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Re-identification of *Aspergillus fumigatus* sensu lato Based on a New Concept of Species Delimitation

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The species concept of *Aspergillus fumigatus* sensu stricto has recently been defined by polyphasic taxonomy. Based on the new concept of species delimitations, 146 worldwide strains of *Aspergillus fumigatus* sensu lato were re-identified. Of those 146 strains, 140 (95.8%) could be identified as *A. fumigatus* sensu stricto, 3 (2.1%) as *A. lentulus*, and the remaining 3 strains as *A. viridinutans* complex, *Neosartorya udagawae*, and *N.* cf. *nishimurae*. Of 98 clinical strains, only 1 from dolphin nostril was identified as *A. lentulus* and not *A. fumigatus* sensu stricto. Random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) with primers PELF and URP1F produced nearly the same band patterns among 136 strains of *A. fumigatus* sensu stricto while discriminated the species from its related species. We also discussed about identification of several atypical *A. fumigatus* strains from clinical environments.

***Keywords*:** *A. fumigatus*, *A. lentulus*, re-identification, species delimitation, multilocus sequence typing

Aspergillus fumigatus Fresenius is an important human pathogen which causes several diseases including allergic bronchopulmonary aspergillosis, aspergilloma, and invasive aspergillosis, particularly in immunocompromised patients. The species also occurs as a common saprophytic fungus playing an essential role in recycling carbon and nitrogen into the soil (Rinyu *et al.*, 1995). Recently, it was reported that some clinical and soil strains included in the old concept of *A. fumigatus* (i.e., *A. fumigatus* sensu lato, which is based mainly on morphology) belonged to separate species from *A. fumigatus* sensu stricto based on multilocus sequence typing results (Balajee *et al.*, 2005a; Hong *et al.*, 2005; Samson *et al.,* 2007). Hong *et al.* (2005) arranged the taxonomy of *A. fumigatus* and related species based on polyphasic taxonomy and divided *A. fumigatus* sensu lato into 5 species; *A. fumigatus* sensu stricto, *A. lentulus* Balajee *et al.*, *A. fumigatiaffinis* Hong *et al.*, *A. novofumigatus* Hong *et al.,* and *A. viridinutans* Ducker & Thrower species complex.

The Centraalbureau voor Schimmelcultures (CBS) (Utrecht, the Netherlands) maintains a world-renowned collection of living filamentous fungi. It holds approximately 150 strains of *A. fumigatus* sensu lato identified based mainly on morphology. Of these strains, 98 originated from clinical environments. An additional 5 atypical isolates of *A. fumigatus* were isolated from clinical environments, but could not be identified and were subsequently considered as being new species (Katz *et al.,* 2005). We thought it would be interesting to re-examine the species composition of CBS strains of *A. fumigatus* sensu lato and to identify species of atypical strains based on the new concept of species delimitation (Hong *et al.,* 2005). In this study, we performed random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) and β-tubulin gene sequence analysis of CBS *A. fumigatus* sensu lato strains to identify their species. Additionally, we compared β-tubulin sequences of Katz's clinical strains (2005) with those of Hong *et al.* (2005 and 2006) to identify their species.

Materials and Methods

Strains examined are listed in Table 1. The 146 CBS *A. fumigatus* sensu lato strains originated from human (90), animal (8), soil (22), indoor environment (12), cotton yarn (1), feed (1), and unrecorded sources (12). All strains preserved in lyophilized ampoules were revived in malt pepton [10 g malt extract (Oxoid L39), 10 g Bacto pepton (Difco 0118-7), 1,000 ml dH_2O] at 25°C for 1 day, transferred into malt extract agar slants, incubated for about 2 weeks at 25°C and then maintained at 15°C.

To examine the genetic variability of *A. fumigatus* sensu lato, RAPD-PCR was performed with PELF and URP1F primers according to the methods of Hong *et al.* (2005). We chose 9 strains, including 3 which produced unusual band patterns, from *A. fumigatus* RAPD-PCR results. Partial β-tubulin genes of the selected strains were sequenced with primers bt2a and bt2b and phenotypic characters including macro- and micro-morphology, growth rate, etc. were also examined as described by Hong *et al.* (2005). A phylogenetic tree of the β-tubulin gene sequence was constructed with strains from this study as well as *A. fumigatus* sensu lato strains from Hong *et al.* (2005), atypical *A. fumigatus* strains from Katz *et al.* (2005), and type strains of additional species in *Aspergillus* section *Fumigati* including its teleomorph, *Neosartorya.*

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Table 1. Strains of *A. fumigatus* sensu lato and related species in section *Fumigati* used in this study

Species	Isolate no.[a]	GB no.[b]	Source	PELF[c]	URP1F[c]
A. fumigatiaffinis	IBT 12703[T]	DQ094885	soil, USA		
A. fumigatus	CBS 487.65	AY685149	human lung, USA	Hong *et al.*[d]	
A. fumigatus	CBS 133.61	AY685150	chicken lung , USA	"	
A. fumigatus	CBS 545.65	AY685151	unknown	"	
A. fumigatus	CBS 457.75	AY685152	soil, India	"	
A. fumigatus	CBS 542.75	AY685153	man, USA	"	
A. fumigatus	CBS 113.26	AY685154	soil, Germany	"	
A. fumigatus	CBS 110.46	AY685155	unknown	"	
A. fumigatus	CBS 120.53	AY685156	human lung, Netherlands	"	
A. fumigatus	CBS 132.54	AY685157	unknown	"	
A. fumigatus	CBS 123.59	AY685158	human sputum, Netherlands	"	
A. fumigatus	CBS 158.71	AY685159	soil, Ukraine	"	
A. fumigatus	CBS 180.76	AY685160	penguin lung	"	
A. fumigatus	CBS 143.89	AY685161	man, France	"	
A. fumigatus	CBS 148.89	AY685162	*Zea mays*, France	"	
A. fum. (→*A. lentulus*)	CBS 153.89	AY685175	citrus grove soil , USA		
A. fumigatus	CBS 488.90	AY685163	man, Netherlands	Hong *et al.*[d]	
A. fumigatus	CBS 287.95	AY685164	unknown	"	
A. fum. (→*A. lentulus*)	CBS 175.97	AY685176	dolphin nostril , Netherlands	"	
A. fumigatus	CBS 10076	AY685165	man, France	"	
A. fumigatus	CBS 109032	AY685166	HIV human, Germany	"	
A. fumigatus	CBS 386.75	AY685168	soil, India	"	
A. fumigatus	CBS 286.95	AY685169	unknown	"	
A. f. (→*N.*cf. *nishimurae*)	CBS 116047	DQ534075	unknown		
A. fumigatus	CBS 116885		tomato field soil, Korea	1	1
A. fumigatus	CBS 116887		soybean field soil , Korea	2	2
A. fumigatus	CBS 117202		human broncho alveolar lavage, Netherlands	3	3
A. fumigatus	CBS 112.33		cow fetus	4	4
A. fumigatus	CBS 113.37		pigeon lung	5	5
A. fumigatus	CBS 114.45		human lung	6	6
A. fumigatus	CBS 115.45		cotton yarn, UK	7	7
A. fumigatus	CBS 113.51		unknown	8	8
A. fumigatus	CBS 121.53	DQ534076	unknown, Netherlands	9	9
A. fumigatus	CBS 113.55		human lung, Netherlands	10	10
A. fumigatus	CBS 114.55		human lung, Netherlands	11	11
A. fumigatus	CBS 115.55		human lung, Netherlands	12	12
A. fumigatus	CBS 312.60		human sputum, Netherlands	13	13
A. fumigatus	CBS 313.60		human sputum, Netherlands	14	14
A. fumigatus	CBS 314.60		human sputum, Netherlands	15	15
A. fumigatus	CBS 315.60	DQ534077	human bronchial secretion, Netherlands		
A. fumigatus	CBS 317.60		human bronchial secretion, Netherlands	16	16
A. fumigatus	CBS 132.61		cow mastitic secretion, Greece	17	17
A. fumigatus	CBS 493.61		human lung, Netherlands	18	18
A. fumigatus	CBS 494.61		human lung, Netherlands	19	19
A. fumigatus	CBS 495.61		human lung, Netherlands	20	20
A. fumigatus	CBS 496.61		human sputum, Netherlands	21	21
A. fumigatus	CBS 497.61		human sputum, Netherlands	22	22
A. fumigatus	CBS 498.61		human ear, Netherlands	23	23
A. fumigatus	CBS 499.61		human sputum, Netherlands	24	24
A. fumigatus	CBS 500.61		unknown	25	25

Table 1. Continued

Species	Isolate no.[a]	GB no.[b]	Source	PELF[c]	URP1F[c]
A. fumigatus	CBS 501.61		human sputum, Netherlands	26	26
A. fumigatus	CBS 502.61		human sputum, Netherlands	27	27
A. fumigatus	CBS 502.62		human lung, Netherlands	28	28
A. fumigatus	CBS 504.62		human sputum, Netherlands	29	29
A. fumigatus	CBS 505.62		human thoracic liquid, Netherlands	30	30
A. fumigatus	CBS 507.62		human sputum, Netherlands	31	31
A. fumigatus	CBS 508.62		human sputum, Netherlands	32	32
A. fumigatus	CBS 509.62		human lung, Germany	33	33
A. fumigatus	CBS 510.62		human lung, Germany	34	34
A. fumigatus	CBS 511.62		man, Netherlands	35	35
A. fumigatus	CBS 512.62		man, Netherlands	36	36
A. fumigatus	CBS 513.62		man, Netherlands	37	37
A. fumigatus	CBS 514.62		human sputum, Netherlands	38	38
A. fumigatus	CBS 515.62		human sputum, Netherlands	39	39
A. fumigatus	CBS 516.62		human sputum, Netherlands	40	40
A. fumigatus	CBS 517.62		human sputum, Netherlands	41	41
A. fumigatus	CBS 518.62		human sputum, Netherlands	42	42
A. fumigatus	CBS 519.62		man, Netherlands	43	43
A. fumigatus	CBS 520.62	DQ534078	human sputum, Netherlands	44	44
A. fumigatus	CBS 521.62		human pleural tissue, Netherlands	45	45
A. fumigatus	CBS 522.62		human sputum, Netherlands	46	46
A. fumigatus	CBS 523.62		human lung secretion, Netherlands	47	47
A. fumigatus	CBS 524.62		human sputum, Netherlands	48	48
A. fumigatus	CBS 525.62		human excrement, Netherlands	49	49
A. fumigatus	CBS 594.63		man, Netherlands	50	50
A. fumigatus	CBS 596.63		human ear, Netherlands	51	51
A. fumigatus	CBS 598.63		human sputum, Netherlands	52	52
A. fumigatus	CBS 601.63		human ear, Netherlands	53	53
A. fumigatus	CBS 602.63		pony lung, Netherlands	54	54
A. fumigatus	CBS 603.63		human sputum, Netherlands	55	55
A. fumigatus	CBS 604.63		man, Netherlands	56	56
A. fumigatus	CBS 605.63		man, Netherlands	57	57
A. fumigatus	CBS 608.63		human lung, Germany	58	58
A. fumigatus	CBS 611.63		human lung, Netherlands	59	59
A. fumigatus	CBS 612.63		human sputum, Netherlands	60	60
A. fumigatus	CBS 613.63		human ear, Netherlands	61	61
A. fumigatus	CBS 615.63		man, Netherlands	62	62
A. fumigatus	CBS 617.63		man, Netherlands	63	63
A. fumigatus	CBS 618.63		man, Netherlands	64	64
A. fumigatus	CBS 619.63		human sputum, Netherlands	65	65
A. fumigatus	CBS 620.63		human sputum, Netherlands	66	66
A. fumigatus	CBS 621.63		human sputum, Netherlands	67	67
A. fumigatus	CBS 623.63		human bronchus, Netherlands	68	68
A. fumigatus	CBS 624.63		human lung, Netherlands	69	69
A. fumigatus	CBS 626.63		human sputum, Netherlands	70	70
A. fumigatus	CBS 627.63		man, Germany	71	71
A. fumigatus	CBS 628.63		human pleura, Netherlands	72	72
A. fumigatus	CBS 630.63		human lung, Netherlands	73	73
A. fumigatus	CBS 631.63		human sputum, Netherlands	74	74
A. fumigatus	CBS 632.63		human sputum, Netherlands	75	75

Table 1. Continued

Species	Isolate no.[a]	GB no.[b]	Source	PELF[c]	URP1F[c]
A. fumigatus	CBS 633.63		human lung, Netherlands	76	76
A. fumigatus	CBS 634.63		man, Netherlands	77	77
A. fumigatus	CBS 419.64		man, USA	78	78
A. fumigatus	CBS 499.64		air, Netherlands	79	79
A. fumigatus	CBS 501.64		air, Netherlands	80	80
A. fumigatus	CBS 502.64		air, Netherlands	81	81
A. fumigatus	CBS 503.64		air, Netherlands	82	82
A. fumigatus	CBS 504.64		air, Netherlands	83	83
A. fumigatus	CBS 505.64		air, Netherlands	84	84
A. fumigatus	CBS 506.64		air, Netherlands	85	85
A. fumigatus	CBS 507.64		air, Netherlands	86	86
A. fumigatus	CBS 508.64		soil, Netherlands	87	87
A. fumigatus	CBS 509.64		soil, Netherlands	88	88
A. fumigatus	CBS 510.64		soil, Netherlands	89	89
A. fumigatus	CBS 511.64	DQ534079	soil, Netherlands		
A. fumigatus	CBS 512.64		soil, Netherlands	90	90
A. fumigatus	CBS 513.64		air, Netherlands	91	91
A. fumigatus	CBS 514.64		air, Netherlands	92	92
A. fumigatus	CBS 515.64		soil, France	93	93
A. fumigatus	CBS 517.64		soil, Netherlands	94	94
A. fumigatus	CBS 518.64		soil, Netherlands	95	95
A. fumigatus	CBS 519.64		air, Netherlands	96	96
A. fumigatus	CBS 192.65		animal feed, Netherlands	97	97
A. fumigatus	CBS 229.65		man, Netherlands	98	98
A. fumigatus	CBS 712.74		unknown, Italy	99	99
A. f. (→*A. viridinutans*)	CBS 458.75	AY685178	soil, India	100	100
A. fumigatus	CBS 549.82		snake skin	101	101
A. fumigatus	CBS 144.89		man, France	102	102
A. fumigatus	CBS 145.89		man, France	103	103
A. fumigatus	CBS 146.89		man, France	104	104
A. fumigatus	CBS 149.89		*Zea mays*, France	105	105
A. fumigatus	CBS 150.89		*Beta vulgaris*, France	106	106
A. fumigatus	CBS 151.89		stone, Germany	107	107
A. fumigatus	CBS 152.89		stone, Germany	108	108
A. fum. (→*N. udagawae*)	CBS 154.89	DQ534080	citrus grove soil, USA	109	109
A. fumigatus	CBS 331.90		man, France	110	110
A. fumigatus	CBS 500.90		man, Netherlands	111	111
A. fum. (→*A. lentulus*)	CBS 612.97	DQ534081	unknown	112	112
A. fumigatus	CBS 100074		man, France	113	113
A. fumigatus	CBS 100075		man, France	114	114
A. fumigatus	CBS 100077		human sinus, France	120	115
A. fumigatus	CBS 100078		human sinus, France	115	116
A. fumigatus	CBS 100079		human ear, France	116	117
A. fumigatus	CBS 101355		unknown	117	118
A. fumigatus	CBS 101639		human lung, France	118	119
A. fumigatus	CBS 101640		human lung, France	119	120
A. fumigatus	CBS 112389	AY685148	indoor, Germany		
A. fumigatus	CBS det315-31	AY685167	unknown	Hong *et al*.[d]	
A. lentulus	CBS 117887[T]	AY738513	man, USA		
A. novofumigatus	IBT 16806[T]	DQ094886	soil, Ecuador		

Table 1. Continued

Species	Isolate no.[a]	GB no.[b]	Source	PELF[c]	URP1F[c]
A. unilateralis	CBS 126.56^T	AF057316	rhizosphere, Australia		
A. viridinutans	CBS 127.56^T	AF134779	rabbit dung , Australia		
A. viridinutans	IMI 182127	AF134777	*Pinus caribea*, SriLanka		
A. viridinutans	IMI 280490	AF134780	soil, Zambia		
N. aurata	CBS 466.65^T	AF057318	jungle soil, Brunei		
N. coreana	KACC 41659^T	AY870758	tomato soil, Buyeo, Korea		
N. fennelliae a	CBS 599.74^T	DQ11428	*Oryctolagus cuniculus* eye ball, USA		
N. fennelliae A	CBS 598.74^T	DQ114127	*Oryctolagus cuniculus* eye ball, USA		
N. fischeri	CBS 544.65^T	AF057322	canned apple		
N. glabra	CBS 111.55^T	AY685134	old tire, USA		
N. hiratsukae	CBS 294.93^T	AF057324	aloe juice, Japan		
N. laciniosa	KACC 41657^T	AY870756	tomato soil, Byeo Korea		
N. multiplicata	CBS 646.95^T	DQ114129	soil, Taiwan		
N. nishimurae	IFM 54133	AB201360	forest soil, Kenya		
N. nishimurae	IFM 53610	AB201361	unknown		
N. pseudofischeri	CBS 208.92^T	AY685142	human vertebrate, USA		
N. quadricincta	CBS 135.52^T	AF057326	cardboard, UK		
N. spathulata MT A	CBS 408.89^T	AF057327	soil, Taiwan		
N. spathulata MT a	CBS 409.89^T	AF057328	soil, Taiwan		
N. spinosa	CBS 483.65^T	AF057329	soil, Nicaragua		
N. stramenia	CBS 498.65^T	AY685166	soil, USA		
N. tatenoi	CBS 407.93^T	DQ114130	soil, Brazil		
N. udagawae	CBS 114217^T	AF132226	soil, Brazil		
N. udagawae	CBS 114218^T	AF132230	soil, Brazil		
A. fum. (→*A. lentulus*?)	MK 245	AY590128	human lung, Australia		
A. fum. (→*A. viridinutans*?)	MK 246	AY590129	cat, Australia		
A. fum. (→*A. viridinutans*?)	MK 284	AY590130	cat, Australia		
A. fum. (→*N. udagawae*?)	MK 285	AY590133	cat, Australia		
A. fum. (→*N. coreana*?)	NSW 3	AY590132	ostrich, Australia		

[a] CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; KACC, Korean Agricultural Culture Collection, Suwon, Korea; IBT, Institute for Biotechnology, Lyngby, Technical University of Denmark; IFM, Research Center for Pathogenic Fungi and Microbial Toxicose, Chiba University, Chiba, Japan; IMI, CAB International Mycological Institute, Kew, UK. ^{T} type strain
[b] GB no., GenBank accession number of partial β-tubulin gene.
[c] PELF and URP1F, RAPD-PCR lane numbers from Figs. 1 and 2.
[d] Hong *et al.*, Results of RAPD previously presented by Hong *et al.* (2005).

Results

Primers of PELF and URP1F produced multiple bands for each strain in RAPD-PCR but the band patterns were similar to each other, except a few strains (Figs. 1 and 2). Only CBS 458.75, CBS 154.89, and CBS 612.97 showed band patterns clearly different from the other strains.

CBS 458.75 clustered with *A. viridinutans* species complex and CBS 154.89 clustered with *A. udagawae* showing 100% similarity with type strains of *N. udagawae* CBS 114217^T in the phylogenetic tree of the β-tubulin gene sequence (Fig. 3). CBS 612.97 clustered into *A. lentulus* strains including CBS 175.97 from Hong *et al.* (2005) and CBS 153.89 which was not analyzed by RAPD-PCR in this study. CBS 116047 clustered with *N. nishimurae* reported recently as heterothallic *Neosartorya* (Takada *et al.*, 2001). CBS 121.53, CBS 520.62, CBS 286.95, CBS 315.60, CBS 511.64, and CBS 112389, which showed typical *A. fumigatus* band patterns in RAPD-PCR, clustered into *A. fumigatus* sensu stricto strains (Fig. 3).

For confirmation of identification, phenotypic characters were examined. Six strains grouped with *A. fumigatus* sensu stricto by β-tubulin phylogeny showed typical morphology of *A. fumigatus*. CBS 612.97 and CBS 153.89 clustered with *A. lentulus*, grew at 10°C, did not grow at 50°C, and had thinner stipes and more globose vesicles than *A. fumigatus* sensu stricto and were therefore identified as *A. lentulus*. CBS 154.89 produced ascomata when mated with *N. udagawae* CBS 114217^T and was subsequently identified as *N. udagawae*. Anamorphic characteristics of CBS 116047 were similar to the original description of *N. nishimurae* (Takada *et al.*, 2001); however, identification of the strain was not confirmed by mating experiments due to unavailability of the other *N. nishimurae* strains. The taxonomic status of CBS 175.97 *A. lentulus,* and CBS 458.75 *A. viridinutans* species complex has

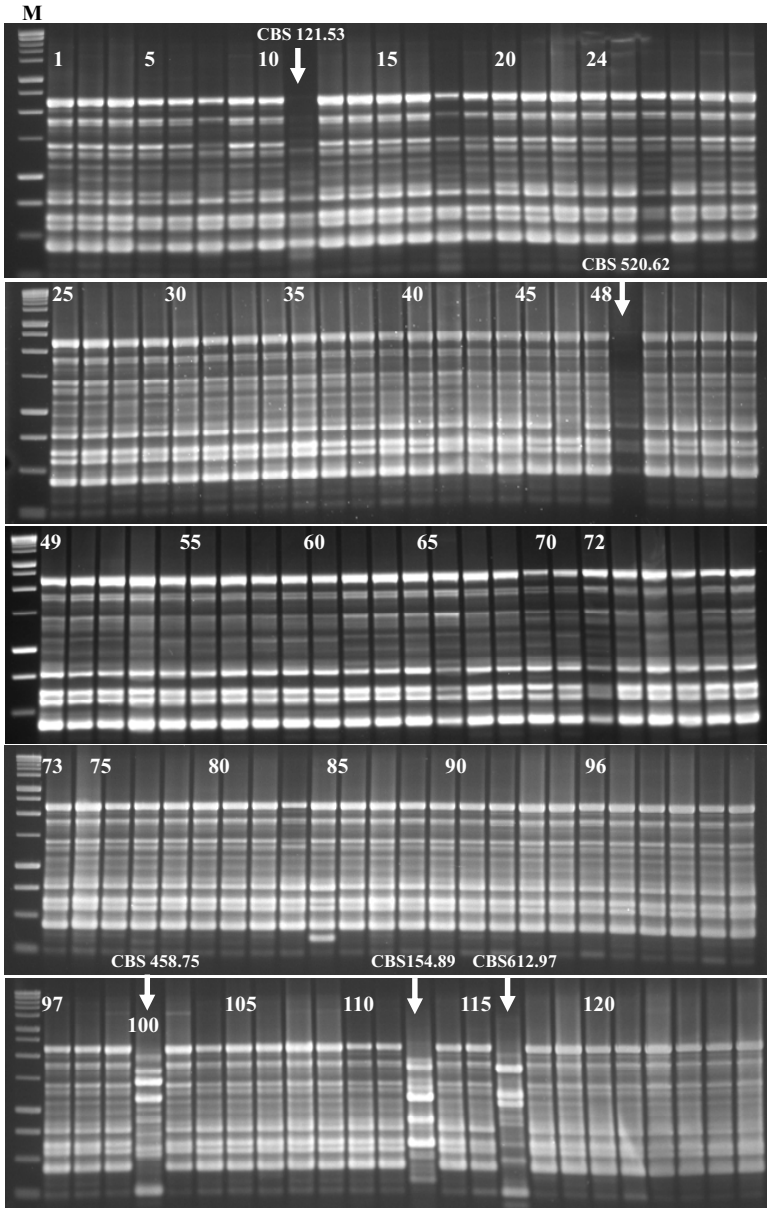

Fig. 1. RAPD-PCR of CBS strains of *A. fumigatus* sensu lato with primer URP1F. The lane number of each strain is recorded as the URP1F value in Table 1.

been previously described (Hong *et al.*, 2005).

As a result of RAPD-PCR and β-tubulin sequence analyses of 146 CBS strains of *A. fumigatus* sensu lato, 140 (95.8%) were identified as *A. fumigatus* sensu stricto, while 3 (2.1%) from dolphin nostril, soil, and unknown sources were identified as *A. lentulus* and the remaining 3 strains were identified as *A. viridinutans* species complex (ex soil), *N. udagawae* (ex soil) and *N*. cf. *nishimurae* (ex unknown source). *A. fumigatiaffinis* and *A. novofumigatus* were not found in CBS strains of *A. fumigatus* sensu lato.

Five clinical strains reported as atypical *A. fumigatus* by Katz *et al.* (2005) clustered with *A. lentulus* (MK245), *Neosartorya* udagawae (MK285), *N. coreana* (NSW3), and *A. viridinutans* complex (MK 246, MK284) (Fig. 3).

Discussion

A. fumigatus infections are usually detected by standard cultural and/or histological methods and the species is identified on the basis of its morphological features (Wang *et al.*, 2000). Clinical isolates can be remarkably different from food- or soil-born isolates, showing more floccose growth with fewer conidia (Leslie *et al.*, 1988). Identification of clinical strains based on morphology has often led to misclassification (Balajee *et al.*, 2005b; Katz *et al.*, 2005). Of 98 clinical strains identified as *A. fumigatus* based mainly on morphology, only 1 strain was re-identified as *A. lentulus*. Furthermore, all 90 human strains from the Netherlands, Germany, France, USA, etc. were identified as *A. fumigatus* sensu stricto. These results

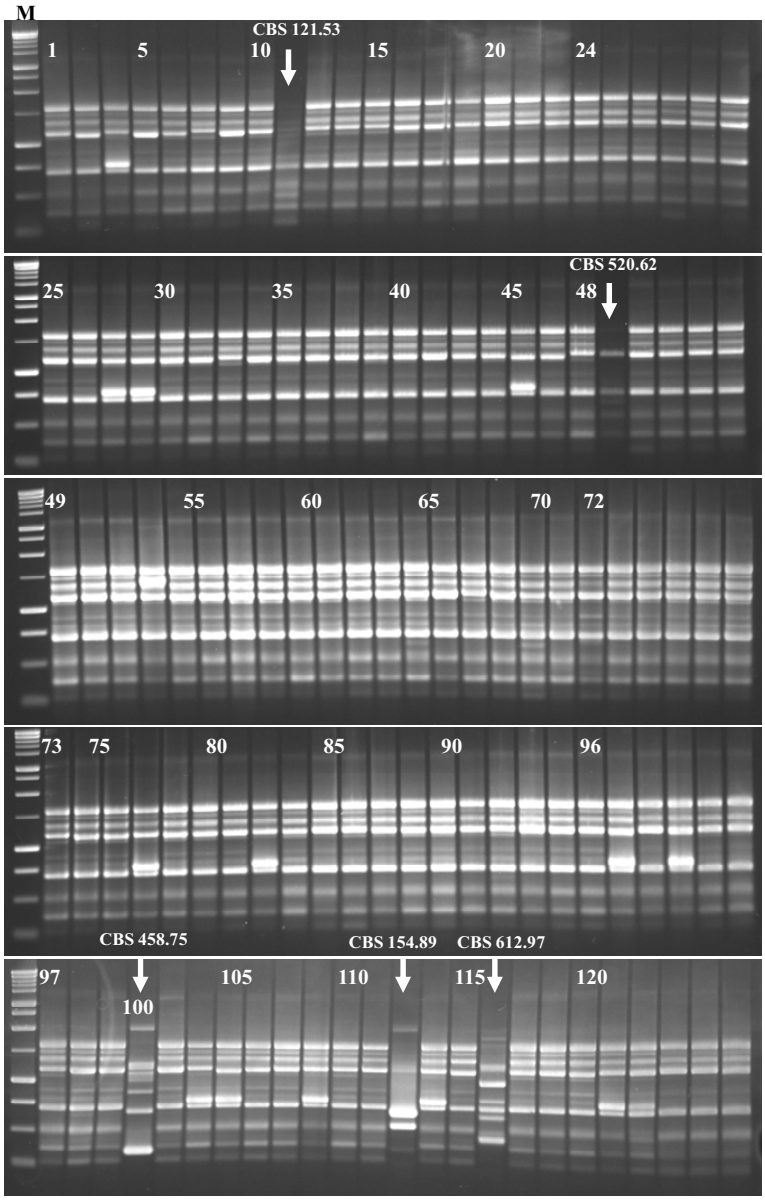

Fig. 2. RAPD-PCR of CBS strains of *A. fumigatus* sensu lato with primer PELF. The lane number of each strain is recorded as the PELF value in Table 1.

suggest that *A. fumigatus* sensu stricto is a predominant species in *Aspergillus* section *Fumigati* in clinical environments while other species may be rarely isolated.

Of the 22 soil strains of CBS *A. fumigatus* sensu lato analyzed, 3 were re-identified as *A. lentulus*, *A. viridinutans* species complex, and *N. udagawae*. Of the 12 air strains of CBS *A. fumigatus* sensu lato evaluated, all were identified as *A. fumigatus* sensu stricto. *A. fumigatiaffinis* and *A. novofumigatus* were not found in air, human, or animal strains. In view of the isolation rate of *A. fumigatus* and related species, it can be assumed that species diversity in air and clinical environments is lower than that in soil environments.

Latge *et al.* (2000) evaluated the availability of RAPD-PCR for genotyping of *A. fumigatus* species. Almost all primers failed to detect variability among *A. fumigatus* strains. Although the primers produced polymorphism among strains, interpretation of the intraspecific variability was difficult. On the contrary, Van Belkum *et al.* (1993) used RAPD-PCR for taxonomy and species identification of *Aspergillus.* In this study and that of Hong *et al.* (2005), 155 strains of *A. fumigatus* sensu lato (including 10 strains of *A. lentulus*, 2 strains of *A. fumigatiaffinis*, and 2 strains of *A. novofumigatus*) were analyzed with PELF and URP1F primers. The primers generated nearly the same band patterns among 141 strains of *A. fumigatus* sensu stricto and among 10 strains of *A. lentulus*, but the band patterns were able to show clear differences among interspecies [Figs. 1-2 and Hong *et al.* (2005)]. Therefore, RAPD-PCR with PELF and URP1F primers can be considered

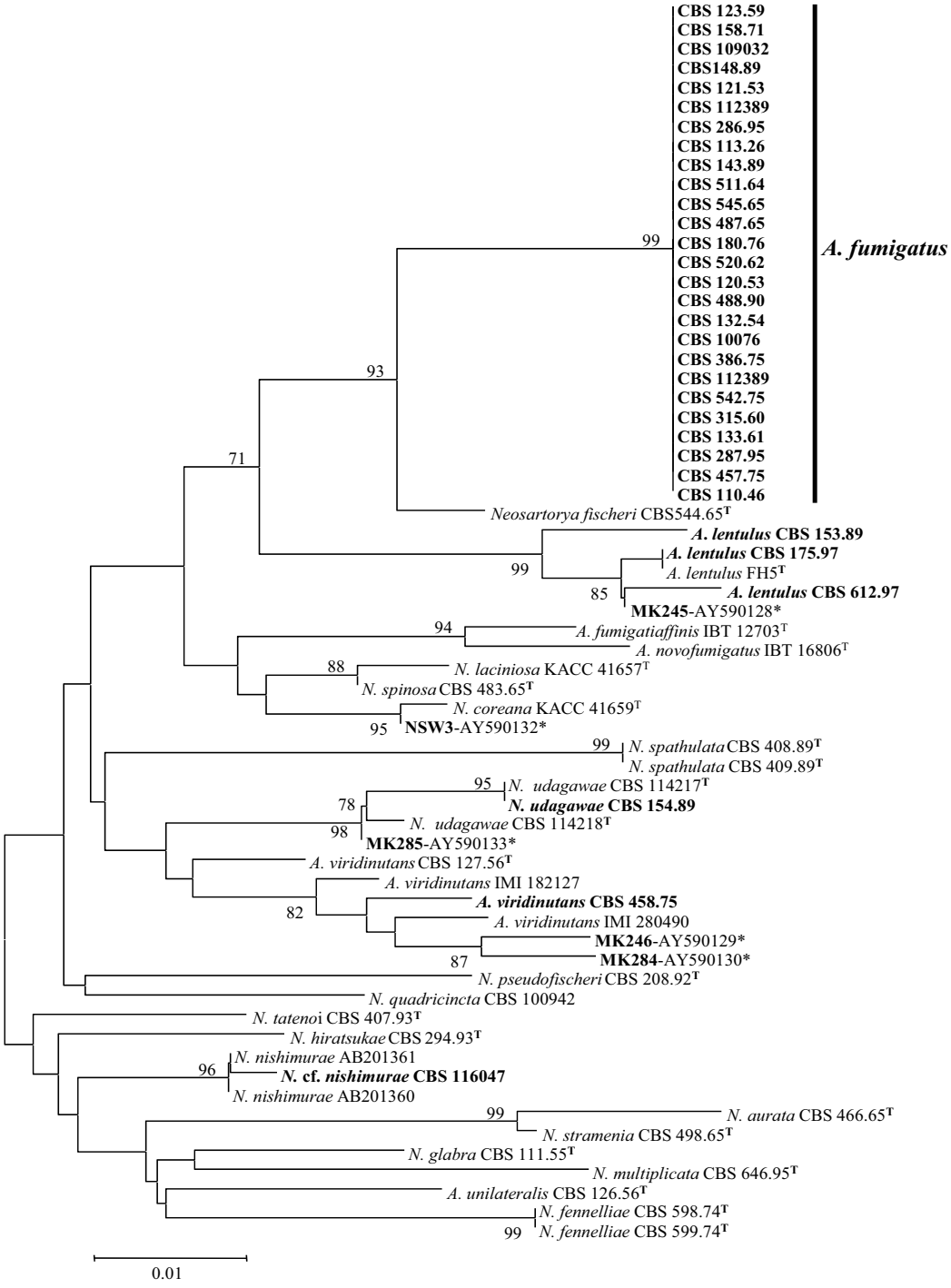

Fig. 3. Taxonomic position of CBS strains of *A. fumigatus* sensu lato inferred from neighbor-joining analysis of partial β-tubulin gene sequences. CBS strains previously identified as *A. fumigatus* based on morphology, were written in bold. Partial β-tubulin gene (primers bt2a and bt2b) sequences analyzed first using the Tamura-Nei parameter distance calculation model with gamma-distributed substitution rates, which was then used to construct the neighbor-joining tree with MEGA version 3.1. Numbers above or below nodes are bootstrap values. Only value >70% are shown. *MK245, 246, 284, 285, and NSW3 sequences were obtained from Katz *et al.* (2005).

a rapid and reliable method to identify *A. fumigatus* and *A. lentulus*.

A total of 141 strains of *A. fumigatus* from clinical, soil, and indoor environments had nearly the same band patterns in RAPD-PCR [Figs. 1-2 and Hong *et al.* (2005)] and 26 strains of them showed nearly identical DNA sequences of β-tubulin (Fig. 3). These results demonstrate that *A. fumigatus* sensu stricto strains are genetically homogeneous regardless of their

source, because URP1F showed fine resolution for genotypes of various microorganisms (Kang *et al.*, 2002). Our findings support the results of Debeaupuis *et al.* (1997) that genetic discrimination in *A. fumigatus* was not determined on the basis of the saprophytic or pathogenic origin of the isolates.

Clinical strains reported as atypical *A. fumigatus* (Katz *et al.*, 2005) clustered with *A. lentulus* (MK245), *Neosartorya udagawae* (MK285), *N. coreana* (NSW3), and *A. viridinutans* species complex (MK 246, MK284) in the phylogenetic tree of β-tubulin gene sequences (Fig. 3). β-tubulin gene sequence was elucidated as a useful tool for species delimitation of *Aspergillus* section *Fumigati* (Geiser *et al.*, 1998; Varga *et al.*, 2000a, 2000b; Hong *et al.*, 2005, 2006). Therefore, it suggests that MK245 may be *A. lentulus*, MK285 may be *Neosartorya udagawae*, NSW3 may be *N. coreana*, and MK246 and MK284 may be *A. viridinutans* complex, although further examination to confirm these identifications are required.

Several species in *Aspergillus* section *Fumigati* were reported to be from clinical environments: *Neosartorya fischeri* (Lonial *et al.,* 1997; Chim *et al.,* 1998; Gori *et al.,* 1998), *N. pseudofischeri* (often as *A. thermomutans* status) (Coriglione *et al.*, 1990; Padhye *et al.*, 1994; Balajee *et al.*, 2005b), *N. spinosa* (Gerber *et al.*, 1973; Summerbell *et al.*, 1992), and *N. fennelliae* (Kwon-Chung and Kim, 1974) [maybe *A. viridinutans* species complex, *N. udagawae* and *N. coreana* (from identification of Katz' atypical strains in this study)], although *A. fumigatus* is the predominant species. The clinical species described above were easily isolated from soil (Hong *et al*., 2010; CBS database at http://www.knaw.nl), and also from indoor environments (Udagawa *et al.*, 1996). Anamorphic states of the mentioned *Neosartorya* species have abundant conidiation. In the case of *A. fumigatus*, a genetic difference was not found between pathogenic and saprophytic isolates. These results support the notion that the natural ecological niche of *Aspergillus* section *Fumigati* strains which cause human and animal aspergillosis is soil, the isolates are dispersed into the air and can become pathogenic if they encounter the appropriate host (Latge *et al.*, 2000).

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