Mycoscience 36: 345–351, 1995

Acremonium-like submerged conidiation in *Paecilomyces nostocoides* and *P. lilacinus*

Gen Okada[1], Noriyoshi Sakai[2] and Michio Yamagishi[2]

[1] Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan

[2] Research Center, Taisho Pharmaceutical Co., Ltd., Ohmiya-shi, Saitama 330, Japan

Accepted for publication 15 August 1995

***Paecilomyces nostocoides*, in which conidia of smaller or larger sizes appear in chains, was newly isolated from Japan. In addition to the typical *Paecilomyces*-type conidiation, the Japanese isolate showed additional *Acremonium*-like submerged conidiation in and/or on some agar media. The submerged conidiation was also observed in the ex-type strains, but not in the type specimens of *P. nostocoides*. The same submerged conidiation was observed in *P. lilacinus*, the species most similar to *P. nostocoides*. The species definitions of *P. nostocoides* and *P. lilacinus* were emended to include the submerged conidiation. *Paecilomyces*-type conidia were uninucleate in both *P. nostocoides* and *P. lilacinus*. *Paecilomyces nostocoides* and *P. lilacinus* had the Q-10(H_2) ubiquinone system.**

Key Words——*Acremonium*-like submerged conidiation; nuclear condition; *Paecilomyces lilacinus*; *Paecilomyces nostocoides*; ubiquinone.

Dunn (1983) described the species *Paecilomyces nostocoides* M. Dunn, isolated from cysts of *Heterodera zeae* Koshy, Swarup et Seth (corn cyst nematode), in which conidia of two sizes appear in chains. Superficially, the conidial chains resemble filaments of blue-green algae belonging to the genus *Nostoc* Vaucher. We isolated a similar hyphomycete from litter in Japan. The isolate showed a similar conidial arrangement to that of *P. nostocoides*, but also exhibited *Acremonium*-like conidiation in and/or on agar media. To confirm the species identity and concept of *P. nostocoides*, we compared the isolate with the type specimens and ex-type/authentic strains of the species. *Paecilomyces lilacinus* (Thom) Samson, one of the closest related species to *P. nostocoides*, was also examined. The nuclear condition of conidia and the ubiquinone system were also studied in these species.

Materials and Methods

Strain and specimen For *P. nostocoides*, JCM 8372 (originally F-5425) was isolated from litter, Sado Isl., Niigata Pref., Japan, Sept. 1989. The following type specimens and ex-type strains of the species were used for comparison: BPI 411288 (holotype, on MYP), BPI 411289 (isotype, on Difco Czapek sol. agar), BPI 411290 and 411291 (paratypes, on MYP and Difco Czapek sol. agar, respectively), JCM 8437 (=ATCC 46608, derived from the holotype), JCM 8438 (=ATCC 46609, derived from the paratype).

For *P. lilacinus*, the following ex-type and authentic strains were used: JCM 9332 (=CBS 284.36, derived from the type), JCM 8369 (=AHU 8021, =ATCC 1123, derived from the type), JCM 8370 (=AHU 8357), JCM 8371 (=AHU 8333).

Medium The strains were cultured on MYP (0.6% malt extract, 0.1% soytone, 0.05% yeast extract and 2% agar; Dunn, 1983). Some additional media were also used: Difco Czapek sol. agar (CzA; pH 6.0), Emerson's medium (YpSs, pH 6.0; Johnston and Booth, 1983), malt extract agar (MEA, Blakeslee's formula, pH 6.0; Johnston and Booth, 1983), Miura's medium (LcA, pH 6.0; Miura and Kudo, 1970), diluted (half strength) Difco oatmeal agar (OA, pH 6.0), potato-carrot agar (PCA, pH 6.0; Johnston and Booth, 1983), potato-dextrose agar (PDA, pH 5.6; Eiken, Tokyo), and Sabouraud agar (SA, pH 6.0; Eiken, Tokyo).

Light microscopy To observe the submerged conidiation with slide preparations, a small agar block was cut from an agar plate and placed upside down on a microscope slide. A thin section was then prepared from the upper surface (originally the bottom) of the agar block using a razor. The section was transferred onto a slide and covered with a drop of mounting fluid and a cover glass. Conidiation in a Petri dish was also observed directly under a long working distance objective (CF SLWD M Plan, Achromat 40×, Working distance 15.0 mm; Nikon, Tokyo).

Scanning electron microscopy For scanning electron microscopy, the fungus was fixed and dehydrated using the methods of Cole and Samson (1979). After critical-point drying with a Hitachi HCP (Hitachi, Tokyo), the material was coated with Pt-Pd in an ion sputter E-1030 (Hitachi, Tokyo) and observed with a Hitachi scanning

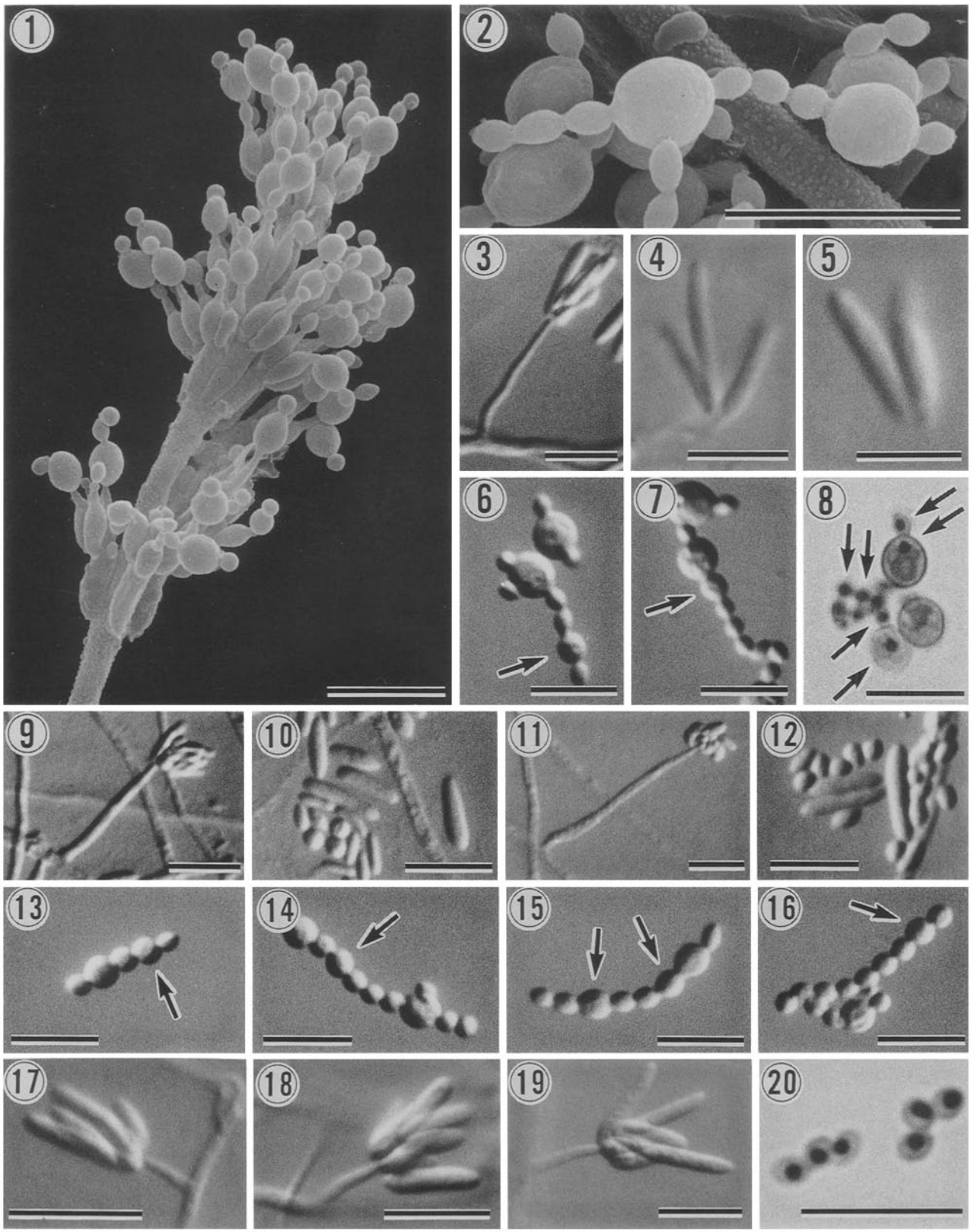

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Table 1. Dimensions (in μm) of the fertile structures of *Paecilomyces nostocoides* observed on the surface of agar media in the isolate (JCM 8372), ex-type strains, the type specimens and the data from Dunn (1983).

Strains/ Specimens/ Reference	Conidiophores		Phialides	Smaller conidia (ellipsoidal to fusiform)	Larger conidia (globose to subglobose)
	Width	Length			
JCM 8372*	1.5-3.8	<435	5.7-16.0×2.1-2.9	1.7-3.7×1.4-2.9	2.9-6.7×2.7-5.7
JCM 8437*[a]	2.0-4.2	<393	4.0-13.0×1.8-4.0	1.7-3.6×1.4-2.5	2.9-5.7×2.7-4.9
JCM 8438*[b]	1.8-3.8	<905	6.0-16.0×2.0-4.0	1.7-3.8×1.3-2.7	2.7-6.5×2.7-5.7
BPI 411288[c]	N.D.	N.D.	N.D.	1.8-3.4×1.6-2.4	2.8-5.5×2.5-4.1
BPI 411289[d]	N.D.	N.D.	N.D.	1.8-3.1×1.4-2.7	2.7-5.3×2.4-4.3
BPI 411290[e]	N.D.	N.D.	N.D.	1.8-3.7×1.4-2.7	2.5-5.1×2.0-4.3
BPI 411291[f]	N.D.	N.D.	N.D.	2.0-3.1×1.8-2.4	2.5-5.7×2.1-4.5
Dunn (1983)	2.0-2.3	<184	7.5-11.5×2.0-2.3	2.3-3.5×1.7-2.3	3.5-4.6×3.2-4.4

*: On MYP. a: Derived from the holotype. b: Derived from the paratype. c: Holotype (on MYP). d: Isotype (on Difco Czapek sol. agar). e: Paratype (on MYP). f: Paratype (on Difco Czapek sol. agar). N.D.: Not detected.

Table 2. Dimensions (in μm) of the submerged fertile structures of *Paecilomyces nostocoides* and *P. lilacinus* in the isolate (JCM 8372) and ex-type strains in agar medium (MYP).

Strains	Conidiophores	Phialides (cylindrical)	Projections from intercalary cells (peg-like)	Conidia
JCM 8372	3.4-59.0×1.0-1.6	6.8-26.4×1.0-1.4	0.6-6.0×0.6-1.0	1.9-23.8×0.9-2.7
JCM 8437[a]	1.0-46.0×1.2-1.8	4.0-22.0×1.0-1.8	0.6-6.0×0.6-1.0	1.7-14.3×0.9-2.9
JCM 8438[b]	2.0-44.2×1.2-2.2	4.8-23.0×1.0-1.9	0.6-5.0×0.6-1.2	1.7-14.0×0.8-2.9
JCM 9332[c]	8.4-53.0×1.2-2.8	4.2-29.0×1.0-2.9	0.6-5.0×0.6-1.0	1.8-14.2×1.0-3.0

a: Derived from the holotype of *P. nostocoides*. b: Derived from the paratype of *P. nostocoides*. c: Derived from the holotype of *P. lilacinus*.

electron microscope S-2500 at 20 kV.

Nuclear staining An air-dried conidial suspension on a microscope slide was treated with formaldehyde-alkaline ethanol (Williams, 1975), and the nuclei in conidia were stained in HCl-Giemsa (Colotelo and Grinchenko, 1962).

Ubiquinone Ubiquinones were extracted and analyzed according to Kuraishi et al. (1985).

Results and Discussion

On the surface of MYP, the morphological features of the Japanese isolate (JCM 8372; Figs. 1, 2, 21) agreed well with the description of *P. nostocoides* by Dunn (1983). However, the following discrepancies also existed: 1) *Acremonium*-like submerged conidiation in and/or on MYP (Figs. 3, 24, 25) and CzA; 2) the dimensions of conidiophores, phialides and conidia (Table 1); and 3) conidia intermediate between smaller and larger conidia (Figs. 6, 7, 22).

Submerged phialides and conidia of our isolate (*Acremonium*-like conidiation) were different in morphology from those on the surface of the agar media (normal *Paecilomyces*-type conidiation). Although we were unable to observe the submerged conidiation in the type specimens (BPI 411288, 411289, 411290, 411291), it was observed in the cultures derived from the type specimens (JCM 8437, 8438; Figs. 9-12; Tables 2, 3). This is the first report of the submerged conidiation of *P. nostocoides*. Table 2 shows the very similar dimensions of the submerged fertile structures in the Japanese isolate (JCM 8372) and the ex-type strains (JCM 8437, 8438) in MYP. Still unknown is the mechanism of the dimorphism between *Paecilomyces*-type and *Acremonium*-like conidiation. The latter submerged conidiation, however, was repeatedly observed even on the surface of agar media when we used diluted media (Figs. 28-31). The same conidiation was also confirmed in a decompressed condition (unpublished data). Further stu-

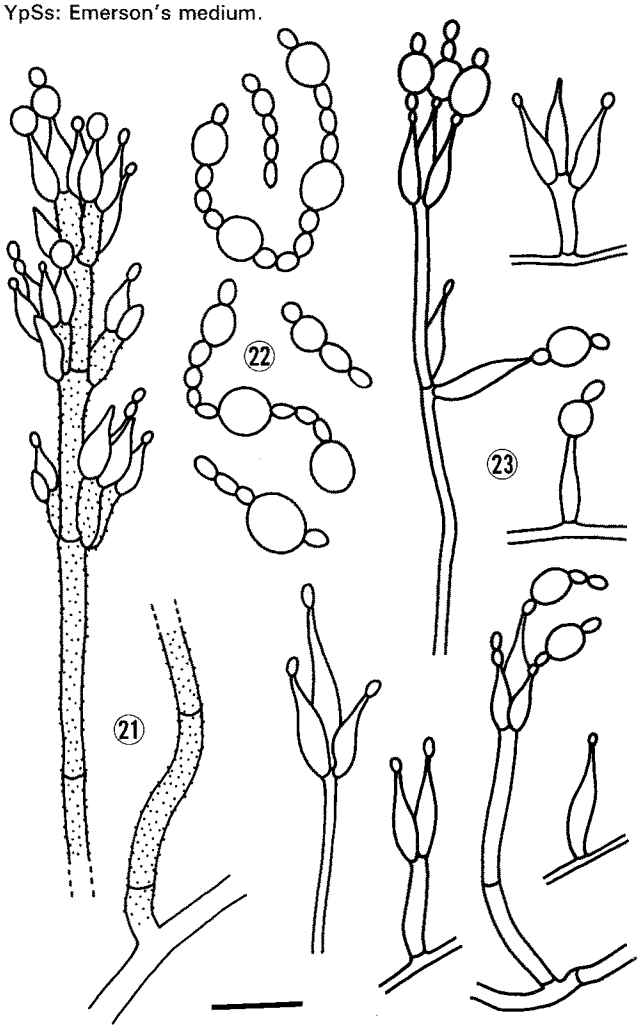
Figs. 1-16. *Paecilomyces nostocoides*.
1, 2. *Paecilomyces*-type conidiation with smaller and larger conidia (JCM 8372). 3-5, 9-12. *Acremonium*-like submerged phialides and/or conidia (3, JCM 8372 in MYP; 4, JCM 8372 in OA; 5, JCM 8372 in PCA; 9 and 10, JCM 8437 in MYP; 11 and 12, JCM 8438 in MYP). 6, 7, 13-16. Smaller, larger and intermediate (arrows) *Paecilomyces*-type conidia (6 and 7, JCM 8372; 13, BPI 411288; 14, BPI 411289; 15, BPI 411290; 16, BPI 411291). 8. Nuclei (arrows) in smaller and larger *Paecilomyces*-type conidia (JCM 8372).

Figs. 17-20. *Paecilomyces lilacinus* JCM 9332.
17-19. *Acremonium*-like submerged phialides and conidia (17, in MYP; 18, in OA; 19, in PCA). 20. Uninucleate conidia. Bars in 1-4, 6-20=10 μm, in 5=5 μm.

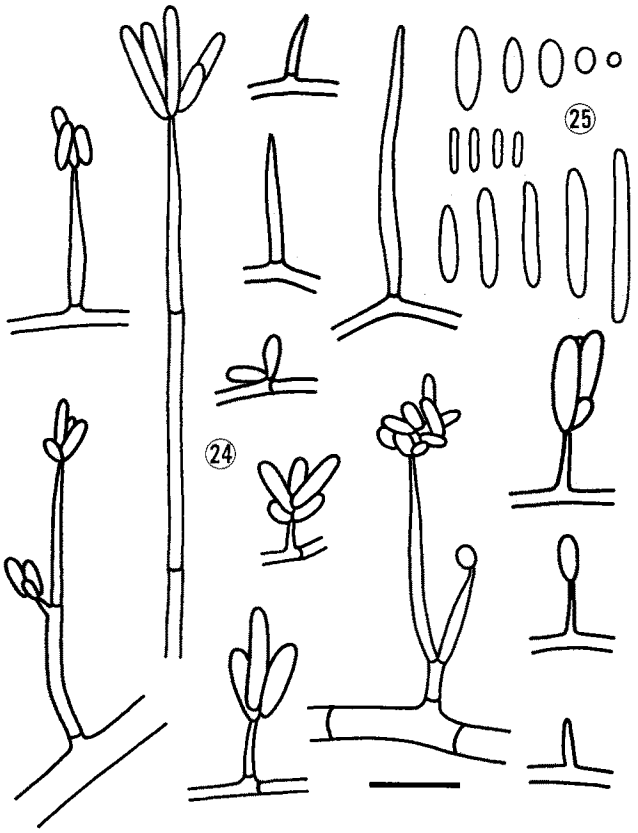
Table 3. Production of the submerged conidia of *Paecilomyces nostocoides* and *P. lilacinus* in different media.

Species/ Strains	Media								
	CzA	MEA	MYP	LcA	OA	PCA	PDA	SA	YpSs
P. nostocoides									
JCM 8372	++	++	++	+	++	+	++	++	+
JCM 8437	++	++	++	++	++	++	++	++	++
JCM 8438	+++	+++	+++	+	++	++	++	+++	+++
P. lilacinus									
JCM 8369	+++	++	++	++	+++	++	++	++	++
JCM 8370	+++	+++	++	++	+++	++	+++	+++	++
JCM 8371	+++	+++	++	++	+++	++	+++	+++	+++
JCM 9332	+++	++	++	+	+++	++	++	+++	+++

+: Scanty. ++: Moderate. +++: Abundant. CzA: Czapek sol. agar. MEA: Malt extract agar. MYP: Dunn's medium. LcA: Miura's medium. OA: Oatmeal agar. PCA: Potato-carrot agar. PDA: Potato-dextrose agar. SA: Sabouraud agar. YpSs: Emerson's medium.



Figs. 21–23. *Paecilomyces nostocoides* JCM 8372 on the surface of MYP.
21. *Paecilomyces*-type conidiation with smaller and larger conidia. 22. Smaller, larger and intermediate *Paecilomyces*-type conidia. 23. Variation of the conidiophore-phialide arrangement. Bar=10 μm.



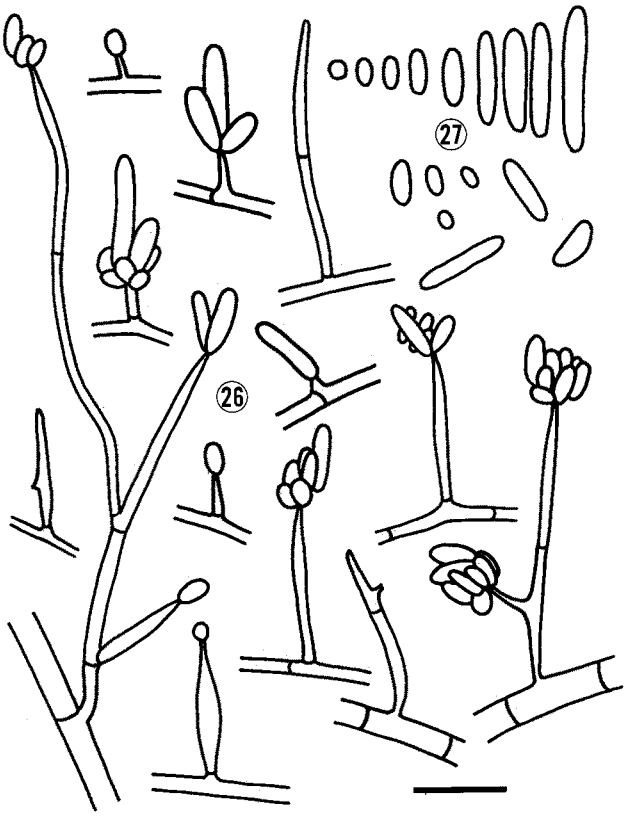
Figs. 24, 25. *Paecilomyces nostocoides* JCM 8372 in/on MYP.
24. *Acremonium*-like submerged conidiophores, phialides and conidia. 25. Variation of *Acremonium*-like submerged conidia. Bar=10 μm.

dies are required on the mechanism of this dimorphism.

In comparison with the data of Dunn (1983), much wider variations in the dimensions of the fertile structures were observed on the surface of agar medium (MYP) in the isolate, ex-type strains and type materials of *P. nostocoides* (Table 1).

The most distinctive specific feature of *P. nostocoides* is the two different sizes and shapes of conidia in the *Paecilomyces*-type conidiation. This feature clearly distinguishes *P. nostocoides* from other *Paecilomyces* species (Dunn, 1983). In the Japanese isolate, however, we sometimes found conidia intermediate in size and shape (Figs. 6, 7), and these were also observed in the holotype, isotype and paratype materials (Figs. 13–16). Therefore, our isolate agrees well with the type materials and the ex-type/authentic strains regarding the above-mentioned characteristics, and thus it is identified as *P. nostocoides*. This is the second record of *P. nostocoides* as far as we know.

The nuclear condition of smaller and larger conidia was studied using the Japanese isolate. In spite of the differences in size, both conidia were uninucleate (Fig. 8). The same nuclear condition was also confirmed by use of the fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI).



Figs. 26, 27. *Paecilomyces lilacinus* JCM 9332 in/on MYP. 26. *Acremonium*-like submerged conidiophores, phialides and conidia. 27. Variation of *Acremonium*-like submerged conidia. Bar=10 μm.

It is also noteworthy that *P. lilacinus*, the species most similar to *P. nostocoides*, also produced similar submerged phialides and conidia in and/or on different agar media (Figs. 17-19, 26, 27, 30, 31; Tables 2, 3). The conidiogenesis of *Acremonium*-like conidiation was obviously phialidic in *P. nostocoides* and *P. lilacinus* (Figs. 28-31). It is worthwhile to consider further the species delimitation between *P. nostocoides*, *P. lilacinus* and *P. marquandii* (Massee) S. Hughes (Domsch et al., 1980; Dunn, 1983). As an incidental result, the culture (JCM 5648, =AHU 8022) originally named as *Penicillium luteum* Zukal was identified as *P. lilacinus* because of the *Acremonium*-like and *Paecilomyces*-type conidiation.

The major ubiquinone of *P. nostocoides* (JCM 8372, 8437, 8438) and *P. lilacinus* (JCM 8369, 8370, 8371, 9332) was Q-10(H_2). According to Samson (1974), *P. nostocoides* and *P. lilacinus* belong to sect. *Isarioidea* Samson. Most of the species in this section, except *P. puntonii* (Vuill.) Nannizzi, have Q-10(H_2) as the major ubiquinone (pers. comm. from H. Kuraishi).

We finally emend here the species definitions of *P. nostocoides* and *P. lilacinus* to include *Acremonium*-like submerged conidiation and give below the species descriptions based mainly on the submerged conidiation in MYP.

Paecilomyces nostocoides M. Dunn, Mycologia **75**: 179. 1983. Figs. 1-16, 21-25, 28, 29

Conidiophores arising from submerged or aerial hyphae, typically with penicillate branching at several levels, up to 905 μm long, 1.5-4.2 μm wide. Phialides borne either in whorls on conidiophores or directly on hyphae, rarely on irregularly branched short conidiophores, 4.0-16.0×1.8-4.0 μm. Conidia basically of two sizes, sometimes of intermediate size, always uninucleate; smaller conidia ellipsoidal to subglobose, rarely globose, 1.7-3.8×1.3-2.9 μm; larger conidia somewhat regularly spaced along the conidial chain, subglobose to globose, 2.5-6.7×2.0-5.7 μm.

In MYP, phialides and conidia of different morphology from those on the surface of the medium. Conidiophores arising from submerged hyphae, straight or simply branched, sometimes integrated with submerged hyphae, 1.0-59.0×1.0-2.2 μm. Phialides borne directly on submerged hyphae or on simple conidiophores, sometimes *Acremonium*-like, cylindrical and tapering toward the apex (4.0-26.4×1.0-1.9 μm), or sometimes reduced to pegs of intercalary cells (0.6-6.0×0.6-1.2 μm). Submerged conidia aggregated into clusters, smooth-walled, hyaline, fusiform, ellipsoidal, subglobose, or rarely globose, 1.7-16.2(-23.8)×0.8-3.0 μm.

Ubiquinone system Q-10(H_2).

Paecilomyces lilacinus (Thom) Samson, Stud. Mycol. **6**: 58. 1974. Figs. 17-20, 26, 27, 30, 31

On the surface of MYP, microscopic morphological characteristics quite similar to those in Samson (1974) and Domsch et al. (1980). Conidia uninucleate (Fig. 20).

In MYP, phialides and conidia of different morphology from those on the surface of the medium. Conidiophores arising from submerged hyphae, straight or simply branched, sometimes integrated with submerged hyphae, 8.4-53.0×1.2-2.8 μm. Phialides sometimes *Acremonium*-like, cylindrical and tapering toward the apex (4.2-29.0×1.0-2.9 μm), or sometimes reduced to pegs of intercalary cells (0.6-5.0×0.6-1.0 μm). Submerged conidia aggregated into clusters, smooth-walled, hyaline, fusiform, ellipsoidal, subglobose, or rarely globose, 1.8-14.2×1.0-3.0 μm.

Ubiquinone system Q-10(H_2).

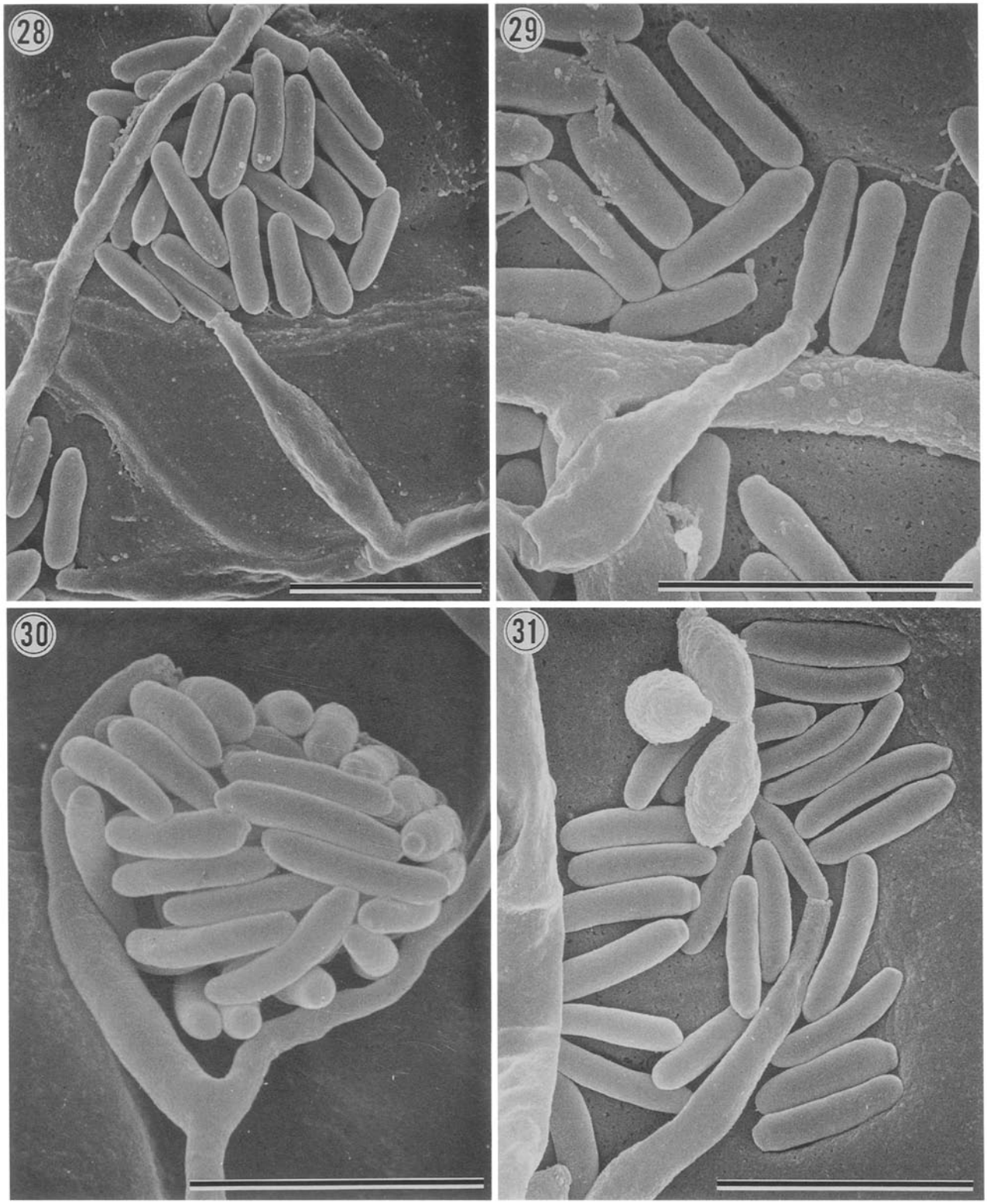
Acknowledgements——For the supply of specimens and cultures, we are grateful to the curators of the BPI herbarium and the ATCC and AHU culture collections. We would also like to thank Drs. K. Ando and H. Kuraishi for information on nuclear staining and quinone systems, respectively. The manuscript was critically reviewed by Drs. K. A. Seifert (Ottawa) and W. Gams (Baarn), and we thank them for their comments.

Literature cited

Cole, G. T. and Samson, R. A. 1979. "Patterns of development in conidial fungi," Pitman, London. 190 p.

Colotelo, N. and Grinchenko, A. H. H. 1962. Growth of *Kabatiella caulivora* on different media. Can. J. Bot. **40**: 439-446.

Domsch, K. H., Gams, W. and Anderson, T.-H. 1980. "Com-



Figs. 28–31. *Acremonium*-like phialidic conidiation on the surface of agar media.
28, 29. *Paecilomyces nostocoides* JCM 8372 on diluted (one-fourth strength) CzA (pH 6.0). 30, 31. *P. lilacinus* JCM 9332 on diluted (half strength) LcA (pH 6.0). Bars = 10 μm.

pendium of soil fungi, Vol. 1," Academic Press, London. 859 p.

Dunn, M. T. 1983. *Paecilomyces nostocoides*, a new hyphomycete isolated from cysts of *Heterodera* zeae. Mycologia **75**: 179–182.

Johnston, A. and Booth, C. (ed.) 1983. "Plant pathologist's pocketbook, 2nd ed.," Commonwealth Mycol. Inst., Kew. 439 p.

Kuraishi, H., Katayama-Fujimura, Y., Sugiyama, J. and Yokoyama, T. 1985. Ubiquinone systems in fungi I. Distribution of ubiquinones in the major families of ascomycetes, basidiomycetes, and deuteromycetes, and their taxonomic implications. Trans. Mycol. Soc. Japan **26**: 383–395.

Miura, K. and Kudo, M. Y. 1970. An agar-medium for aquatic Hyphomycetes. Trans. Mycol. Soc. Japan **11**: 116–118. (In Japanese.)

Samson, R. A. 1974. *Paecilomyces* and some allied hyphomycetes. Stud. Mycol. **6**: 1–119.

Williams, P. G. 1975. Evidence for diploidy of a monokaryotic strain of *Puccinia graminis* f. sp. *tritici*. Trans. Br. Mycol. Soc. **64**: 15–22.