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Short Communication

Identification of *Penicillium* sp. NR 6564 and taxonomic notes on *P. janthinellum*

Shoko Matsukuma, Yuri Nishizuka, Junko Watanabe and Toru Okuda

Department of Microbiology and Taxonomy, Nippon Roche Research Center, 200 Kajiwara, Kamakura 247, Japan

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The fungal strain NR 6564, which produces a new series of antifungal antibiotics, Ro 09-1470 and its congeners, was identified as *Penicillium janthinellum* from its cultural and morphological properties. The identification was confirmed by analysis of the ubiquinone system and DNA-DNA hybridization.

Key Words——DNA-DNA hybridization; *Penicillium janthinellum*; *P. simplicissimum*; ubiquinone.

Penicillium sp. NR 6564, a strain producing a new series of antifungal antibiotics comprising Ro 09-1470 and its congeners, was isolated from a soil sample collected in Hong Kong in 1987 by a serial dilution method on a modified malt extract agar (Matsukuma et al., 1992). Ro 09-1470 is the first natural antifungal compound to inhibit the P450 lanosterol C-14 demethylase of fungi (Aoki et al., 1992).

We examined cultural and morphological properties of NR 6564 according to Pitt (1979, 1988), in comparison with several reference strains. The reference strains used were *P. janthinellum* Biourge IMI 40238, IMI 71625, FRR 1893, FRR 1962, FRR 513, IMI 68213 and *P. simplicissimum* (Oudem.) Thom IMI 40032, IMI 166578, FRR 1859, FRR 89, FRR 725. These strains were obtained from the following culture collections: ATCC (American Type Culture Collection, Rockville, MD, U.S.A), FRR (CSIRO, Division of Food Processing, North Ryde, N.S.W., Australia), and IMI (International Mycological Institute, Egham, Surrey, U.K.).

Table 1 shows the cultural properties of strain NR 6564 and six strains of *P. janthinellum*. After we had revived the sporulation ability of IMI 40238 (Nishizuka et al., 1993), we found that NR 6564 formed characteristic colonies on CYA and MEA, dull red soluble pigments, darker reverse side color, and no germination of conidia at 5°C on CYA, indicating a closer afffinity to IMI 40238 (a strain derived from the neotype) than to the other reference strains.

Table 2 shows the morphological properties of NR 6564 and six other strains of *P. janthinellum.* NR 6564 characteristically formed smooth, thin-walled stipes. The penicilli were biverticillate. The metulae were mostly divergent but sometimes compact and parallel. The phialides were ampulliform, gradually tapering to collula that were usually long and slender but occasionally very short. The conidia were most often subglobose to broadly ellipsoidal, sometimes pyriform with apiculate ends, with smooth to finely roughened walls (Fig. 1). Although there were considerable variations in penicillus-type, phialide neck length, and conidial shape and surface ornamentation among the strains examined, NR 6564 most closely resembled IMI 40238. In addition, the morphological and cultural characteristics of NR 6564 clearly differed from those of *P. simplicissimum* (data not shown).

As described above, NR 6564 showed closer resemblance to *Penicillium janthinellum* IMI 40238 and IMI 71625 than to the other references strains. However, discrepancies were considerable from strain to strain in this species. Pitt (1979, 1988) described *P. janthinellum* as being one of the *Penicillium* species that is difficult to define, because it shows great variation. The nomenclature treatment is also confusing. *Penicillium janthinellum* has been treated as a synonym for *P. simplicissimum* (Oudem.) Thom (Stolk and Samson, 1983; Fassatiová and Kubatová, 1990; Frisvad and Filtenborg, 1990).

Fig. 1. *Penicillium* sp. NR 6564 (Bar: 10 μm).

Table 1. Cultural properties of *Penicillium* sp. NR 6564 and *P. janthinellum*.

	Penicillium sp. NR 6564	*Penicillium janthinellum*					
		IMI 40238	IMI 71625	FRR 1893	FRR 1962	FRR 513	IMI 68213
CYA 25°C*	39-43	25-26	18-20	34-37	35-37	23-29	22-26
MEA 25°C*	43-45	38-42	27-30	39-41	49-50	60-62	31-37
G25N 25°C*	7-11	10-11	7-9	16-17	17-18	14-15	12-13
5°C on CYA*	0	0	0	7	5-6	germination	germination
37°C on CYA*	germination	12-13	germination	12-13	10-13	4-5	13
CYA							
Conidial mass color	light graysh green	not observed	pale blue green	pale bluish green	pale blue green	not observed	grayish green
Mycelial color	pale pink	white-cream	white	white	white	pale yellowish brown	white
Reverse color	dard reddish brown	dark yellow	brownish olive	light yellow	yellowish brown	dull yellowish orange	yellowish gray
Soluble pigment	dull red	none**	none**	none	none	none	none
MEA							
Conidial mass color	pale blue green	pale blue green	poor	greenish gray	grayish green	pale blue green	pale green
Mycelial color	white-pale yellow	white	white	white	white	white	white
Reverse color	brownish olive	dull yellow	dull yellow	pale yellowish green	yellowish white	pale yellow	yellowish white
Soluble pigment	none	none	none	none	none	none	none

* Colony diam are expressed in mm after 7 days. ** Dull red pigmentation appeared after 14 days.

We, therefore, applied chemotaxonomic and molecular taxonomic methods to support our identification.

Ubiquinone analysis by high-performance liquid chromatography was carried out by the method of Kuraishi et al. (1985). In the ubiquinone system, Kuraishi et al. (1991) report that almost all the species belonging to the subgenus *Furcatum* have Q-9, whereas the species belonging to the subgenus *Biverticillium*, except for the section *Coremigena*, have Q-10(H_2). We confirmed that NR 6564 belongs to the subgenus *Furcatum*, because it contained Q-9 as a major ubiquinone type.

DNA-DNA hybridization was performed by the microtiter plate method according to Ezaki et al. (1989). The hybridization temperature was determined as 45°C based on the Tm. Tm values of the DNAs purified from NR 6564, *P. janthinellum* FRR 1893, IMI 68213, and *P. simplicissimum* FRR 725 were 90°C, 90°C, 88°C, and 89°C, respectively.

Table 2. Morphological properties of *Penicillium* sp. NR 6564 and *P. janthinellum*.

	Penicillium sp. NR 6564	*Penicillium janthinellum*					
		IMI 40238	IMI 71625	FRR 1893	FRR 1962	FRR 513	IMI 68213
Penicillus	biverticillate to irregular biverticillate	monoverticillate to irregular biverticillate with a few metulae	monoverticillate to irregular biverticillate with a few metulae	monoverticillate to irregular biverticillate with a few metulae	monoverticillate to irregular biverticillate with a few metulae	monoverticillate	monoverticillate, rarely irregular biverticillate with a few metulae
Phialide							
Size (μm)	10.0-16.5×2.5-4.0	8.5-11.0×2.0-3.0	7.5-9.0×2.0-3.0	7.0-9.5×2.0-3.0	6.5-10.0×2.0-2.5	8.0-12.0×2.5-3.0	7.0-11.0×2.5-3.5
Shape	ampulliform	ampulliform	ampulliform	ampulliform	ampulliform	ampulliform	ampulliform
Neck	long	long	long	short	short	long	long
Wall	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Conidium							
Size (μm)	3.0-4.0×2.5-3.0	3.0-4.0×2.5-3.5	2.5-3.0×2.5-3.0	3.0-3.5×3.0-3.5	2.5-3.0×2.5-3.0	3.0-4.0×2.5-3.5	3.0-4.0×3.0-3.5
Shape	ellipsoidal with apiculate ends	ellipsoidal with apiculate ends	ellipsoidal with apiculate ends	spherical	oval	ellipsoidal	spherical
Wall	smooth	smooth	smooth	rough	smooth	spinose	spinose

Table 3. DNA relatedness between *Penicillium* sp. NR 6564 and *P. janthinellum*.

Species	Strains	% Homology with photobiotin labeled DNA						
		NR 6564	IMI 40238	IMI 71625	FRR 1893	FRR 1962	FRR 513	IMI 68213
Penicillium sp.	NR 6564	100						
P. janthinellum	IMI 40238	84	100					
P. janthinellum	IMI 71625	64	75	100				
P. janthinellum	FRR 1893	26	28	32	100			
P. janthinellum	FRR 1962	31	33	30	53	100		
P. janthinellum	FRR 513	11	13	12	9	13	100	
P. janthinellum	IMI 68213	22	34	29	33	35	14	100

Table 4. DNA relatedness between *Penicillium* sp. NR 6564 and *P. simplicissimum*.

Species	Strains	% Homology with photobiotin labeled DNA					
		NR 6564	IMI 40032	IMI 166578	FRR 1859	FRR 89	FRR725
Penicillium sp.	NR 6564	100					
P. simplicissimum	IMI 40032	34	100				
P. simplicissimum	IMI 166578	47	47	100			
P. simplicissimum	FRR 1859	25	36	38	100		
P. simplicissimum	FRR 89	29	28	34	88	100	
P. simplicissimum	FRR 725	17	5	17	28	24	100

Table 3 and 4 show the DNA-DNA relatendness between *Penicillium* sp. NR 6564 and *P. janthinellum*, and between *Penicillium* sp. NR 6564 and *P. simplicissimum*, respectively. NR 6564 showed high homology with *P. janthinellum* IMI 40238 and IMI 71625 but low homology with the other strains of *P. janthinellum* and all examined strains of *P. simplicissimum*.

Therefore, the results obtained support our identification by chemotaxonomical and molecular taxonomical methods.

Some strains, however, showed low DNA-DNA homology values within species, indicating that the species of *P. janthinellum* also varied widely at the genetic level. Although DNA-DNA relatedness by hybridization is considered a reliable method in bacterial taxonomy, it has not been so frequently used in the taxonomy of deuteromycetes. It has been speculated that the wider species concept in some deuteromycetes would make such comparisons difficult. As more data on molecular taxonomy are accumulated, such difficulties should be resolved. From our studies, however, it is clear that chemotaxonomy and molecular taxonomy provide effective tools for identifying deteriorated or poorly sporulated strains or for comparing similar strains.

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