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Species delimitation in the genus *Lipomyces* by nuclear genome comparison

Maudy Th. Smith[1,2], G.A. Poot[1], Wilma H. Batenburg-van der Vegte[2] & J.P. van der Walt[3]
[1] *Centraalbureau voor Schimmelcultures, Yeast Division,* [2] *Delft University of Technology, Kluyver Laboratory, Julianalaan 67, 2628 BC Delft, The Netherlands;* [3] *Department of Microbiology & Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300, South Africa*

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Abstract

Species delimitation in *Lipomyces* was attempted by nuclear genome comparison in conjuction with the re-evaluation of 48 physiological characters of 65 strains.

High intraspecific (> 75%) and low interspecific (< 28%) similarity values established that *L. japonicus, L. lipofer* and *L. tetrasporus* are genetically isolated, and also distinct from *L. kononenkoae* and *L. starkeyi*.

Ambiguous similarity values were obtained with *L. kononenkoae* and *L. starkeyi*. Strains previously assigned to *L. kononenkoae* constitute two related clusters. While similarity values within each cluster range from 76–99%, representatives of the two clusters reassociate for only 47%. Since these clusters are differentiated by their ecologically relevant maximum growth temperature, *L. kononenkoae* is subdivided. Strains previously assigned to *L. starkeyi* resolve into four closely related clusters. While similarity values within each cluster range from 78–100%, representatives of the four clusters reassociate for only 59–69%. Since these four clusters are poorly differentiated, the subdivision of *L. starkeyi* does not appear possible without recourse to other criteria.

Four unassigned strains constitute a further two clusters. Reassociation within these clusters is of the order of 91–100%, while reassociation between them occurs only at 59%. Reassociation of representatives of these clusters with those of the *L. kononenkoae* and *L. starkeyi* complexes is around 40% and 31%, respectively. These two clusters consequently appear to be intermediate between *L. kononenkoae* and *L. starkeyi*, and will, as such, have to be considered in any delimitation of these two species. A key to the taxa of *Lipomyces* and related genera of the Lipomycetaceae is given.

Introduction

Smith et al. (1994), by their introduction of the genus *Babjevia* Van der Walt et M.Th. Smith (1994) based on *B. anomala* (Bab'evia & Gorin) Van der Walt et M.Th. Smith 1994, restricted the genus *Lipomyces* Lodder & Kreger-van Rij (1952) to *L. japonicus* Van der Walt et al. (1989), *L. lipofer* Lodder & Kreger-van Rij ex Slooff (1970), *L. starkeyi* Lodder & Kreger-van Rij (1952) and *L. tetrasporus* Nieuwdorp et al. (1974).

The identification of these five species, and particularly of more recent isolates from sub-Saharan localities, nevertheless remains tedious, and is often inconclusive. With the exception of *L. japonicus*, the species are, by and large, indistinguishable in terms of their guanine + cytosine content (mol % G + C) of their nDNA. Their phenotypic differentiation is also constrained. The utilization of the conventional carbon sources is often either variable, or non-differential. Ascospore topography though discriminative of species producing conspiciously ornamented spores, does not distinguish between taxa forming smooth spores (Smith & Batenburg-van der Vegte 1984).

More recent revisions of the genus were based either on the use of rather limited numbers of characters (Phaff & Kurtzman 1984), or were limited mainly to the study of type strains (Barnett et al. 1990), features that do not take variability of the species into account. Thus some of these species might either be hetero-

geneous (Spencer-Martins 1983; Yamazaki & Goto 1985) or not biologically distinct (Kurtzman & Liu 1990). Therefore, the delimitation of the five currently accepted species was attempted by nDNA reassociation procedures in conjuction with the reassessment of 48 physiological characters of the 65 strains held by the Yeast Division of the Centraalbureau voor Schimmelcultures.

Materials and methods

Cultures examined
Strains along with their source and country of isolation and their mol % G + C are listed in Table 1.

DNA analysis
All strains were grown for 2 days at 25° C on a rotary shaker at 125 rpm in 2 L YM broth (Wickerham 1951) in 1 L flat-bottom flasks. Isolation and purification of DNA, determination of DNA base composition and DNA-DNA reassociations were performed according the procedures described by Golubev et al. (1989).

Nutritional physiology
Utilization of 43 carbon sources was tested at least in duplicate according to the methods of Van der Walt & Yarrow (1984). Cultures were grown in liquid medium in test tubes at 25° C for 4 weeks and shaken continuously at 30 rpm.

Growth capacity at five different temperatures was tested.

Ascospore fine structure
For ultra-thin sectioning, material was prepared according to methods previously described by Smith & Batenburg-van der Vegte (1984).

Results

The resolution of the genus in terms of the nDNA base composition (mol % G + C) of the strains

The strains examined resolved into two groups (Table 1):

Group 1 comprises strains with mol % G + C values ranging from 41.5–42.2%, and includes the type strain of *L. japonicus* and all strains identified as representatives of this taxon.

Group 2 is characterized by mol % G + C values ranging from 47–49% and includes the type strains of *L. kononenkoae, L. lipofer, L. starkeyi, L. tetrasporus*, strains previously assigned to these species and a number of strains that, to some extent, differ phenotypically from the type strains.

The recognition of specific and infraspecific taxa in terms of DNA-DNA similarity of the strains

Similarity data of interspecific and intraspecific reassociations are presented in Table 2, and Fig. 1.

Interspecific reassociation between species of Groups 1 and 2

Interspecific reassociation between the type strains of *L. japonicus* (Group 1) and of *L. starkeyi* (Group 2) is 20% (Table 2), the average value between the three strains of this taxon and the type strain of *L. starkeyi* being 24% (Fig. 1), irrespective of 6% difference in their mol % G + C. Since differences of more than 2% in the nDNA composition of strains are assumed to preclude their conspecificity (Price et al. 1978), nDNA complementarity values of 24% or less probably serve to discern between genetically isolated species in *Lipomyces*.

Intraspecific and interspecific reassociations

Strains assigned to L. japonicus *(Group 1: G + C% 41.5–42.2)*
The two additional strains assigned to the species reassociated with the type strain, CBS 7319, for 99 and 100% from which it is inferred that there is little genetic divergence (Fig. 1). The species appears to be widely distributed, with isolates known from Japan and Southern Africa. It is easily recognized by its characteristic physiological properties (Table 3), and its distinctive globose ascospores which by TEM appear to be alveolate or reticulate with a loose undulate outer membrane (Fig. 2a).

Strains assigned to L. lipofer *(Group 2: G + C% 46.9–48.5)*
Similarity values among *L. lipofer* strains including the type strain, CBS 944, range from 92–100% (Fig. 1), which also implies little genetic divergence. The type strain reassociates with the type strains of *L. kononenkoae, L. starkeyi* and *L. tetrasporus* from 5–

Table 1. Strains of *Lipomyces* examined in this study

Species and strain designation	Source	mol% G+C ± S.D.
L. japonicus		
CBS 7319	garden soil, Kanagawa Prefecture, Japan; J.P. van der Walt. Type	41.5 ± 0.3
CBS 7549	uncultivated soil, Bronkhorstspruit District, Transvaal, S.A.;	42.2 ± 0.1
CBS 7550	uncultivated soil, Frankfort, Orange Free State, S.A.; E.L. Jansen van Rensburg	41.6 ± 0.4
L. kononenkoae spp. *kononenkoae*		
CBS 2514	soil of citrus orchard, Los Lomas, Trinidat; J.H. Becking. Type	47.9 ± 0.5
CBS 7535	soil, Magoeba's Kloof, Transvaal, S.A.; J.P. van der Walt	47.0 ± 0.1
CBS 8113	soil, S.A.; J.P. van der Walt	48.0 ± 1.0
CBS 8114	soil, S.A.; J.P. van der Walt	48.0 ± 0.4
IGC 4983	soil, S.A.; I. Spencer-Martins	n.d.
L. kononenkoae spp. *spencermartinsiae*		
CBS 5608	soil, Nigeria; E. Drouhet. Type	47.5 ± 0.8
CBS 7534	soil, Pretoria district, Transvaal, S.A.; J.P. van der Walt	47.4 ± 0.5
CBS 7543	soil, Kwa-Mbonambi State Forest Reserve, Natal, S.A.; J.P. van der Walt	47.7 ± 0.7
CBS 7681	soil, Bronkhorstspruit District, Transvaal, S.A.; J.P. van der Walt	n.d.
CBS 7682	soil, Bronkhorstspruit District, Transvaal, S.A.; J.P. van der Walt	48.9 ± 0.4
L. lipofer		
CBS 944	garden soil, The Netherlands; L.E. den Dooren de Jong. Type	46.9 ± 0.6
CBS 2513	soil botanic garden Leiden, The Netherlands; J.H. Becking	48.0 ± 0.4
CBS 5841	unknown; A.C. Thaysen	48.5 ± 0.8
CBS 5842	soil, Wales, U.K.; D. Jones	n.d.
CBS 7602	cultivated podzolic soil, Biological Station of Perm University, USSR, I.P. Bab'eva	n.d.
CBS 7603	cultivated meadow soil, Kirghiz, ASSR; I.P. Bab'eva	47.2 ± 0.2
L. starkeyi sensu stricto		
CBS 1807	soil, USA; R.L. Starkey. Type	48.0 ± 0.4
CBS 1809	dry mutant ex CBS 1807; R.L. Starkey	48.0 ± 0.5
CBS 2512	soil Wageningen, The Netherlands; J.H. Becking	46.6 ± 0.5
CBS 6047	soil Haren, The Netherlands; N.J.W. Kreger-van Rij	n.d.
CBS 7537	soil Thornton, Canada; J.P. van der Walt	n.d.
CBS 7537	soil Botanic Garden, Cape Town, S.A.; J.P. van der Walt	47.3 ± 0.3
CBS 7544	soil Mount Sheba Forest Reserve, Transvaal, S.A.; J.P. van der Walt	n.d.
CBS 7545	soil Mount Sheba Forest Reserve, Transvaal, S.A.; J.P. van der Walt	46.3 ± 0.5
CBS 8064	lemons, France; F. Seigle-Murandi	47.3 ± 0.5
L. starkeyi cluster α		
CBS 7661	soil, Drakensberg, Natal, S.A.; E.E. Pretorius. Type	47.0 ± 0.3
CBS 7600	uncultivated soil, Mt-aux-Sources Plateau, Natal, S.A.; E.L. Jansen van Rensburg	48.0 ± 0.6
CBS 7601	uncultivated soil, Mt-aux-Sources Plateau, Natal, S.A.; E.L. Jansen van Rensburg	47.4 ± 0.1

Table 1. Continued

Species and strain designation	Source	mol% G+C ± S.D.
CBS 7605	uncultivated soil, Phuthaditjhaba, Orange Free State, S.A.; E.L. Jansen van Rensburg	47.7 ± 0.3
CBS 7737	forest soil, Nature's Valley, Southern Cape Province, S.A.; J.P. van der Walt	47.1 ± 0.5
L. starkeyi cluster β		
CBS 7542	soil, Kwa-Mbonambi State Forest Reserve, Natal, S.A.; J.P. van der Walt	47.7 ± 0.7
CBS 2516	soil, Magalies Mountains, Transvaal, S.A.; J.P. van der Walt	48.4 ± 0.5
L. starkeyi cluster γ		
CBS 7729	forest soil, Knysna district, Southern Cape Province, S.A.; J.P. van der Walt	46.3 ± 0.6
CBS 7731	forest soil, Knysna district, Southern Cape Province, S.A.; J.P. van der Walt	46.7 ± 0.9
CBS 7732	forest soil, Knysna district, Southern Cape Province, S.A.; J.P. van der Walt	n.d.
CBS 7733	forest soil, Knysna district, Southern Cape Province, S.A.; J.P. van der Walt	46.6
CBS 7734	forest soil, Knysna district, Southern Cape Province, S.A.; J.P. van der Walt	n.d.
IGC 4985	soil, S.A.; I. Spencer-Martins	n.d.
L. tetrasporus		
CBS 5910	soil, USSR; I.P. Bab'eva. Type	48.1 ± 0.8
CBS 1808	soil, USA; R.L. Starkey	n.d.
CBS 1810	soil, Ottawa, Canada; Harmsen	n.d.
CBS 2511	soil, Monaco; J.H. Becking	n.d.
CBS 5607	soil, Reunion; M. Dommerques	n.d.
CBS 5910.1	Schernozom soil, Orenburg district, USSR; I.P. Bab'eva	n.d.
CBS 5911	forest soil, Tula district, USSR; I.P. Bab'eva	n.d.
CBS 6048	soil; J.C.G. Ottow	n.d.
CBS 6049	soil, USSR; I.P. Bab'eva	n.d.
CBS 6050	soil, USSR; I.P. Bab'eva	n.d.
CBS 6051	soil, USSR; I.P. Bab'eva	47.3 ± 0.4
CBS 6132	soil; J. Pullen	48.6 ± 0.2
CBS 7656	uncultivated surface soil, Transvaal, S.A.; J.P. van der Walt	49.1 ± 0.7
CBS 7728	forest soil, Knysna district, Southern Cape Province, S.A.; J.P. van der Walt	n.d.
CBS 7730	forest soil, Knysna district, Southern Cape Province, S.A.; J.P. van der Walt	n.d.
CBS 7735	forest soil, Nature's Valley, Southern Cape Province, S.A.; J.P. van der Walt	n.d.
CBS 7736	forest soil, Nature's Valley, Southern Cape Province, S.A.; J.P. van der Walt	n.d.
IGC 4728	culture contaminant, Oeiras, Portugal; C. Cabeça-Silva	n.d.
IGC 4977	soil, Alentejo, Portugal; I. Spencer-Martins	n.d.
IGC 4980	soil, Oeiras, Portugal; I. Spencer-Martins	n.d.

Table 1. Continued

Species and strain designation	Source	mol% G+C ± S.D.
Lipomyces Cluster A		
CBS 7557	soil near Curepipe, Mauritius; J.P. van der Walt. Type	47.5 ± 1.0
CBS 7558	soil neal Vacoas, Mauritius; J.P. van der Walt	47.8 ± 0.3
Lipomyces Cluster B		
CBS 7532	soil, Helspoort, Eastern Cape Province, S.A.; J.P. van der Walt. Type	47.9 ± 0.4
CBS 7533	soil, Hogs Back, Eastern Cape Province, S.A.; J.P. van der Walt	46.9 ± 0.4

CBS = Centraalbureau voor Schimmelcultures
IGC = Centro de Biologia, Institute Gulbenkian de Ciencia, Oeiras, Portugal
SD = Standard deviation of at least two determinations

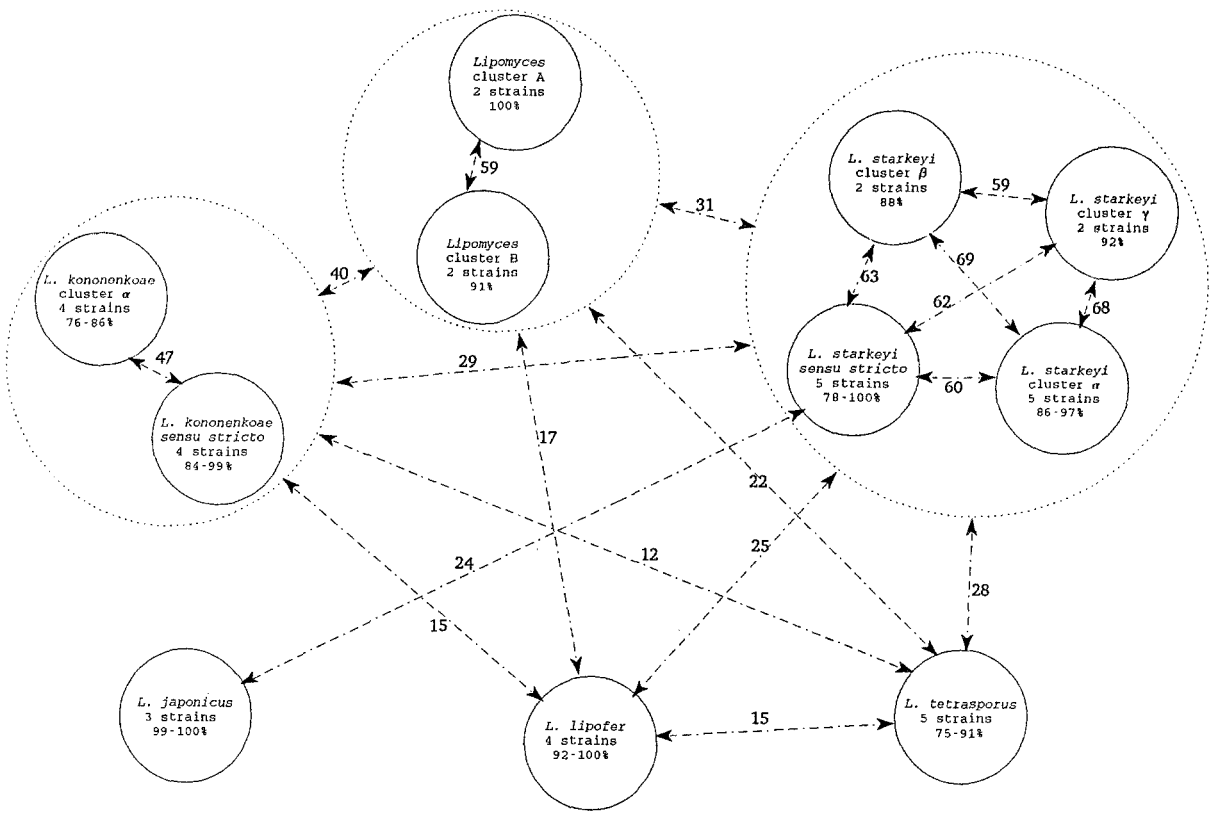

Fig. 1. Relationships of taxa of *Lipomyces* based on nDNA similarity. Distances not proportional to degree of relatedness. Standard deviation of intraspecific reassociations $\leq$ 5%. Values are averages of at least two determinations. Standard deviation of reassociations between taxa $\leq$ 8%.

20%: thus *L. lipofer* is genetically separate from these three taxa (Table 2). The presence of Coenzyme Q-10 system distinguishes *L. lipofer* from all other members of the genus with CoQ 9 (Yamada et al. 1986; Billon-Grand 1987). The species is currently known only from isolates from temperate zones of the Northern Hemisphere (The Netherlands, United Kingdom and Russia), and none of the strains grows at 35° C (Table 3). All examined strains form smooth ascospores (Fig. 2b).

Table 2. DNA similarity (%) between type strains and representatives of *Lipomyces* taxa. Averages of at least two determinations (SD $\leq$ 5%).

		CBS 7319	CBS 944	CBS 5910	CBS 2514	CBS 5608	CBS 1807	CBS 7661	CBS 7542	CBS 7729	CBS 7557	CBS 7532
L. japonicus	CBS 7319	—										
L. lipofer	CBS 944	nd	—									
L. tetrasporus	CBS 5910	nd	18	—								
L. kononenkoae ssp. *kononenkoae*	CBS 2514	nd	5	15	—							
L. kononenkoae ssp. *spencermartinsiae*	CBS 5608	nd	22	10	51	—						
L. starkeyi	CBS 1807	20	20	22	11	23	—					
L. starkeyi cluster α	CBS 7661	nd	23	31	26	32	60	—				
L. starkeyi cluster β	CBS 7542	nd	34	26	27	34	67	68	—			
L. starkeyi cluster γ	CBS 7729	nd	27	25	23	36	58	68	66			
Lipomyces cluster A	CBS 7557	nd	22	26	35	43	27	34	29	21	—	
Lipomyces cluster B	CBS 7532	nd	8	15	38	37	33	25	38	nd	59	—

nd = not determined

Strains assigned to L. tetrasporus *(Group 2: G + C% 47.3–49.1)*

Strains of *L. tetrasporus* including the type strain, CBS 5190, reassociate from 75–91%, suggesting that this population likewise shows little genetic divergence (Fig. 1). The type strain reassociates with the types of *L. kononenkoae, L. lipofer,* and *L. starkeyi* from 15–22% from which it is concluded that *L. tetrasporus* is genetically apart from these three taxa (Table 2). The species appears to be widely distributed and isolates are currently known from Russia, North America, Europe and Southern Africa. The species is easily recognized by its distinctive, longitudinally grooved or sulcate ascospores (Fig. 2c).

Strains assigned to L. kononenkoae *(Group 2: G + C% 47.0–48.9)*

The eight strains previously assigned to *L. kononenkoae* resolve into two distinct clusters (Fig. 1). The first cluster, *L. kononenkoae sensu stricto,* of four strains centres around the type strain, CBS 2514. Reassociation values within this cluster range from 84–99%. The second cluster, *L. kononenkoae* α, of four strains centres around CBS 5608. Similarity values within this cluster were from 76–86%. Strains CBS 2514 and CBS 5608 reassociate at 51% (Table 2), but the average reassociation between both clusters is 47% (Fig. 1), from which it is inferred that *L. kononenkoae* according to its previous delimitation, comprises genetically divergent strains. These two clusters are differentiated by their ability to grow at 40° C (Table 3). The phenotypic discontinuity of CBS 2514 and CBS 5608 was first observed by Hossack & Spencer-Martins (1978), and notably Spencer-Martins (1983), who drew attention to differences between the two strains in cell size, lipid composition, amylolytic complexes, optimal growth temperature and the utilization of β-cyclodextrin and D-deoxyglucose. Both clusters are known mainly from sub-Saharan isolates. The type strain CBS 2514, however, derives from Trinidad which implies that *L. kononenkoae* probably has a much wider distribution. The type of *L. kononenkoae,* CBS 2514, reassociates with the types of *L. lipofer, L. tetrasporus* and *L. starkeyi* at 5–15% and the reassociation of CBS 5608 with these three type strains ranges from 10–23% (Table 2). Both clusters consequently appear to be genetically separate from *L. lipofer, L. tetrasporus* and *L. starkeyi.* The ascospores of both taxa are smooth (Fig. 2d).

Strains assigned to L. starkeyi *(Group 2: G + C% 46.3–48.4)*

Reassociation of the type strain, CBS 1807, with 14 somewhat phenotypically similar strains, established

Table 3. Characteristics of strains of Lipomyces taxa. Numbers of strains analyzed in DNA similarity (R) and physiology (P) studies are indicated per taxon.

nr. of strains in:	Ja	Li	Te			KoKo		KoSp	St				Stα		Stβ	Stγ		LipA	LipB
R	3	4	4	1	0	3	1	4	2	2	1	1	4	1	2	2	1	2	2
P	3	6	13	5	2	4	1	5	4	3	1	1	4	1	2	5	1	2	2
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
L-Sorbose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
D-Glucosamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Ribose	-	-	V	V	-	-	-	-	+	V	-	-	-	-	-	-	-	-	-
D-Xylose	+	V	+	+	+	V	-	-	+	+	V	-	-	+	+	V	+	-	-
L-Arabinose	+	V	+	+	+	-	-	-	+	V	-	-	-	+	+	V	+	-	-
D-Arabinose	-	V	+	+	+	V	v	V	+	V	-	-	-	-	-	-	-	-	-
L-Rhamnose	-	-	V	V	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Maltose	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
α, α Trehalose	-	+	+	V	V	+	+	V	+	+	+	+	V	+	+	-	+	+	+
methyl-α-Glucoside	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Cellobiose	+	+	V	+	+	V	-	V	+	+	+	+	-	+	+	+	+	+	+
Salicin	V	+	V	+	+	V	-	V	+	V	-	+	-	+	V	+	+	+	+
Arbutin	-	+	V	V	+	V	-	V	+	V	-	-	-	+	V	-	v	+	+
Melibiose	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	V	V	+	+	-	-	-	V	-	-	-	-	+	+	-	-	-	-
Raffinose	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Melezitose	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Inulin	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Starch	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+
Glycerol	+	V	V	+	+	V	+	+	+	+	-	-	-	+	-	V	-	-	-
meso-Erythritol	-	+	+	+	+	-	-	-	+	+	-	+	-	-	+	-	+	-	-
Ribitol	+	V	+	+	+	-	-	-	+	V	-	-	-	+	-	-	-	-	-
Xylitol	+	+	+	+	+	+	+	+	+	+	+	+	-	+	V	+	+	+	+
L-Arabinitol	+	V	+	+	+	-	-	-	+	V	-	v	-	+	V	V	v	-	-
D-Glucitol	+	+	+	+	+	+	+	+	+	+	+	+	V	+	+	V	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	V	+	+	+	+	+	+
Galactitol	-	V	+	V	+	V	-	-	+	+	-	+	-	+	+	V	+	+	-
myo-Inositol	-	-	V	V	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-
Glucono-δ-lactone	V	+	+	+	+	+	+	+	+	V	+	+	-	+	+	+	+	+	+
D-Gluconate	-	+	+	+	-	V	-	V	-	-	+	-	-	+	-	V	-	+	+
D-Glucuronate	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Galacturonate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DL-lactate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Succinate	-	$+^w$	V	+	+	V	-	-	w	w	v	v	+	v	V	V	+	-	-
Citrate	+	$+^w$	w	w	W	V	+	V	w	w	v	v	+	+	V	-	-	V	+
Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol	V	$+^w$	+	+	+	+	+	V	+	+	+	+	V	-	V	+	+	+	+
Propane 1,2 diol	-	-	+	-	-	-	-	-	V	-	-	-	-	-	V	-	-	-	-
Butane 2,3 diol	+	+	+	+	+	-	-	-	+	+	-	+	-	v	V	V	+	-	-
Galactonate	V	+	+	+	+	+	-	V	V	V	-	-	-	-	-	-	-	-	-

Table 3. Continued

nr. of strains in:	Ja	Li	Te			KoKo		KoSp	St				Stα		Stβ	Stγ		LipA	LipB
R	3	4	4	1	0	3	1	4	2	2	1	1	4	1	2	2	1	2	2
P	3	6	13	5	2	4	1	5	4	3	1	1	4	1	2	5	1	2	2
25° C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30° C	+	V	+	+	+	+	+	+	+	+	+	+	V	+	+	+	+	+	+
35° C	-	-	V	V	+	V	-	+	V	V	-	-	-	-	V	V	?	-	+
37° C	-	-	-	-	-	V	-	+	-	-	-	-	-	-	-	-	-	-	-
40° C	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

V = Variable within species W = weak growth
v = variable within strains ? = not determined
Ja = *L. japonicus*; Li = *L. lipofer*; Te = *L. tetrasporus*; KoKo = *L. kononenkoae* ssp. *kononenkoae*; KoSp = *L. kononenkoae* ssp. *spencermartinsiae*; St = *L. starkeyi*; Stα = *L. starkeyi* cluster α; Stβ = *L. starkeyi* cluster β; Stγ = *L. starkeyi* cluster γ; Lip. A = *Lipomyces* cluster A; Lip. B = *Lipomyces* cluster B

that this group shows considerable genetic divergence. Four distinct clusters of reassociating strains are recognizable (Fig. 1). The first cluster, *L. starkeyi sensu stricto*, of five strains centres around the type strain, CBS 1807. Similarity within this cluster was 79–100%. The second, *L. starkeyi* cluster α, comprising five strains centres around CBS 7661 and shows reassociation values of 86–97%. The third, *L. starkeyi* cluster β, is based on CBS 7542 reassociating with the other strain, CBS 2516, for 88%. The fourth, *L. starkeyi* cluster γ, centres around CBS 7729 reassociating with CBS 7731 for 92%. As shown in Table 2, the reassociation values of the type strain CBS 1807 with the aforecited strains typifying clusters α, β and γ range from 58–68%. These four cluster are phenotypically very similar and, moreover, rather variable physiologically (Table 3). Interspecific reassociations of CBS 1807, CBS 7542, CBS 7729 and CBS 7661 with the types of *L. kononenkoae sensu stricto, L. kononenkoae* cluster α, *L. lipofer* and *L. tetrasporus* are from 11–36% (Table 2).

Whereas *L. starkeyi sensu stricto* is known from isolates from North America, Europe and Southern Africa, strains constituting *L. starkeyi* clusters α, β and γ all derive from Southern Africa. The ascospores of the four *L. starkeyi* clusters are as a rule verrucose, though frequently smooth in the same strain (Fig. 2e).

Unassigned Lipomyces *strains*

nDNA reassociation of the type strains of *L. kononenkoae sensu stricto, L. kononenkoae* cluster α, *L. lipofer, L. tetrasporus* and *L. starkeyi sensu stricto, L. starkeyi* clusters α, β and γ, with four unassigned strains helped to demarcate yet another two genetic entities. The first, *Lipomyces* cluster A, comprises two strains CBS 7557 and CBS 7558 that reassociate at 100% (Fig. 1), and derive from two localities on Mauritius. The second, *Lipomyces* cluster B, is constituted by CBS 7532 and CBS 7533 that reassociate at 91%, and derive from two localities in Southern Africa. Strains CBS 7557 of *Lipomyces* cluster A and CBS 7532 of *Lipomyces* cluster B reassociate at 59% (Table 2). Clusters A and B are phenotypically very similar and differentiate only in terms of their utilization of galactitol (Table 3). Strains CBS 7557 and CBS 7532 reassociate with the type strains of *L. lipofer* and *L. tetrasporus* at 8–26%, from which it is inferred that *Lipomyces* clusters A and B are individually distict from both these species (Table 2). Reassociation of these strains with types of the *L. starkeyi* complex ranges from 21–38%, and with the strains of the *L. kononenkoae* complex in the order of 35–43% (Table 2). The ascospores of both strains appear to be more or less smooth by TEM (Fig. 2f).

Discussion

Kurtzman (1987) in an attempt to predict 'biological relatedness' among sexually-reproducing yeasts from DNA reassociation data, suggested, but not without additional qualification, that reassociation values of 65–70% might be the lower limit to support conspecificity. The determination of relatedness beyond presumed sibling species was not considered possible with this method. Reassociation values of less than 65–70% were considered to be indicative of either varieties or

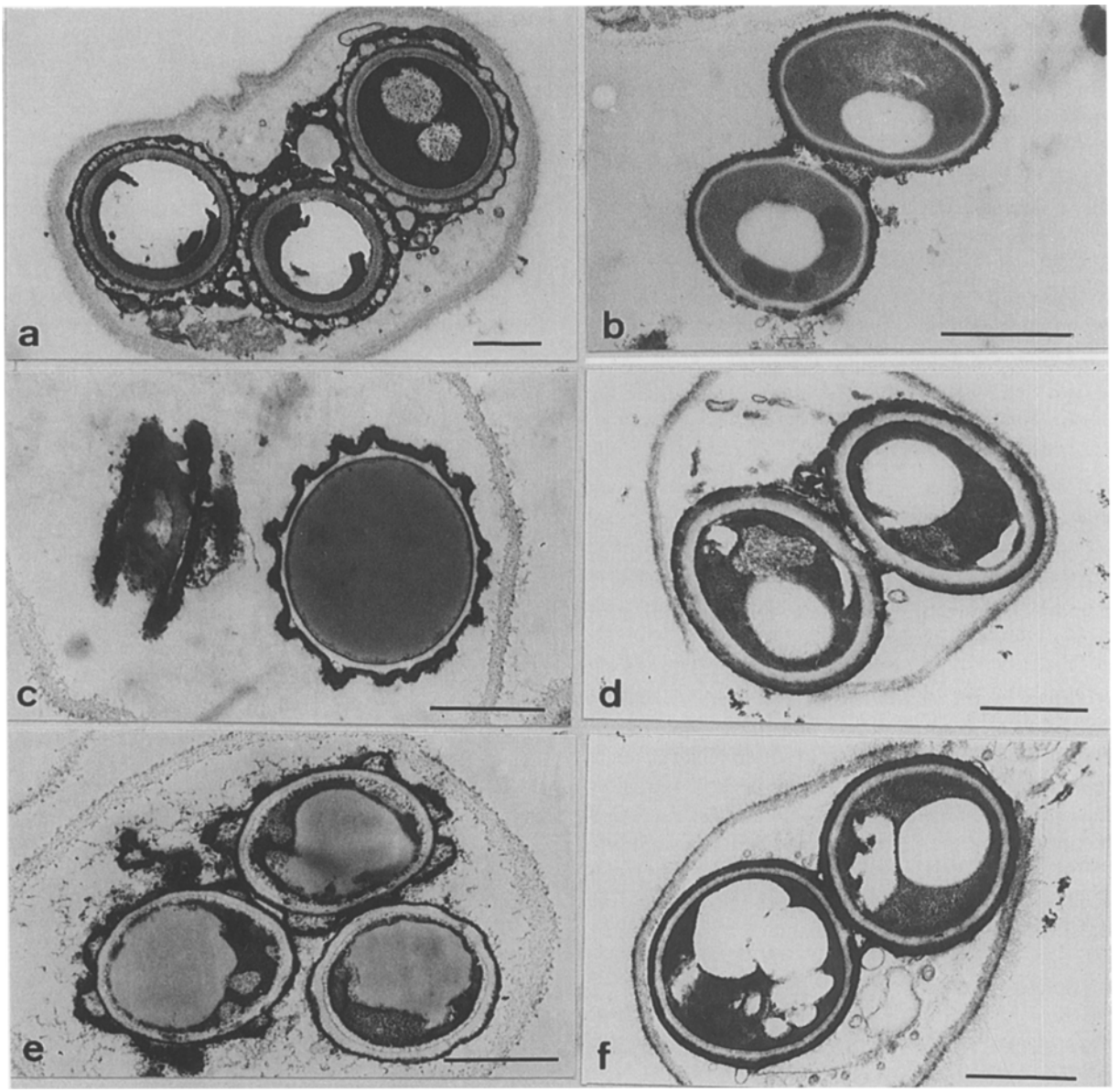

Fig. 2. Transmission electron micrographs of ascospores of a. *L. japonicus* CBS 7319; b. *L. lipofer* CBS 5842; c. *L. tetrasporus* CBS 5910; d. *L. kononenkoae* CBS 2514; e. *L. starkeyi* CBS 8064; f. *Lipomyces*-cluster A CBS 7557. Bar represents 1 μm. Glutaraldehyde-potassium permanganate fixation.

of discrete species, 'depending on what we perceive of their chromosomal make-up and other genetic factors'. Jahnke (1987), however, studying heterothallic Basidiomycetes accepted nDNA similarities of 90–95% as criterion for conspecificity, and values of 30–70% as indicative of different species, but did not interpret values ranging from 70–90%. Consequently, the interpretation of nDNA reassociation data is not invariably clear-cut and inevitably hinges on the number of strains examined.

Taxonomic consequences

Lipomyces japonicus, L. lipofer and *L. tetrasporus* are genetically discrete (Fig. 1).

Lipomyces kononenkoae

This species comprises two genetically discernable entities, the species *L. kononenkoae* (based on CBS 2514), and *L. kononenkoae* cluster α (based on CBS 5608). The two taxa are, moreover, phenotypically distinguishable, particularly by their optimal and maximum growth temperatures (Spencer-Martins 1983). Since the average reassociation of the clusters typified by CBS 2514 and CBS 5608 is 47% (Fig. 1), it is proposed to subdivide *L. kononenkoae*:

- *Lipomyces kononenkoae* Nieuwdorp et al. 1974 subspecies *kononenkoae*;
- *L. kononenkoae* Nieuwdorp et al. subspecies *spencermartinsiae* Van der Walt et M.Th. Smith ssp n.

Differt a *L. kononenkoae* ssp. *kononenkoae* potentia crescendi ad temperaturam 40° C. Typus cultura CBS 5608 in collectione zymotica Centraalbureau voor Schimmelcultures (Delphis Batavorum, in Hollandia) in sicco et vivo praeservatus.

It differs from *L. kononenkoae* ssp. *kononenkoae* by its ability to grow at 40° C and nDNA data. *Type*: culture CBS 5608, isolated from soil in Nigeria, maintained in the dried and living state in the Centraalbureau voor Schimmelcultures, Baarn and the Yeast Collection of the Centraalbureau voor Schimmelcultures, Delft, (The Netherlands), respectively. The subspecies is named after I. Spencer-Martins who first pointed out the divergent position of this taxon.

Lipomyces starkeyi

This species comprises four genetically discernable entities, the centre based on CBS 1807, and *L. starkeyi* clusters α, β, and γ based on strains CBS 7661, CBS 7542 and CBS 7729, respectively. These four, genetically demarcated entities reassociate mutually in the range of 59–69% (Fig. 1). Given

- the taxonomic ambiguity of such values, and
- the lack of phenotypic differentiation of the reassociating groups, no further formal subdivision of the *L. starkeyi* complex appears possible without recourse to other criteria.

It can be speculated that the *L. starkeyi* clusters α, β and γ represent initial stages of speciation.

The fact that the type strains of *L. starkeyi* and *L. tetrasporus* reassociate for only 22% does not support the hypothesis that these two species are taxonomically identical as was inferred from small differences in the partial 26S rRNA base sequences (Kurtzman & Liu 1990).

Taxa represented by Lipomyces *clusters A and B*

The taxonomic status of the four strains assigned to these two additional clusters remains uncertain. While reassociation values within each entity ranges from 91–100%, representatives of the two entities reassociate at only 59%. Interspecific reassociation values of 17–22% show these two entities to be genetically different from *L. lipofer* and *L. tetrasporus* (Fig. 1). However, the *Lipomyces* clusters A and B reassociate with the type strains of *L. kononenkoae* ssp. *kononenkoae* and *L. kononenkoae* ssp. *spencermartinsiae* and the *L. starkeyi* complex to a greater extent, in the order of 40% and 31%, respectively (Fig. 1). Although these reassociation values are ambiguous, the observed similarities, nevertheless, suggest that clusters A and B might be intermediate to the *L. starkeyi* and *L. kononenkoae* complexes. Because low nDNA reassociation values do not a *priori* preclude genetic exchange among sexually-reproducing ascomycetous yeasts (Johannsen 1980), these ambiguous values raise the question whether or not clusters A and B possibly represent natural hybrids of *L. starkeyi* and *L. kononenkoae* or, alternatively, constitute taxa from which the *L. starkeyi* and *L. kononenkoae* complexes may have evolved. The demarcation of both *L. starkeyi* and *L. kononenkoae* consequently requires consideration of their connection with the interrelated clusters A and B.

The detection of the generic divergence in *L. starkeyi* and *L. kononenkoae* depends largely on the fact that the present study included numerous strains of these species originating from different localities. Such divergence might well occur in other soil-borne genera, e.g. *Williopsis* Zender and *Debaryomyces* Lodder & Kreger-van Rij, where reassociation studies based exclusively on type strains could lead to oversimplified perceptions of genetically complex, infrageneric taxa. Current perceptions of *L. starkeyi* and *L. kononenkoae* complexes are for the greater part based on the study of isolates from sub-Saharan soils. These perceptions may change, once information on populations that might have evolved in other geographically isolated areas, becomes available. A sharper delimitation of the *L. starkeyi* and *L. kononenkoae* complexes has to await the availability of strains of different origin.

A composite key to the *Lipomycetaceae*

1.	a. Reproduction by septate hyphae	2
	b. Reproduction by multilateral budding	3
2.	a. Growth on lactose -	*Dipodascopsis uninucleatus*
	b. Growth on lactose	*Dipodascopsis tothii*
3.	a. Growth on melibiose -	4
	b. Growth on melibiose +	18
4.	a. Growth on melezitose -	5
	b. Growth on melezitose +	14
5.	a. Growth on sucrose -	6
	b. Growth on sucrose +	13
6.	a. Growth at 37° C -	7
	b. Growth at 37° C +	*Myxozyma sirexii**
7.	a. Growth on meso-erythritol -	8
	b. Growth on meso-erythritol +	*Myxozyma nipponensis**
8.	a. Growth on D-gluconate -	9
	b. Growth on D-gluconate +	11
9.	a. Growth on propane, 1,2 diol, D-galactonate -	10
	b. Growth on propane, 1,2 diol, D-galactonate +	*Zygozyma arxii*
10.	a. Growth on D-ribose, α,α trehalose -	*Babjevia anomala*
	b. Growth on D-ribose, α,α trehalose +	*Myxozyma lipomycoides*
11.	a. Growth on cellobiose, salicin, arbutin -	12
	b. Growth on cellobiose, salicin, arbutin +	*Zygozyma suomiensis*
12.	a. Growth on D-ribose, D-galactonate -	*Zygozyma oligophaga*
	b. Growth on D-ribose, D-galactonate +	*Myxozyma geophila*
13.	a. Growth on D-galactose, lactose -	*Zygozyma smithiae*
	b. Growth on D-galactose, lactose +	*Lipomyces japonicus*
14.	a. Growth on raffinose, soluble starch -	15
	b. Growth on raffinose, soluble starch +	*Lipomyces tetrasporus*
15.	a. Growth on myo-inositol -	16
	b. Growth on myo-inositol +	17
16.	a. Growth on α,α trehalose -, at 35° C +	*Myxozyma mucilagina*
	b. Growth on α,α trehalose +, at 35° C -	*Myxozyma vanderwaltii*
17.	a. Growth at 37° C -	*Myxozyma neotropica*
	b. Growth at 37° C +	*Myxozyma udenii*
18.	a. Growth at 40° C -	19
	b. Growth at 40° C +	*Lipomyces kononenkoae* ssp. *spencermartinsiae*
19.	a. Growth on melezitose -	20
	b. Growth on melezitose +	22
20.	a. Growth on D-galactose, lactose -	*Lipomyces starkeyi* cluster α
	b. Growth on D-galactose, lactose +	21
21.	a. Growth on sucrose, α,α trehalose -	*Myxozyma melibiosi*
	b. Growth on sucrose, α,α trehalose +	*Myxozyma kluyveri*
22.	a. Growth on meso erythritol -	23
	b. Growth on meso erythritol +	29
23.	a. Growth on lactose -	24
	b. Growth on lactose +	*Lipomyces starkeyi* cluster α
24.	a. Growth on soluble starch -	*Lipomyces starkeyi sensu stricto*
	b. Growth on soluble starch +	25

continued

25.	a. Growth on α,α trehalose -	*Lipomyces starkeyi* cluster γ
	b. Growth on α,α trehalose +	26
26.	a. Growth on D-galactonate -	27
	b. Growth on D-galactonate +	*Lipomyces kononenkoae* ssp. *kononenkoae*
27.	a. Growth at 35° C -	28
	b. Growth at 35° C +	*Lipomyces* cluster B
28.	a. Growth on cellobiose, salicin, arbutin -	*Lipomyces kononenkoae* ssp. *kononenkoae*
	b. Growth on cellobiose, salicin, arbutin +	*Lipomyces* cluster A
29.	a. Growth on raffinose -	*Myxozyma monticola*
	b. Growth on raffinose +	30
30.	a. Growth on D-gluconate -	31
	b. Growth on D-gluconate +	37
31.	a. Growth on glycerol -	32
	b. Growth on glycerol +	34
32.	a. Growth on lactose -	33
	b. Growth on lactose +	*Lipomyces starkeyi* cluster β
33.	a. Growth on L-arabinose -	*Lipomyces starkeyi sensu stricto*
	b. Growth on L-arabinose +	*Lipomyces starkeyi* cluster γ
34.	a. Growth on L-rhamnose -	35
	b. Growth on L-rhamnose +	*Lipomyces starkeyi sensu stricto*
35.	a. Growth on lactose -	*Lipomyces starkeyi sensu stricto*
	b. Growth on lactose +	36
36.	a. Growth on D-galactonate -	*Lipomyces starkeyi sensu stricto*
	b. Growth on D-galactonate +	*Lipomyces tetrasporus*
37.	a. Growth on propane 1,2 diol -	38
	b. Growth on propane 1,2 diol +	*Lipomyces tetrasporus*
38.	a. Ascospores without ridges, CO-Q 10	*Lipomyces lipofer*
	b. Ascospores with ridges, CO-Q 9	*Lipomyces tetrasporus*

Physiological data of species marked with an asterisk are from the literature (Spaaij et al. 1992, 1993).

The widely distributed genus *Lipomyces* probably provides a model taxon to demonstrate the demarcation of the typological species in terms of geographically and reproductively isolated populations (van der Walt 1987).

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