

The identity of the lipstick mould of cultivated mushrooms, *Agaricus bisporus*

Martmari van Greuning and Albert Eicker¹

Department of Botany, University of Pretoria, Pretoria 0002, Republic of South Africa

(Received November 24, 1990; Accepted December 4, 1990)

Abstract. Lipstick mould or red geotrichum, as it is commonly known among mushroom growers, is a competitive weed mould of *Agaricus bisporus*. In literature lipstick mould is associated with either *Geotrichum* sp., *G. candidum*, *Sporendonema* sp. or *S. purpurascens*. Certain authors regard the two genera as synonyms. Studies of spore ontogeny by means of light and electron microscopy showed clear distinctions between *Geotrichum* spp. and *Sporendonema* spp.. Conidia of *Sporendonema* spp. are formed entero-arthrally, whereas *Geotrichum* spp. form exo-arthric conidia. Isolates of the lipstick mould were positively identified as *Sporendonema purpurascens*.

Key words: *Agaricus bisporus*; Lipstick mould; Weed mould.

Introduction

Lipstick mould or red geotrichum is well known among commercial mushroom growers as a competitive and inhibitory weed mould. Its presence may be an indication of relatively high nitrogen levels in the compost. Growth of this mould is favoured by excessively wet compost and casing soil (Betterley, 1983; Geels *et al.*, 1988). On South African mushroom farms lipstick mould occurs more often on the casing soil which indicates improper pasteurization of the soil. However, it was also noticed in the compost.

Lipstick mould appears as a thick, white to yellowish mouldy fluff between the lumps of the casing soil from where it spreads over the sporocarps, causing their death. The infected mushrooms turn brownish and eventually cherry red, hence the name 'lipstick mould'. Growth of the lipstick mould is more restricted than that of cobweb mould (*Cladobotryum mycophilum*), which it closely resembles.

Apparently much confusion prevails concerning

the identity of lipstick mould. Several authors regard the fungus as *Geotrichum candidum* Link, whereas others only refer to the genus *Geotrichum* (Kneebone and Merek, 1959; Wuest, 1965). In 1949 this mould was assigned by the Commonwealth Mycological Institute (CMI) to the genus *Sporendonema* (Anonymous, 1949). Wood (1957) was the first to suggest that the spore type rather resembles *Sporendonema purpurascens* (Bon.) Mason & Hughes. This was accepted by Fletcher (1989) and Geels *et al.* (1988). Some authors regarded *S. purpurascens* as synonymous with the genus *Geotrichum* (Betterley, 1983; Harvey, 1982; Sinden, 1971; Vedder, 1978).

Isolates of *Geotrichum* spp. are variable in morphology and cultural characters which led to the description of several species. Carmichael (1957) listed 55 synonyms for *G. candidum* (the type species), including *Coprotrichum purpurascens* Bon. and *G. purpurascens* (Bon.) Sacc.. Sigler and Carmichael (1976) suggested that *G. purpurascens* should rather be included in the genus *Sporendonema*. Consequently *C. purpurascens* and *G. purpurascens* are regarded synonymous of *S. purpurascens* (Sigler and Carmichael, 1976).

Both *G. candidum* and *S. purpurascens* produce

¹ To whom correspondence should be addressed.

arthroconidia and are classified in the subdivision Deuteromycotina. *Geotrichum candidum* is the anamorph of the genus *Dipodascus*, a hemiascomycete and member of the family Dipodascaceae. No teleomorph of *S. purpurascens* is known (Domsch *et al.*, 1980; Sigler and Carmichael, 1976).

In an attempt to clarify the uncertainty about the identity of lipstick mould, a comprehensive study of the taxonomic history of both species, *G. candidum* and *S. purpurascens*, was undertaken. The micromorphology, conidial ontogeny and cultural characters of isolates of both species, as well as a local isolate from lipstick mould were studied. The results are reported in this paper.

Materials and Methods

Materials

Four isolates designated as lipstick mould, as well as a sample obtained from fresh material were investigated. The isolates, their origin, as well as their culture collection numbers are given in Table 1.

Methods

Cultural characteristics were studied by growing the fungi on potato dextrose agar (PDA) incubated for seven days at 25°C. A modified slide culture technique was used to make preparations of fungi for light microscopical study (Coetzee and Eicker, 1990).

A cryoscanning technique was applied to observe the fungi electron microscopically by using a modified

procedure suggested by Hamilton-Atwell and Jooste (1988). Special aluminium stubs (15 mm in diameter) were machined in a cup shape (Fig. 1a). The stubs were autoclaved and a thin, 1 mm layer of agar medium aseptically poured into each cup and inoculated. To ensure a humid environment, the stubs were incubated in petri dishes with agar from which a square piece of agar was removed from the centre to serve as a seat for the stub (Fig. 1b). After sufficient growth the stub was fixed to the end of a transfer rod by means of which the stub with the fungus was immersed into liquid nitrogen (less than -196°C). The frozen specimen was transferred to the preparation chamber, evacuated, kept at a temperature of -160°C, and sputter coated with gold. The specimen was then moved through a gate valve to the cooled stage for scanning electron microscopy, observation and photography. A Jeol JSM 840 SEM with Bio Rad E7400 sputter coater was used.

Results

Cultural Characteristics

Colonies of the isolate from Hauser and the isolate DC 36(a) were fast growing and reached an average diameter of 40 mm within five days. Colonies remained white on PDA. The consistency was butyrous and the surface velvety. When disturbed, even the driest appearing colonies looked slimy.

Sporendonema purpurascens, as well as isolate DC 36(b) grew very slowly, the colonies reaching 11 mm in diameter after seven days. Colony surface covered with

Table 1. Investigated isolates, their origin and isolate numbers

Fungus designated as	Origin	Isolate number	PRU(M)* number
<i>Geotrichum</i> sp.	Pennsylvania ¹	DC 36(a)	28
<i>G. candidum</i>	Hauser ²	NA*	220
<i>Geotrichum</i> sp.	Pennsylvania	DC 36(b)	156
<i>Sporendonema purpurascens</i>	Baarn ³	CBS 425.51	366
<i>S. purpurascens</i>	Pretoria	Studied from fresh material	

*PRU(M) Number = isolate number of the culture collection at the University of Pretoria; NA = isolate number not available.

¹Department of Plant Pathology, The Pennsylvania State University, Pennsylvania, USA.

²Hauser Champignonkulturen AG, Gossau-Zürich, Switzerland.

³Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

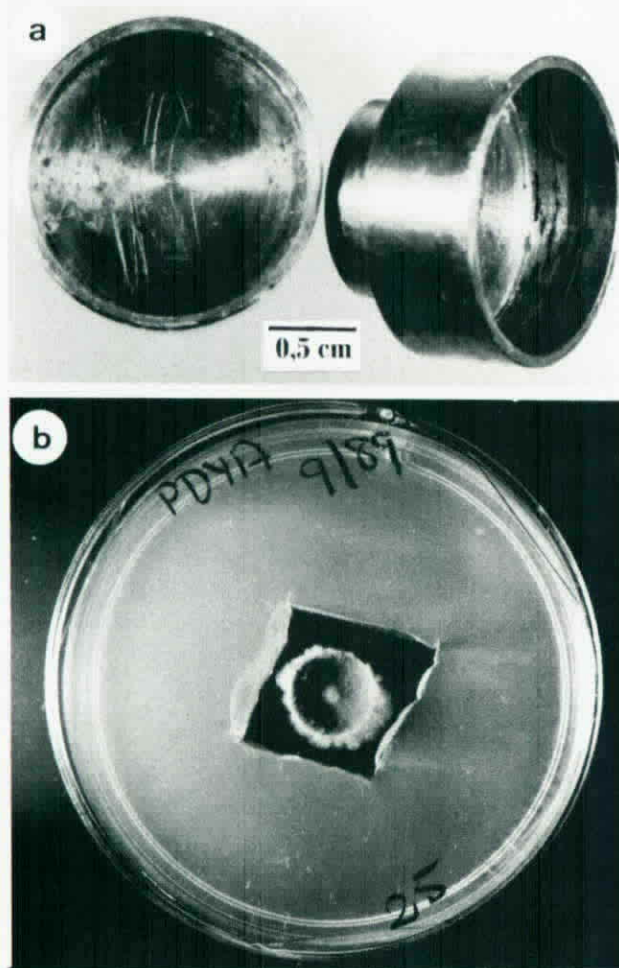


Fig. 1. a. Aluminium stubs to grow samples for electron microscopical observation; b. Fungus inoculated in a stub containing a thin layer of agar and incubated in a petri dish with agar.

white to yellowish cottony mycelium which slowly turned light rose-pink and more dense after 15 days' incubation.

Microscopic Characters

Isolates DC 36(a) and Hauser were characterized by the segmentation of hyphae to produce chains of hyaline arthroconidia. Formation of arthroconidia was exogenous in which case the outer wall of the hyphae broke down with the formation of transverse septa. Conidia remained cylindrical in shape or became barrelshaped (Fig. 2a and b).

Arthroconidia of *S. purpurascens*, isolate DC 36(b)

and the Pretoria isolate, developed endogenously within the outer wall of the hypha. A distinguishing characteristic of enteroarthric development is the conversion of particular hyphal compartments into conidia, while other cells undergo autolysis (Fig. 2c and d). Conidia are released by breakdown of the outer wall of the empty compartments.

Conclusion

No record could be found in the literature of the isolation of *Geotrichum* species from mushroom beds. Isolates of *Geotrichum* were obtained from many other sources such as milk, cheese, skin, faeces, soil etc., while isolates of *Sporendonema* were obtained from mushroom beds (Carmichael, 1957; Sigler and Carmichael, 1976). It does not seem appropriate to refer to *Geotrichum candidum* as lipstick mould because it remains white in pure culture. In contrast, *S. purpurascens* initially produces cottony white mycelium which later turns rose-pink. The epithet *purpurascens* refers to the original description of Bonorden in 1851 of the purple or pinkish hyphae and conidia.

Geotrichum candidum produce arthroconidia exo-arthrically and differs from *S. purpurascens* in which case conidium ontogeny is described as entero-arthric (Cole and Kendrick, 1969; Cole and Samson, 1979). Microscopic characters and conidium ontogeny of the culture of *S. purpurascens* from Baarn correspond with the descriptions given for this species by Cole and Kendrick (1969). The isolate DC 36(b) fit the descriptions given for *S. purpurascens* rather than *G. candidum* as it was designated. The fungus studied as lipstick mould from fresh material was positively identified as *S. purpurascens*. Isolate DC 36(a) and the isolate from Hauser resembles *G. candidum*, a fungus which is not associated with mushroom crops. This only proves the confusion which is probably attributable to its earlier incorrect identification as a *Geotrichum* sp. and secondly to the common name 'red geotrichum' adopted by some mushroom growers. Since the correct identification for lipstick mould is *S. purpurascens*, the name 'red geotrichum' is misleading and its use should be stopped.

Acknowledgements. Dr. G. C. A. van der Westhuizen is thanked for critically reading the manuscript. The South African FRD and the University of Pretoria is thanked for financial support.

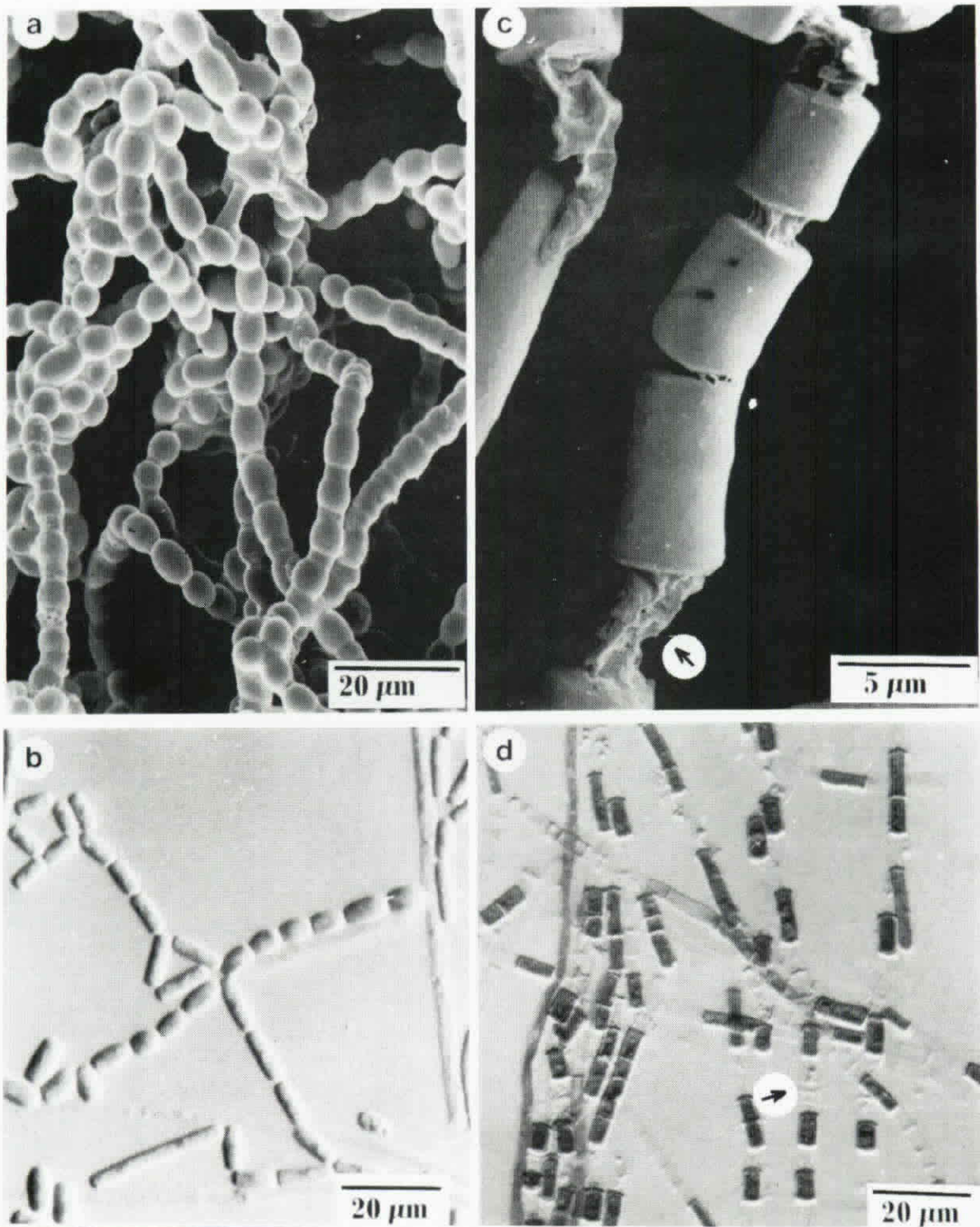


Fig. 2. (a and b) *Geotrichum candidum*. a. An electron micrograph illustrating exogenous formation of chains of arthroconidia in which case the outer wall of the hyphae breaks down with the formation of transverse septa; b. Conidia remain cylindrical or become barrel-shaped or ellipsoidal. (c and d) *Sporendonium purpurascens*. c. Endogenous development of arthroconidia within the outer wall of the hypha; d. Certain hyphal compartments are converted into conidia, while other cells undergo autolysis (arrow).

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洋菇栽培上紅地霉之鑑定

Martmari van Greuning and Albert Eicker

Department of Botany, University of Pretoria
Pretoria 0002, Republic of South Africa

蕈類培育者所熟知的紅地霉為洋菇的競爭性有害黴菌之一。在文獻上，紅地霉或歸類於 *Geotrichum* sp., *G. candidum*, *Sporendonema* sp., 或 *S. purpurascens*。某些作者將這兩屬視作同物異名。以光學顯微鏡和電子顯微鏡研究其孢子之個體發育，發現 *Geotrichum* (地霉屬) 和 *Sporendonema* (絲內霉屬) 有明顯的差異。絲內霉屬的分生孢子具內生關節，而地霉屬則產生具外生關節的分生孢子。所以，分離出來的紅地霉毫無疑問地應為 *Sporendonema purpurascens*。