

NEOSARTORYA PRIMULINA, A NEW SPECIES OF FOOD-
BORNE ASCOMYCETESShun-ichi UDAGAWA¹, Noritsuna TOYAZAKI²

and

Haruo TSUBOUCHI³

- 1 Nodai Research Institute, Tokyo University of Agriculture, 1-1-1, Sakuragaoka, Setagaya-ku, Tokyo 156, Japan
- 2 Public Health Research Institute of Kobe City, 4-6, Minatojimanaka-machi, Chuo-ku, Kobe 650, Japan
- 3 Nagoya City Health Research Institute, 1-11, Hagiya-cho, Mizuho-ku, Nagoya 467, Japan

ABSTRACT

A new species of *Neosartorya*, *N. primulina* Udagawa, Toyazaki et Tsubouchi, isolated from a canned oolong tea beverage in Japan, is described and illustrated. This fungus is characterized by its restricted growth on Czapek agar, chalky-buff ascomata, and lenticular ascospores with a very irregular ornamentation composed of several narrow crests and verrucose hemispheres.

Recently some widely-distributed species of *Neosartorya* have been incriminated as spoilage agents in commercially processed fruit products (Kavanagh et al., 1963; Beuchat, 1986; Conner and Beuchat, 1987; Scott and Bernard, 1987; Gomez et al., 1989; Nielsen et al., 1989; Samson, 1989; Splittstoesser and Churey, 1989; Samson et al., 1992). In an earlier paper (Udagawa et al., 1991), *Neosartorya hiratsukae* Udagawa, Tsubouchi et Horie was reported as the new causal agent of spoilage of pasteurized aloe beverage in Japan. A variety of commercial pasteurized beverages which were undergoing spoilage were

selectively surveyed for further isolation of heat resistant. Pasteurized beverages (packaged in cans) by commercial processors for fungal isolation. Fungal mycelia were removed from the beverage by straining through a sterile membrane filter by aseptically transferred to chloramphenicol (100 µg/l)-potato dextrose agar.

During the course of this survey, several heat resistant fungi were encountered. Among them one isolate representing a hitherto undescribed species of *Neosartorya* (Malloch and Cain, 1972; Kozakiewicz, 1989; Peterson, 1992) was found which is herein described and illustrated.

Neosartorya primulina Udagawa, Toyazaki et Tsubouchi,
sp. nov. (Figs. 1, 2)

St. Anam. *Aspergillus primulinus* Udagawa, Toyazaki et
Tsubouchi, anam. sp. nov.

Coloniae in agar *Czapekii paulo restrictae*, aliquantum floccosae, plus minusve radiatim sulcatae, laxae, flavo-albae; conidiogenesis sparsa; reversum incoloratum vel pallide flavum. Coloniae in "Czapek yeast extract agar (CYA)" celeriter crescentes, velutinae, conspicue sulcatae, saepe zonatae, ex coacto mycelio basali tenuiter constantes, numerosis ascomatibus formantes, granulares, flavo-albae vel pallide flavae vel primulinae; conidiogenesis varians, viridi-grisea vel avellanea; reversum pallide flavum vel rubro-brunneum vel brunneo-aurantiacum. Coloniae in agar maltoso effusae, floccosae, parum zonatae, tenues, laxae, abundantibus ascomatibus formantes, pallide flavae; conidiogenesis inconspicua; reversum incoloratum vel pallide flavum.

Ascomata non-ostiolata, superficialia, dispersa, cretacea vel bubalina, globosa vel subglobosa, 100–250 µm diam, hyphis brunneis laxè intricatis circumdata, lente maturescentia; peridium membranaceum, "textura epidermoidea" et "textura angularis"; stratum externum ex cellulis brunneis, irregularibus, incrassatis, 5–7.5 µm latis compositum. Asci octospori, brevi-catenati, subglobosi vel plus minusve pyriformes, 12–15(–17.5) x 11–13(–15) µm, evanescentes. Ascosporae hyalinae, lenticulares, sine cristis 4.5–5(–5.5) x 3.8–5 µm, cristis numerosis angustatis irregulariter ornatae.

Capitula conidica parva, brunneo-grisea, laxè radiantia; conidiophora ex mycelio basali vel interdum hyphis aeriis ascendentes; stipites brunneoli, 200–600 x 2.5–5(–6) µm, leves et incrassati, saepe septati; vesiculae brunneae, ampulliformes, 7.5–16 µm diam. Aspergilla uniserialia; phialides cylindricae, 5–7.5 x 2–2.5 µm, in sum-

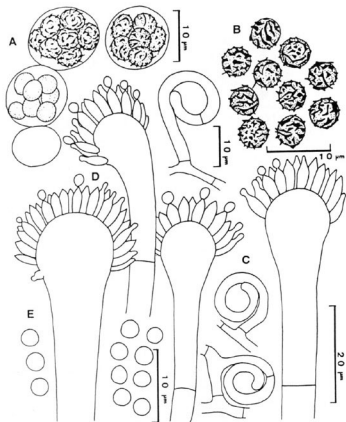


Figure 1. *Neosartorya primulina*, SUM 3014.
 A. Asci. B. Ascospores. C. Ascomatal initials.
 D. Aspergilla. E. Conidia.

ma 1/2-2/3 vesicula insidentes. Conidia hyalina, globosa vel subglobosa vel parum ovoidea, 2-3(-4.5) x 2-2.5 μ m, levia.

Holotypus SUM 3014, colonia exsiccata in cultura ex aqua decocta thea, Okinawa in Japonia, 22.vii.1991, isolata et ea in collectione fungorum Musei et Instituti Historiae Naturalis Chiba (CBM) conservata.

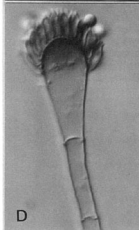
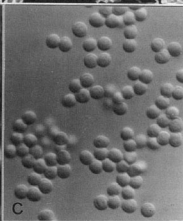
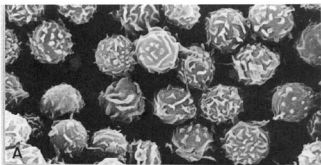
Etymology: from Latin, *primulinus*=primrose, referring to the colony color.

Colonies on Czapek agar growing rather restrictedly, attaining a diameter of 27 mm within 7 days at 25°C, somewhat floccose, more or less furrowed in a radial pattern, loose-textured, yellowish white (M. 4A2 after Kornerup and Wanscher, 1978); conidiogenesis sparse, inconspicuous; reverse uncolored to pale yellow (M. 4A3) or Straw (Rayner, 1970). Colonies on CYA growing rapidly, attaining a diameter of 46 mm within 7 days at 25°C, or 85 mm within 7 days at 37°C, velvety, deeply furrowed, often zonate, consisting of a thin basal felt intermixed with numerous ascumata in a granular appearance at 25°C, yellowish white (M. 4A2) to pale yellow (M. 3A3-4A3) or Primrose (Rayner); conidiogenesis limited or profuse at 37°C, in the latter case greenish gray to brownish orange (M. 29D2-6C4) or Hazel (Rayner); reverse light yellow to reddish brown (M. 4A4-8E5) or Dark Brick (Rayner). Colonies on malt extract agar (MEA) spreading broadly, attaining a diameter of 54-56 mm within 7 days at 25°C, or 85 mm within 7 days at 37°C, floccose, plane or slightly zonate, thin, loose-textured, characterized by abundant ascumata in granular appearance, pale yellow (M. 4A3) or Buff (Rayner); conidiogenesis moderate at 37°C but not sufficiently produced to influence the colony appearance; reverse uncolored to pale yellow (M. 4A2-3) or Pale Luteous (Rayner).

Ascomata non-ostiolate, superficial, scattered, chalky to buff, globose to subglobose, 100-250 μ m in diam, covered loosely with brownish encrusted hyphae, maturing within 21 days at 25°C; peridium 10-12.5 μ m thick, membranaceous, textura epidermoidea, two-layered; outer layer composed of brownish, irregular-shaped, thick-walled cells measuring 5-7.5 μ m wide; inner layer of hyaline, thin, flattened, angular cells measuring 6-12.5 x 4.5-7.5 μ m.

Figure 2. *Neosartorya primulina*, SUM 3014.

- A. Ascospores (SEM), x 2500. B. Asci, x 1300.
C. Conidia, x 1300. D, E. Aspergilla, x 1000 and x 1300, respectively.



Ascomatal initials developing as short branches of hyphae with large curled tips measuring ca. 12.5 μm in diam. Asci 8-spored, produced in short chains, subglobose to more or less pyriform, 12-15(-17.5) $\times$ 11-13(-15) μm , evanescent at maturity. Ascospores hyaline, lenticular, spore body 4.5-5(-5.5) $\times$ 3.8-5 μm , provided with several narrow crests measuring ca. 0.5 μm wide and with irregularly verrucose hemispheres. Conidial heads intermixed with ascomata, small, brownish gray, loosely radiate; conidiophores arising from the basal mycelium or sometimes from the aerial hyphae, stipes brownish, 200-600 $\times$ 2.5-5(-6) μm , smooth and thick-walled, often several times septate; vesicles brownish, flask-shaped, 7.5-16 μm in diam. *Aspergilla* uniseriate, phialides cylindric, 5-7.5 $\times$ 2-2.5 μm , covering the upper half to two-thirds of the vesicle. Conidia hyaline, globose to subglobose or slightly ovoidal, 2-3(-4.5) $\times$ 2-2.5 μm , smooth-walled. Maximum growth temperature: 47°C. Pasteurization at 82°C for 15 min is effective.

Specimen examined: SUM 3014 (holotype), a dried culture of an isolate from a canned oolong tea beverage, Okinawa-Pref., Japan, 22.vii.1991. The specimen studied and living culture derived from the type are preserved in the Natural History Museum and Institute, Chiba (CBM), Japan.

Neosartorya primulina can be readily recognized by its chalky-buff colored ascomata, short-stipitate initials of the ascomata, and morphology of the ascospores. The scanning electron micrograph (SEM) of the ascospores provided a much clearer differentiation between *N. primulina* and all other members of *Neosartorya* (Samson et al., 1990, 1992); the ascospore ornamentation in this species is characterized by several narrow crests in the equatorial area and irregularly verrucose hemispheres (Fig. 2). *Neosartorya hiratsukae* somewhat resembles this species in the restricted growth on Czapek agar and in the formation of pale yellow ascomata, but differs in having ascospores with convex walls bearing numerous closely anastomosing ridges arranged in a fine reticulate ornamentation (Udagawa et al., 1991).

Acknowledgement: We thank Prof. D. Malloch of the University of Toronto for reviewing this paper.

Literature cited

- Beuchat, L. R. 1986. Extraordinary heat resistance of *Talaromyces flavus* and *Neosartorya fischeri* ascospores in fruit products. *J. Food Sci.*, 51: 1506-1510.

- Conner, D. E. and Beuchat, L. R. 1987. Heat resistance of ascospores of *Neosartorya fischeri* as affected by sporulation and heating medium. *Intl. J. Food Microbiol.*, 4: 303-312.
- Gomez, M. M., Busta, F. F. and Pflug, I. J. 1989. Effect of the post-dry heat treatment temperature on the recovery of ascospores of *Neosartorya fischeri*. *Lett. Appl. Microbiol.*, 8: 59-62.
- Kavanagh, J., Larchet, N. and Stuart, M. 1963. Occurrence of a heat resistant species of *Aspergillus* in canned strawberries. *Nature (Lond.)*, 198: 1322.
- Kornerup, A. and Wanscher, J. H. 1978. *Methuen Handbook of Colour*. 3rd ed. Eyre Methuen, London. 252p.
- Kozakiewicz, Z. 1989. *Aspergillus* species on stored products. *CMI Mycological Papers No.* 161: 1-188.
- Malloch, D. and Cain, R. F. 1972. The Trichocomataceae: Ascomycetes with *Aspergillus*, *Paecilomyces*, and *Penicillium* imperfect states. *Can. J. Bot.*, 50: 2613-2628.
- Nielsen, P. V., Beuchat, L. R. and Frisvad, J. C. 1989. Growth and fumitremorgin production by *Neosartorya fischeri* as affected by food preservatives and organic acids. *J. Appl. Bacteriol.*, 66: 197-207.
- Peterson, S. W. 1992. *Neosartorya pseudofischeri* sp. nov. and its relationship to other species in *Aspergillus* section *Fumigati*. *Mycol. Res.*, 96: 547-554.
- Rayner, R. W. 1970. *A Mycological Colour Chart*. Commonwealth Mycological Institute, Kew and the British Mycological Society.
- Samson, R. A. 1989. Filamentous fungi in food and feed. *J. Appl. Bacteriol. Symp. Suppl.*, 67: 27S-35S.
- Samson, R. A., Nielsen, P. V. and Frisvad, J. C. 1990. The genus *Neosartorya*: Differentiation by scanning electron microscopy and mycotoxin profiles. In: *Modern Concepts in Penicillium and Aspergillus Classification*. Ed. Samson, R. A. and Pitt, J. I. Pp. 455-467. Plenum Press, New York.
- Samson, R. A., Hocking, A. D., Pitt, J. I. and King, A. D. (ed.) 1992. *Modern Methods in Food Mycology*. Elsevier, Amsterdam. 388p.
- Scott, V. N. and Bernard, D. T. 1987. Heat resistance of *Talaromyces flavus* and *Neosartorya fischeri* isolated from commercial fruit juices. *J. Food Prot.*, 50: 18-20.
- Splitstoeser, D. F. and Churey, J. J. 1989. Effect of low concentration of sorbic acid on the heat resistance and viable recovery of *Neosartorya fischeri* asco-

spores. J. Food Prot., 52: 821-822.

Udagawa, S., Tsubouchi, H. and Horie, Y. 1991. *Neosartorya hiratsukae*, a new species of food-borne Ascomycetes. Trans. Mycol. Soc. Japan, 32: 23-29.