humicola* sp. (Von Klopotek, 1962). In particular, types with long chains of spores were found to be dominant in mushroom compost by Cailleux (1973), Olivier & Guillaumes (1976), Eicker (1977) and Straatsma et al. (1989). Austwick (1976) transferred *Torula thermophila* to *Scytalidium thermophilum* sensu Ellis (1971), but without providing a rationale for this nomenclatorial change.

To clarify the taxonomy and nomenclature, more than 30 thermophilic isolates in the *Torula–Humicola* complex were examined morphologically, and their ability to stimulate the extension rate of *Agaricus bisporus* mycelium in sterile mushroom compost was tested.

MATERIALS AND METHODS

Isolation

Beginning in 1986, we isolated thermophilic fungi belonging to the *Torula–Humicola* complex from samples of straw, horse droppings, stable bedding, mushroom compost in various stages, drainage of compost yard, and pig pen litter of sawdust and wood chips. Samples were blended in water and dilutions were plated in yeast–glucose agar containing penicillin G (50 mg l⁻¹) and streptomycin (50 mg l⁻¹) before gelling. Also, blended samples were poured on sieves and vigorously washed with water, and particles between 1 and 2 mm were plated. Plates were incubated at 45 °C in the dark. Representative isolates were kept in a collection on yeast–
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glucose agar slants at room temperature and subcultured twice a year. Two isolates have been lost due to degenerative slow growth.

Cultures examined


Other cultures from collections: *H. grisea var. thermoidea*, IMI 131012 = CBS 624-91; CBS 225-63; CBS 226-63; CBS 227-63; CBS 183-64; CBS 184-64, isolated from mushroom compost by C. L. Fergus; *H. insolens*, IMI 121649 = CBS 628-91; CBS 147-64 = ATCC 22082, isolated from mushroom compost by C. L. Fergus; *S. thermophilum*, ATCC 183-81, isolated from soil by M. R. Tansey, Indiana, U.S.A.; *Thermomyces lanuginosus* Tsiklnyák, ATCC 16455 = CBS 632-91, isolated from rotting guayule shrub by D. G. Cooney, California, U.S.A.; ATCC 22083 = CBS 631-91, isolated from mushroom compost by C. L. Fergus, Switzerland; ATCC 38905, isolated from compost by R. Jeffrey.


Cultivation

Isolates were grown in 9 cm diam. Petri dishes on yeast-glucose, 2% malt extract, oatmeal, and potato–carrot agar and on sterile compost (autoclaved at 120° for 2 h on 2 successive days), and were incubated in the dark at 40° or 45°. Mesophilic isolates were grown at 24°.

Test for growth promotion of *A. bisporus*

The ability of the isolates to stimulate the extension rate of *A. bisporus* ‘Horst® U1’ mycelium was studied according to Straatsma et al. (1989): 25 spawn grains of *A. bisporus* were placed at the bottom of culture tubes as an inoculum and then sterile compost colonized by a fungal isolate was added. Growth of *A. bisporus* was marked at 2–3 d intervals. When growth of *A. bisporus* was finished, the marks were measured and growth rates were calculated.

Isolates unable to grow on sterile compost were tested in compost agar: dried and milled (0·5 mm) compost was dispersed in water at 75 g l–1 and heated to 100° for thorough moistening. Agar was added at 12 g l–1 and 20 ml aliquots of the medium were sterilized at 120° for 1 h and poured in 9 cm Petri dishes. A dish culture of the isolate to be tested was blended in water and 1 ml was inoculated to compost agar before gelling. Dishes were inoculated with one spawn grain of *A. bisporus* at the edge of the dish.

RESULTS AND DISCUSSION

Growth promotion of *Agaricus bisporus*

All isolates of *S. thermophilum sensu lato* stimulated *A. bisporus* to grow at about 7 mm d–1, while growth of *A. bisporus* on sterile controls was only 3·2±0·7 mm d–1 (n = 26). None of the isolates of the thermophilic species *Humicola hyalothermophila, Thermomyces lanuginosus* and *T. stellatus* stimulated *A. bisporus*. Isolates of the mesophilic species *H. fuscoatra* var. *longispora* and *H. grisea* stimulated *A. bisporus* to grow at 5 mm d–1. *S. thermophilum* CBS 183-81 and CBS 183-64, *S. indonesiacum* CBS 259-81, *H. nigrescens* ATCC 22714 and *H. fuscoatra* ATCC 22721 grew erratically or not at all on sterile compost and were tested in compost agar. The two *S. thermophilum* isolates stimulated *A. bisporus* strongly, *S. indonesiacum* and *H. fuscoatra* were moderately stimulating, and *H. nigrescens* was inhibitory. In the case of *S. indonesiacum* the situation was complex since *A. bisporus* grew irregularly, with individual sectors extending at rates of 7 mm d–1.

Tests on another 29 thermophilic fungal species (Straatsma et al., unpublished data) showed that four of these promoted growth of *A. bisporus* as much as *S. thermophilum* and six were moderately growth-promoting. Growth promotion of *A. bisporus* is an interesting additional taxonomic character, since it is not generally found among thermophilic fungi.

*S. thermophilum* isolates will be further tested for growth on...
Figs 1–6. Five-day-old colonies of *Scytalidium thermophilum* on yeast-glucose agar at 45°C. Fig. 1. M7'5'1 (CBS 623.91), colony with regular growth and heavy sporulation. Fig. 2. Reverse side of the colony of Fig. 1, showing radial pattern. Fig. 3. 170-2-2 (CBS 621-91), colony with rather regular but lobed growth. Fig. 4. Reverse side of the colony of Fig. 3 showing lobed pattern. Fig. 5. CBS 226-63, colony with regular growth and fluffy overgrowth. Fig. 6. 15'1 (CBS 619-91), colony with irregular, lobed and somewhat slower growth.

Pasteurized compost, and selected isolates will be inoculated into compost at (semi) industrial scale.

**Observations of Scytalidium thermophilum sensu lato**

Colonies of most isolates grew rapidly, attaining a diameter of 9 cm within 5 d at 40°C on yeast-glucose agar. When young, margins of most isolates were lobed, but later they became even. Some isolates grew faster and had regular margins from the beginning. Fresh isolates mostly became dark green-grey to black quickly due to sporulation. Most isolates had a white to light grey mycelial overgrowth, especially at the margin (Figs 1–6).

In two experiments 11 months apart, more than 40 isolates were subcultured simultaneously. They were grouped according to macroscopical characteristics, but grouping was very different on the two occasions.

From the subculture of the type of *Torula thermophila* ATCC 16463, light-coloured as well as darker-coloured segregates were isolated (Figs 7–8). These segregates were rather stable after continued subculturing. Colonies of many fresh isolates were not dark any more after continued subculturing but green-grey or yellowish grey, and some had also a purple tint. Shortly after isolation, mature colonies of CBS 183-81 and 15-8 (= CBS 671-88; used in experimental work for over 5 years) were very dark, but CBS 183-81 is now...
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Figs 7–10. Five-day-old colonies of *Scytalidium thermophilum* on yeast-glucose agar at 45°. Fig. 7. Type culture of *Torula thermophila*, ATCC 16463, richly sporulating and rather dark colony. Fig. 9. T49·5·1 (CBS 620·91), colony with deteriorating development, characterized by purple-coloured, slow growth. This colony was subcultured simultaneously from the same culture as that shown in Fig. 10. Fig. 10. T49·5·1 (CBS 620·91), green-grey-coloured colony with rather slow irregular growth, subcultured simultaneously from the same culture as the colony in Fig. 9.

 Completely white and 15·8 is green-grey. We have the impression that isolates of type 2 (see below) are particularly unstable.

Some isolates grew slowly, and mature colonies were green-grey with a light-coloured mycelial overgrowth directly on isolation (Fig. 10). Some subcultures had a very slow atypical deteriorating development, becoming intensely purple-coloured (Fig. 9). On subsequent subculturing, reversion to the original appearance occurred.

The mycelium extends at or in the surface of the agar and gives rise to aerial hyphae directly behind the margin. Aerial hyphae may outgrow the margin and revert to slower-growing substrate hyphae. Consequently the margin becomes irregular. The vegetative aerial hyphae are hyaline and (2-)4–6 μm wide, and smooth-walled.

In submerged mycelium of all isolates, chains of thick-walled dark swollen cells were seen by direct observation through the agar from the reverse side of cultures, and also in slide preparations. The chains did not secede easily and the cells were cylindrical or globose, and mostly became darkly pigmented (Figs 11–15). These cells are sometimes referred to as chlamydospores or chlamydoconidia, but we prefer to follow Hughes (1985) and call them conidia.

Conidia are also produced on the surface of the substrate and in the aerial mycelium, about 1 cm behind the colony margin. All isolates formed thick-walled globose to ellipsoidal, smooth conidia mostly 8–12 μm in diameter. Conidia occurred singly, or in short or long chains, and were formed in terminal or intercalary positions. Two extreme types were recognized: type 1 having single very dark spores borne on short lateral hyphal branches (Figs 17–19) (representative of *H. grisea* var. *thermoidea* type) and type 2 having intercalary, slightly pigmented spores in chains (Fig. 16) (representative of *S. thermophilum* type). Within type 2, some isolates also have short terminal chains of conidia (Figs 20–21; similar to Fig. F in plate iv of Awao & Otsuka (1974)), intermediary between types 1 and 2. These isolates can only be recognized when cultures are young. After ageing chains grow longer, and separation from the common type 2 isolates becomes impossible. The slow-growing, green-grey isolates that show the atypical purple development belong to type 2 also.

Pigment is deposited in the vegetative mycelium or sometimes in the thick-walled conidia (Figs 22–23). In atypical purple colonies of these isolates no pigmented conidia are formed, but parts of the vegetative mycelium are filled with dark purple pigment. Only a few thick-walled hyaline conidia are produced which occur in chains or clusters, sometimes singly.

Type 1 isolates are *H. grisea* var. *thermoidea* ATCC 16453, *H. insolens* ATCC 16454, *H. grisea* var. *thermoidea* IMI 131012, CBS 225·63, CBS 226·63, CBS 184·64, *H. insolens* IMI 121649, M9·5·3, 77·7·8 (CBS 622·91), and T49·3·1. Type 2 isolates are *T. thermophila* ATCC 16463, CBS 227·63, CBS 183·64, CBS 227·63, CBS 183·64, CBS 227·63, CBS 183·64.
Figs 11–16. Light micrographs of conidial development in *Scytalidium thermophilum* (slide preparations except Fig. 16; scale bars = 10 μm). Fig. 11. Type culture of *Torula thermophila* ATCC 16463, chains of intercalary conidia (type Fig. 2). Fig. 12. ATCC 16463, young intercalary conidia. Fig. 13. ATCC 16463, young conidia, also solitary. Fig. 14. ATCC 16463, paler intercalary conidia which become darkly pigmented with age; chains of dark conidia. Fig. 15. 170'2'1 (CBS 621'91), development of dark conidia and subhyaline widened hyphae. Fig. 16. Direct view on colony surface of CBS 147'64, long chains of dark conidia.

147'64, *S. thermophilum* CBS 183'81, M7'7, M7'8, M7'5'2, M9'6, T104'1'1, 19'8 (CBS 671'88), 122'2, 144'4a, 170'1'1, 170'2'1, 178'1 and T49'6'1; type 2 also having short terminal chains of conidia are M7'5'1 (CBS 623'91), 244'5'3, 15'1 (CBS 619'91), 170'2'2 and T49'6'1; type 2 showing the atypical purple development are 244'2'5, 201'1'1 (CBS 618'91), 275'6 and T49'5'1 (CBS 620'91).

No phialides or phialoconidia of the *Humicola* type were observed in any isolate of *S. thermophilum sensu lato*. In addition no teleomorph has been found either in individual cultures on compost or on agar media, or in crossing experiments on yeast–glucose agar. Isolates CBS 183'81, IMI 121649 and M7'5'1 (CBS 623'91) grew well on malt agar (pH 5'6); possibly they have a low pH optimum. M7'5'1 grew very rapidly on sterile compost and on yeast–glucose agar and might have an intrinsic high growth rate. *S. thermophilum* grows quickly at elevated temperatures up to 50 °C, it prefers a substrate pH of about 7, and it is cellulolytic (Rosenberg, 1975, 1978; Satyanarayana, Johri & Klein, 1992).

Our observations of a large number of isolates of *Torula thermophila, Humicola grisea var. thermoidea* and *H. insolens* confirm previous reports that colony morphology varies widely and intergrades. Macroscopical and microscopical features were not correlated among the isolates. Although many isolates (types 1 and 2) may be separated on the basis of the single character of conidia in the aerial mycelium, intermediate isolates are also found (Figs 20–21). This character is insufficient for separation at the species level.
Figs 17–23. Light micrographs of conidial development in *Scytalidium thermophilum* (slide preparations except Figs 20–21; scale bars = 10 μm except Figs 20–21 = 50 μm). Fig. 17. Type culture of *Humicola grisau* var. *thermoidea* ATCC 16453, single conidia (type 1). Fig. 18. M9:3 single conidia (type 1). Fig. 19. M9:3, single conidia together with conidial chains. Figs 20–21. Direct view on colony surface of 15:1 (CBS 622:91), short lateral chains of conidia. Figs 22–23. T49:5:1 (CBS 620:91), atypical development (see Fig. 9) showing chains of hyaline conidia; some conidia contain purple pigmentation.
Therefore we conclude that all isolates represent one single variable species, or a morphologically indistinguishable species complex. The variation in the progeny of one isolate of *S. thermophileum* (apparently of our type 1) was also recently described by Rodrigues et al. (1991).

**Generic position**

Isolates of the *Torula–Humicola* complex have been placed in the genera *Humicola*, *Torula* and *Scytalidium*. The type species of these genera (*Humicola fusca, Torula herbarum* and *Scytalidium lignicola*) are mesophilic fungi. *H. fusca* forms both phialoconidia and aseuroconidia and has hyaline vegetative hyphae (Traaen, 1914; De Bertoldi, Lepidi & Nutti, 1973; Nicoli & Russo, 1974). Dimorphic conidia are also present in the mesophilic *H. grisea* (Traaen, 1914; Fergus, 1964) but are absent in the thermophilic isolates. *T. herbarum* forms warted blastic phragmoconidia (Ellis, 1971). Austwick (1976) placed *Torula thermophila* in *Scytalidium sensu Ellis* (1971), who emphasized the dark-pigmented arthroconidia of *Scytalidium*. However, Pesante (1957) described the type species, *S. lignicola*, as having two morphs: one with dematiaceous intercalary conidia and a second with bacilliform hyaline arthroconidia. Sigler & Carmichael (1976) followed Ellis (1971), and characterized the anamorph-genus *Scytalidium* as resembling *Geotrichum* and *Mauginiella* but differing in being dematiaceous. This generic concept was adopted by several authors describing new taxa (Campbell & Mulder, 1977; Hedger, Samson & Basuki, 1982; Narain, Srivastava & Mehrutra, 1983; Morgan-Jones, Gints & Rodriguez-Kabana, 1984; Udagawa, Tominaga & Hamaoka, 1986). Recently Sigler & Wang (1990) reinstated the taxonomic value of the two anamorphs and considered the hyaline arthroconidial synanamorph as the most distinct and stable form. According to these authors, the production of the dematiaceous conidia is variable in *S. lignicola*, depending on the isolate and medium used. Sigler & Wang (1990) stated that *Scytalidium* has become quite heterogeneous, including forms having two synanamorphs and forms producing strictly dematiaceous arthroconidia, and in need of reassessment. The characters of *S. thermophileum* do not fully fit the generic concept of *Scytalidium* as proposed by Pesante (1957) and adapted by Sigler & Wang (1990), but we propose to retain *S. thermophileum* in *Scytalidium* for the time being, having no better-suited genus available for it.

As nomenclator we propose:


≡ *Torula thermophila* Cooney & Emerson, Thermophilic Fungi p. 92 (1964) (basionym)

≡ *Humicola insolens* Cooney & Emerson, Thermophilic Fungi p. 78 (1964).


Colonies growing rapidly, attaining a diameter of 9 cm within 5 d at 40°, first white, soon becoming dark green-grey to black with a white to light grey mycelial overgrowth. Vegetative aerial hyaline hyphae, (2) 4–6 μm wide, smooth walled. Conidia globose to ellipsoidal, 8–12 μm, smooth walled, thick walled, mostly darkly pigmented, borne singly or in short chains on short lateral branches, or intercalary in chains. In submerged mycelium conidia are produced as chains of thick-walled, darkly pigmented, globose to cylindrical, swollen cells. Teleomorph unknown. Thermophilic. Variation may occur, and slow-growing atypical strains are sometimes found.

*S. indonesiacum* (Hedger et al., 1982) resembles *S. thermophileum*, but differs by more irregularly shaped conidia. Both taxa are thermophilic and produce similar dark conidia by fragmentation of undifferentiated hyphae. *S. indonesiacum* does not grow on compost but seems to be growth-promoting towards *A. bisporus* on compost agar like *S. thermophileum*. *S. thermophileum* is such a variable species that the position of *S. indonesiacum* should be re-evaluated. However, the only isolate of *S. indonesiacum* available, isolated from Indonesian soil, is deteriorating, and such a re-evaluation must await new isolates.

*S. thermophileum* can be easily distinguished from another common thermophile *Thermomyces lanuginosus* (≡ *Humicola lanuginosa*). This species grows rather slowly, and colonies have a reddish-brown colour. Conidia are thick walled, dark and ornamented, and are produced as solitary blastic terminal propagules on short differentiated stalk cells. Isolates CBS 153·75 and CBS 152·75 belonging to the unpublished taxon *Humicola brevis* var. *thermoidea* and *H. brevispora*, proved to be identical with *T. lanuginosus*. *T. lanuginosus* mostly occurs early in the fungal succession in mushroom compost, it does not promote growth of *A. bisporus*, its temperature optimum is rather high and it is not cellulolytic.

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**REFERENCES**


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