

ASPERGILLUS PERSII: A NEW SPECIES IN CIRCUMDATI SECTION

Mirca ZOTTI and Aurora MONTEMARTINI CORTE

Dipartimento per lo Studio del Territorio e delle sue Risorse (DIP.TE.RIS.)
Università degli Studi di Genova - Corso Dogali 1/M Genova, Italy
E-mail: Mirca.Zotti@klaatu.com.dist.unige.it, amontem@unige.it

Key Words: *Aspergillus*, opportunistic fungi, onychomycosis.

Abstract

The paper deals with a new species of *Aspergillus* section *Circumdati*, an important group within the genera *Petromyces* (Malloch & Cain, 1972) and *Neopetromyces* (Frisvad & Samson, 2000) and within the anamorph genus *Aspergillus*. This species was isolated from a woman affected by onychomycosis. Some of the characters observed, such as the sclerotia and club-like enlargements, are quite peculiar and suggested that a new species *A. persii*, should be proposed.

Furthermore, colonies were cultivated on five different media and kept at different temperatures. Their morphological characters were analyzed and compared with those of the *Terrei* and *Flavipedes* sections.

Introduction

The incidence of opportunistic fungal infections, as in the case of onychomycosis, has increased drastically due, for instance, to resistance to antifungal agents. It has enabled the existence of new species of fungi never observed before to be highlighted. The isolation of a new species and the study of its morphological characteristics are very interesting issue, not only from a taxonomic point of view, but especially for determining its sensitivity towards some antifungals.

This paper discusses a species of *Aspergillus* isolated by Agostino Persi in January 1999 from a patient who was affected by onychomycosis of the great toe nail.

The original culture was grown on modified Sabouraud medium (see below). The general morphological characters matched those of genus *Aspergillus* (Raper and Fennell, 1977; Christensen, 1982; Klich and Pitt, 1988). The fungus was cultivated on different media and at different temperatures. For each medium employed, morphological elements and their sizes were noted. Our observations carried out on this strain suggest that it belongs in section *Circumdati* (Gams et al. 1985) and is probably a new species. The assignment of a new species to a section *Circumdati* could be temporary, since this section is under revision, based both on biomolecular techniques (Varga et al. 2000a, Varga et al. 2000b) and on profiles of secondary metabolites. Using the latter kind of analysis, J.C. Frisvad (personal communication) confirmed that our strain is a new species, close to *A. sclerotiorum* Huber and to *A. melleus* Yukawa (= *A. quercinus* (Bainier) Thom & Church, Raper and Fennell, 1977).

Material and methods

The fungus was isolated at the Section of Tropical Dermatology-University of Genoa and repeatedly obtained in pure culture (five times through three years).

Development at three temperatures (18-25-37 °C) was studied on the following media (Samson and Pitt, 1985): Czapek Agar (Cz), Czapek Yeast Extract Agar (CYA), Czapek Yeast Extract Agar with 20 % sucrose (CY20S), Malt Extract Agar (MEA); modified Sabouraud (Sab): 15% agar, 10% glucose, 5% neopepton. The most significant morphological elements were measured. The sizes are reported using three numbers corresponding, respectively, to the minimum, the average and the maximum values of a set of measurements. The color notations reported in brackets were taken from Kornerup and Wanscher (1978).

Description

Cz

Colonies 28-30-32 mm in diameter after growth for 10 days at 24-26 °C, in natural light, were medium-sized, plane, floccose with low subsuperficial margins and sparse white mycelium. They sporulated heavily and were characterized by a pale yellow to light yellow (4 A3-4) color. A light amber to yellow exudate was sometimes present. The reverse side of the colony was brownish orange (5 C4-6). Odor not distinctive.

Conidial heads 49-61.5-71 µm in diameter, radiate, but sometimes loosely columnar, reddish yellow (4 B6-7).

Conidiophores 232-388.8-637x4.4-5.5-6.9 μm , longer near the edges of the colony, slightly thick-walled, sometimes with pigmented protoplasm, echinulate, occasionally with fairly well developed bubble-like concretions.

Club-like enlargements 3-5-6x5-7.2-8 μm of the mycelium (3-3.6-4.5 μm in diameter) were observed after ten days. These enlargements resemble the "en raquette" mycelium of *Microsporium* as described by Sabouraud (1910).

Vesicle 10-15-17 μm in diameter, subglobose.

Metulae 5.5-5.7-7x3.2-3.8 μm consistently present, crowded and covering at least the upper third of the vesicle, non-septate, cylindrical to truncated conical with phialides on the larger face.

Phialides often 3-5 per metula, 6.3-6.7x2-3-3.2 μm .

Conidia smooth to delicately roughened, globose, diameter 2.7-2.8-3 μm .

Sclerotia globose, 450-540-600 μm in diameter, few or scattered, pastel to pale yellow (3 A3-4, 4 A3) thick-walled in a web of hyphae, internal composed by sclerenchyma like thick-walled cells. Sclerotia well developed after 10 days at 37°C and 15 days at room temperature.

No evidence of asci throughout a 10 weeks period.

CYA

Colonies 28-30-33 mm in diameter after 10 days at 24-26°C, deep, with few shallow radial furrows, subsuperficial irregular edges and floccose white mycelium. Sporulating abundantly from the basal mycelium and from aerial hyphae, pastel yellow to light yellow (3 A3-4, 4 A3-4). Exudate absent. Color of the reverse side of the colony brownish yellow to light or golden brown (5 B7, 5 C7). Odor not distinctive.

Conidial heads loosely columnar, sometimes radiate, pale yellow, approximately 44-48.3-49 μm , light to grayish/reddish yellow (4 A4, 4 B6-7).

Conidiophores 379-520-673x6.3-6.7-7.3 μm , in general slightly thick-walled, occasionally with light to golden brown (5 B6-7) pigmented protoplasm and with bubble-like concretions.

Club-like enlargements 4.9-6-6.5x7.3-9.6-11 μm , were observed on mycelium measuring 4.4-4.7-5.6 μm .

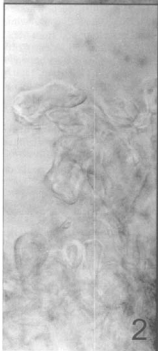
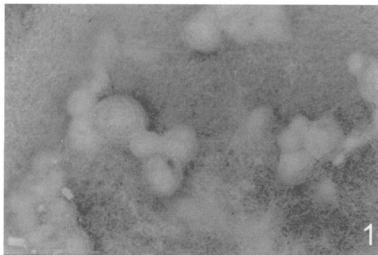
Vesicle 17-20.3-23.3 μm in diameter, subglobose.

Metulae 5.5-7.5-9x3.5-4.3-6 μm , non-septate, cylindrical to truncated conical in shape with phialides on the larger face.

Phialides 2-5 per metula, 5-5.4-7.5x2-2.1-3 μm .

Conidia globose, smooth, 2.5-3.3 μm in diameter, mostly near 2.7.

Sclerotia numerous after three weeks (Fig.1) at 37 °C, either scattered or slightly aggregated, with sizes ranging from 400 to 730 μm , cottony, with thick-walled cells similar to hülle cells (Fig.2). No evidence of asci throughout a 4-month period.



After ten days the appearance of the colonies on CYA kept at a constant temperature of 37°C was similar to colonies kept at 24-26°C, with zones characterized by white to yellowish-white mycelium (4 A2) and sparse conidial heads. Four weeks later, at room temperature, the cultures had abundant sclerotia. These were round and clustered into groups of 4-5. Their appearance was globose or subglobose (440-770 µm), relatively hard and the color was pale greyish yellow or greyish orange (4 B3-4, 5 B3-4).

After 10 days, on CY20S at 25°C, the cultures had the same features as those grown on CYA, although most of the morphological elements seemed to be more developed. Furthermore, it was possible to observe conspicuous conidiophores and a more abundant sporulation (Fig.4).

Cultures on Sab at 37°C were also examined: their development was similar to those on CYA with the exception that variance in conidial size was greater (1.5-3.1-4.9 µm).

MEA

Colonies 2.5-3-3.5 mm in diameter, after 10 days at 24-26°C, low, plane, floccose, with heavy sporulation and low, subsuperficial, transparent edges. The colonies and their reverse side were light yellow to pale yellow (4 A3-4) and grayish yellow to reddish yellow (4 B5-7) in color, respectively.

Conidial heads 50-56.3-66µm loosely columnar to loosely radiate (Fig.6).

Conidiophores 294-394-514x3.2-4.8-6.1 µm, slightly thick-walled (1µm), sometimes with small bubble-like concretions (Fig.6).

Club-like enlargements 6.1-7.7-9.4x8.6-9.4-11 µm were observed on the mycelium measuring 4.9-5.7-7.3 µm (Fig.6).

Vesicle 12-16-25 µm in diameter, subglobose to globose.

Metulae 6-7.2-8.5x 3-3.8-4.5 µm.

Phialides 6-6.5-7.5x2-2.1-2.5 µm, 3-5 per metulae (Fig.6).

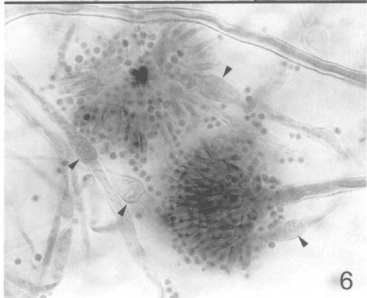
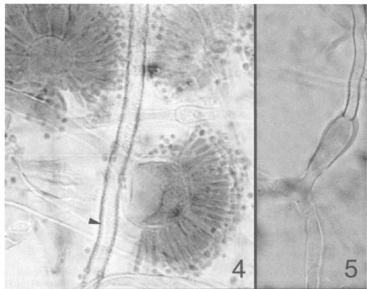
Conidia globose, 2.3-2.7-3.4 µm in diameter.

Sclerotia abundant after three weeks but scattered, cottony, slightly less hard than those observed on other media, 400 to 850 µm in diameter (Fig.3). It should be noted that there were sometimes small cottony tufts that could be considered a sort of sclerotia that had not yet developed. Growth of the colony on MEA was observed for eleven days for cultures kept at three different temperatures, specifically 35, 25, and 18 °C. Fig. 7 reports the

Fig. 1 Sclerotia in CYA after ten days at 37°C and then kept at room temperature for four weeks. (x25).

Fig. 2 Thick-walled cells from sclerotia on CYA.(x500).

Fig. 3 Not fully developed sclerotia on MEA (kept at room temperature) and loosely columnar and radiate conidial heads.(x20).



growth behavior as a function of time at the three previously mentioned temperatures.

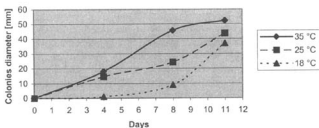


Fig.7 Growth of the colonies plotted against time for three different temperatures.

Secondary metabolites

The secondary metabolites produced by *A. persii* are penicillic acid, xanthomegnin, viomellein, vioxanthin and cyclopenol. Chiefly cyclopenol and some other unknown secondary metabolites make this new species different from other species in Circumdati section (Frisvad, personal communication).

Discussion

Based on a first observation of the colony, the colours of this *Aspergillus* are typical not only of the Circumdati section, but also characteristic of the Terrei section (Gams et al. 1985), specifically of the species *A. terreus* var. *aureus* Thom and Raper, and *A. terreus* var. *africanus* Fennell and Raper (Raper and Fennell, 1977). As concerns *A. terreus* var. *africanus*, the latter has no proper sclerotia, only sclerotium-like (pseudosclerotia) bodies, while *A. terreus* var. *aureus* has no sclerotia at all. The presence of sclerotia as well as the colour of the culture indicated that this species belongs to the section Circumdati (Raper and Fennell, 1977; Christensen, 1982; Gams et al., 1985). It should be remarked that the abundance of sclerotia varied depending on the medium employed, on the temperature and culture time.

Fig. 4 Conidial heads, club-like enlargements and conidiophores with bubble-like concretions (arrow) CY20S. (x1000).

Fig. 5 Club-like enlargements near a foot-cell on Sab. (x1000).

Fig. 6 Conidial heads, club-like enlargement (arrow) and phialides, 2-5 per metula, on MEA. (x1000).

In particular, the *Aspergillus* species under study is similar to *A. melleus*, *A. sclerotiorum* and *A. sulphureus* var. *crassus* Rai, Agarwal & Tewari.

As far as *A. melleus* is concerned, the colonies and sclerotia are similarly sized, and the surface colour of the colonies is also similar. *A. persii* resembles *A. sclerotiorum* as regards the sometimes concentric arrangement of the sclerotia. Our *Aspergillus* has thick-walled cells similar to those of *A. sulphureus* var. *crassus*, which however has larger colonies. Unlike these three species, our fungus has smaller conidiophores, conidial heads and vesicles, the elements are similar in size to those of *A. flavipes* section *Flavipedes*, the anamorph of *Fenellia flavipes* (Wiley and Simmons, 1973). Nevertheless, the presence of sclerotia and typical color of colony (yellow shade) further confirm placement in section *Circumdati*. Besides the revised section *Circumdati* of Varga et al. (2000a) includes *A. melleus*, *A. sclerotiorum* and *A. sulphureus*.

Moreover, the club-like enlargements on the large hyphae is another distinctive feature. They can be regarded as sort of not fully developed conidiophores, since one was observed near a foot cell (Fig.5). The presence of these enlargements on all media suggested that they are typical of the species and not simply a morphological alteration of an opportunistic fungus. Other species in *Aspergillus* have been recorded in onychomycosis (Gupta and Elewski, 1996; Midgely and Moore, 1996; Tosti et al., 2000;) thus confirming the opportunistic nature of this genus.

The species of *Aspergillus* causing onychomycosis are seldom identified. Often, the fungi are simply referred to as "*Aspergillus* spp.". In the literature, the *Aspergillus* species usually involved in onychomycosis and that have been identified are: *A. terreus* (Onsberg et al., 1978;), *A. niger* (Tosti and Piraccini, 1998; Ng et al., 1999;), *A. versicolor* (Torres-Rodriguez et al.1998) and *Emericella nidulans* (Ng et al., 1999 as the anamorph *A. nidulans*); none of them in *Circumdati* section.

Briefly, small size of conidial heads, vesicles and club-like enlargements are unique aspects, together with a red brown or orange brown colony reverse on Cz, CYA and MEA. These features and the secondary metabolite analysis, carried out by J.C. Frisvad, indicate that isolate represents a new species in the *Circumdati* section, with the following Latin diagnosis:

A.persii A. Montemartini Corte et M. Zotti

Coloniae in agar *Czapekii* 28-32 mm diam. post dies 10 sub calore 24-26 °C crescentes; bene fructum ferentes, sufflaviae vel luteolae (4 A3-4), facies coloniarum flocculosa vel leviter funiculosa; exudatum exiguum luteolum vel sucinum; pars postica aurea-fuscula (5 C4-6); nihil olentes.

Capitula conidica 49-71 µm diam., radiantia, sed aliquoties subcolumnaria, cinerea-luteola vel crocea (4 B6-7), biseriata; **conidiophori** plerumque 230-640 x 4.4-6.9 µm, sed paulo longiores in limite coloniarum,

membranis crassis et perlucidis interdum carunculis pustulosis increscentibus praeditis et protoplasma aliquoties pigmento praedito.

Nonnulli tumores manifesti in micelio 3-4.5 µm, claviformes, qui admonent micelium "en raquette" Microsporii (Sabouraud, 1910).

Vesiculae subglobosae 10-17 µm diam.

Metulae non septatae, cylindratae ad leviter dilatatae in culmine 5.5-7 x 3-3.8 µm.

Phialides plerumque 3-5 in una quaque metula insidentes: 6.3-7 x 2-3.2 µm.

Conidia laevia vel leviter granosa, globosa 2.7-3 µm.

Sclerotia disseminata vel leviter congregata, 450-600 µm luteola (3 A3,4, 4A 3) orientes ab hyphis coactis in capsulis sub conclavis temperie, pariete crassa lutea-fuscula et in intima parte repleta coacervatis "hulle" cellulis. Sclerotia frequentiora sunt in capsulis servatis aliquamdiu sub calore 37°C.

Hic Aspergillus suis sclerotiis et colore (flavo) pertinet ad sectionem Circumdati. Proximus est A. melleo, A. sclerotiorum et A. sulphureo var. crasso a quibus differt quod habet capitula conidica, conidiophoros et vesiculas minutiores. A. sulphureus var. crassus dissimilis est etiam mensurae maioris coloniae.

Tumores claviformia sunt alia proprietas A. persii.

Cultura tipica: MUCL³ 41970, ianuario 1999 isolata ex ungue pedis matronae a claro viro Agostino Persi.

Acknowledgements

The authors thank J.C.Frisvad for his studies on secondary metabolites, and for the reviewing of the manuscript, M.Christensen for her valuable suggestions and Silvia Della Loggia Dominici for correcting the Latin diagnosis.

References

- CHRISTENSEN M., 1982. The *Aspergillus ochraceus* group: two new species from western soils and a synoptic key. *Mycologia*, 74:210-225.
- FRISVAD J.C., SAMSON R.A., 2000. *Neopetromyces* gen. nov. and overview of teleomorphs of *Aspergillus* subgenus *Circumdati*. *Stud. Mycol.*, 45:201-207.
- GAMS W., CHRISTENSEN M., ONIONS A.H., PITT J.I. and SAMSON R.A., 1985. Infrageneric taxa of *Aspergillus*. pp.55-62. In SAMSON R.A. and PITT J.I eds.: *Advances in Penicillium and Aspergillus Systematics*. Nato ASI Series A. Vol.102; Plenum Press, New York.
- GUPTA A.K., ELEWSKI B.E., 1996. Nondermatophyte causes of onychomycosis and superficial mycoses. *Curr. Top. Med. Mycol.* 1:87-97.

3 MUCL: Mycothèque de l'Université Catholique de Louvain la Neuve; preservation: LY, FR 140, MO.

- KLICH M.A. and PITT J.I., 1988.** A laboratory guide to common *Aspergillus* species and their teleomorphs. CSIRO, North Ryde, Australia.
- KORNERUP A., WANSCHER J. H., 1978.** Methuen Handbook of Color. Ed. Eyre Methuen, London.
- MALLOCH D., CAIN R.F., 1972.** The *Trichocomaceae*: ascomycetes with *Aspergillus*, *Paecilomyces* and *Penicillium* imperfect states. Can. J. Bot. 50:2613-2628.
- MIDGLEY G., MOORE M.K., 1996.** Nail infection. Dermatol. Clin. 14:41-49.
- NG K.P., SAW T.L., MADASAMY M., SOO HOO T., 1999.** Onychomycosis in Malaysia. Mycopathologia 147:29-32.
- ONSBURG P., STAHL D., VEIEN N.K., 1978.** Onychomycosis caused by *Aspergillus terreus*. Sabouraudia 16:39-46.
- RAPER K.B., FENNELL D.I., 1977.** The genus *Aspergillus*. R.E. Krieger. Malabar, Florida.
- SABOURAUD R., 1910.** Les Tignes. pp.658-665. Masson Editeurs, Paris.
- SAMSON R.A., 1994.** Current systematic of the genus *Aspergillus*. pp.261-276. In POWELL K.A., RENWICK A., PEBERDY J.F. eds., The genus *Aspergillus*. Plenum Press New York.
- SAMSON R.A., PITT J.I., 1985.** General Recommendations. pp.455-460. In Advances in *Penicillium* and *Aspergillus* Systematic. Nato ASI Series A. Vol.102. Plenum Press, New York.
- TORRES-RODRIGUEZ J.M., MADRENYS-BRUNET N., SIDDAT M., LOPEZ-JODRA O., JIMENEZ T., 1998.** *Aspergillus versicolor* as cause of Onychomycosis: report of 12 cases and susceptibility testing to antifungal drugs. Exp. Clin. Mycol. 11:25-31.
- TOSTI A., PIRACCINI B.M., 1998.** Proximal subungual onychomycosis due to *Aspergillus niger*: report of two cases. Br. J. Dermatol. 139:156-7.
- TOSTI A., PIRACCINI B.M., LORENZI S., 2000.** Onychomycosis caused by nondermatophytic molds: clinical features and response to treatment of 59 cases. J. Am. Acad. Dermatol. 42:217-24.
- VARGA J., TOTH B., RIGO K., TEREN J., HOEKSTRA R.F., KOZAKIEWICZ Z., 2000a.** Phylogenetic analysis of *Aspergillus* section *Circumdatis* based on sequences of the internal transcribed spacer regions and the 5.8 S rRNA gene. Fungal Gen. Biol. 30:71-80.
- VARGA J., TOTH B., KEVEI E., PALAGYI A., KOZAKIEWICZ Z., 2000b.** Analysis of genetic variability within the genus *Petromyces*. Antonie van Leeuwenhoek 77:83-89.
- WILEY B.J., SIMMONS E.G., 1973.** New species and a new genus of *Plectomycetes* with *Aspergillus* states. Mycologia 65:935-939.