Hanseniaspora meyeri sp. nov., *Hanseniaspora clermontiae* sp. nov., *Hanseniaspora lachancei* sp. nov. and *Hanseniaspora opuntiae* sp. nov., novel apiculate yeast species

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Fourteen apiculate yeast strains isolated from various sources in South Africa, North America and the Hawaiian islands were found to be genetically divergent from other *Hanseniaspora–Kloeckera* species by using randomly amplified polymorphic DNA (RAPD)-PCR. After cluster analysis of the RAPD-PCR fingerprints, five groups were recognized. DNA reassociation values among representatives of these groups and strains of *Hanseniaspora–Kloeckera* species revealed that the strains represent five novel species. Four are described here as novel species of *Hanseniaspora*: *Hanseniaspora meyeri* sp. nov. (type CBS 8734^T), *Hanseniaspora clermontiae* sp. nov. (type CBS 8821^T), *Hanseniaspora lachancei* sp. nov. (type CBS 8818^T) and *Hanseniaspora opuntiae* sp. nov. (type CBS 8733^T). The fifth novel species, which is represented by only a single strain, CBS 8772, is not introduced as a new taxon. Phylogenetic analyses of the D1/D2 region of the 26S rDNA and internal transcribed spacer (ITS) regions with 5·8S rDNA sequences placed *H. meyeri*, *H. clermontiae*, *H. lachancei*, *H. opuntiae* and strain CBS 8772 close to *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii*. The key characteristics for standard physiological identification of *H. clermontiae* and *H. lachancei* were respectively maximal growth temperature and assimilation of 2-keto-D-gluconate. However, physiological characteristics did not allow the distinction of *H. opuntiae* and strain CBS 8772 from *H. guilliermondii* or *H. meyeri* from *H. uvarum*. These three novel taxa can be identified by either ITS sequencing or PCR-RFLP of ITS regions using restriction enzymes *Mbo*II and *Hin*fI.

INTRODUCTION

In a previous study (Cadez *et al.*, 2002), the genetic diversity of apiculate yeasts belonging to the genera *Hanseniaspora* Zikes and *Kloeckera* Janke was determined using randomly amplified polymorphic DNA (RAPD)-PCR analysis. Cluster analysis of the combined fingerprints of 74 strains segregated the strains into six major clusters, each representing a currently recognized species in the genus *Hanseniaspora* (Smith, 1998a), and one separate cluster of five strains that were physiologically indistinguishable from *Hanseniaspora uvarum* (Cadez *et al.*, 2002). Because the origins of these five strains were natural and man-made environments in North America and Africa only, nine additional strains from Hawaii and Mexico were included in rapid genomic fingerprinting. Eleven strains sharing five distinct RAPD-PCR banding patterns were subjected to further studies involving DNA–DNA reassociation determinations. In addition, the D1/D2 and internal transcribed spacer (ITS) regions (ITS1, ITS2) and the 5·8S rRNA genes were sequenced to determine phylogenetic relationships. Because standard physiological identification tests did not allow the separation of all *Hanseniaspora* and *Kloeckera* species, a combination of phenotypic and molecular tests is required for accurate identification of these novel *Hanseniaspora* species.

Abbreviations: ITS, internal transcribed spacer; RAPD, randomly amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are AJ512451–AJ512461 (26S rDNA D1/D2 domain) and AJ512427–AJ512443 (ITS region and 5·8S rDNA).

Detailed DNA–DNA hybridization data for the novel strains, a D1/D2-based phylogenetic tree and results of PCR-RFLP of the ITS region are available as supplementary material in IJSEM Online.

METHODS

Yeast strains and their characterization. The strains examined and their origin, location and DNA G+C contents are listed in

Table 1. Physiological characteristics of the yeasts were determined according to the methods of Yarrow (1998). Utilization of carbon compounds was tested in liquid media after incubation at 25 °C in tubes for 4 weeks with continuous shaking at 30 r.p.m. Assimilation of nitrogen compounds was examined by the auxonographic method after 1 week.

RAPD-PCR analysis. Genomic DNA was isolated as described by Möller *et al.* (1992). Three microsatellite primers, $(ATG)_5$, $(GTG)_5$ and $(GACA)_4$, were used in PCRs as described before (Cadez *et al.*, 2002). A Perkin Elmer 2400 thermal cycler was programmed for 35 cycles of 1 min at 94 °C, 1 min at 48 °C [primer $(ATG)_5$], 52 °C [$(GTG)_5$] or 43 °C [$(GACA)_4$] followed by 2 min at 72 °C. PCR products were separated on 2 % agarose gels in 1 × TAE buffer. Ethidium bromide-stained gels were documented by GelDoc 2000 (Bio-Rad).

The RAPD-PCR profiles of each strain were combined in a composite fingerprint and a binary matrix was generated manually by scoring the absence (0) or presence (1) of each fragment. Further analyses were performed using NTSYS software package version 2.0 (Applied Biostatistics). Similarity values were calculated using the Dice coefficient and cluster analysis was performed using the UPGMA algorithm.

Genomic DNA analyses. For DNA extraction, strains were grown for 2 days at 25 °C on a rotary shaker at 125 r.p.m. in 2 l yeast extract/malt extract broth (YM; Difco) using four 1 l flat-bottom flasks. Isolation and purification of DNA, determination of DNA base composition and DNA–DNA reassociations were performed according to Golubev *et al.* (1989). Strains used in DNA–DNA reassociations are indicated in Table 1.

rDNA sequencing and phylogenetic analyses. The D1/D2 domain of 26S rDNA was amplified using primers NL-1 and NL-4 (O'Donnell, 1993) and the ITS (ITS1, ITS2) and 5·8S rRNA gene regions were amplified using primers ITS1-F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990). Amplification was performed under the following conditions: an initial denaturing step of 5 min at 94 °C, followed by 35 cycles of 40 s at 94 °C, 40 s at 55 °C and 30 s at 72 °C, with a final extension step of 7 min at 72 °C. The PCR products were purified using the Wizard PCR purification kit (Promega) according to the manufacturer's instructions and sequenced directly by using a Thermo Sequenase Cy5 Dye Terminator kit (Amersham Pharmacia Biotech). The primers used to sequence both strands of the D1/D2 domain and ITS1–5·8S rDNA–ITS2 regions were the same as for PCR amplification. The thermal cycling conditions were as follows: 25 cycles of 30 s at 94 °C, 30 s at 50 °C and 1 min at 72 °C. Purified sequencing reactions were loaded on ALFexpress II automatic DNA sequencer (Amersham Pharmacia Biotech).

The D1/D2 and ITS1–5·8S rDNA–ITS2 sequences were aligned automatically using the multiple sequence alignment program CLUSTAL X 1.8 (Thompson *et al.*, 1997) and were adjusted manually. Positions where gaps existed in any of the aligned sequences were excluded. Further phylogenetic analyses employed the PHYLIP software package version 3.6 (Felsenstein, 2000). A distance matrix was obtained using DNADIST with Kimura's two-parameter method (Jin & Nei, 1990) and phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987). Stability of branches was assessed by bootstrap analysis (Felsenstein, 1985) in which 1000 replicates were used.

PCR-RFLP analysis of ITS. The ITS1–5·8S rDNA–ITS2 regions were amplified using primers ITS1 and ITS4 as described previously (Cadez *et al.*, 2002) and were digested directly with restriction enzymes *Dde*I (Promega), *Hin*fI (Roche) and *Mbo*II (Promega) according to the instructions of the manufacturers. The digests were separated on 3 % agarose gels in 1 × TAE buffer.

The molecular sizes of the ITS and 5·8S rDNA digests were determined by comparison to a DNA molecular marker (100 bp ladder; Gibco-BRL) using the Quantity One 4.0.3 (Bio-Rad) computer program.

RESULTS AND DISCUSSION

Genetic diversity

Initially, the genetic diversity of 14 strains of the genus *Hanseniaspora* isolated from various sources in Africa, North America and Hawaii was analysed using RAPD-PCR analysis in comparison with the type and other representative strains of *Hanseniaspora* and *Kloeckera* species (Table 1). UPGMA clustering of the combined RAPD-PCR fingerprints obtained with microsatellite primers $(ATG)_5$, $(GTG)_5$ and $(GACA)_4$ is shown in Fig. 1. The 14 strains segregated into five distinct groups, which clustered with the other *Hanseniaspora* and *Kloeckera* species at a similarity level below 40 %. On the basis of previous correlation between similarity values derived from RAPD-PCR analysis and DNA–DNA reassociations (Messner *et al.*, 1994; Molnár *et al.*, 1995), we predicted that the five groups were genetically divergent from currently accepted species of *Hanseniaspora*.

DNA relatedness

Subsequently, DNA–DNA reassociation analysis of strains of the five genetically divergent groups (I–V) was performed. Of group I, four selected isolates from South Africa, Hawaii and Georgia (USA) exhibited DNA relatedness values ranging from 91 to 100 %. Relatedness of 100 % was observed within groups II and III, the latter consisting of two strains, CBS 8818^T and CBS 8819, isolated from a traditional tequila fermentation in Mexico and referred to by Lachance (1995) as atypical *Hanseniaspora guilliermondii* strains because of their lack of assimilation of 2-keto-D-gluconate. Among the Hawaii isolates, CBS 8733^T and CBS 8820 of group IV, a relatedness value of 97 % was observed. Group V included only a single strain, CBS 8772, isolated from orange juice concentrate in Georgia (USA). Detailed DNA relatedness data for strains of groups I–V are available as supplementary material in IJSEM Online.

In Table 2, the mutual DNA relatedness values of the five groups and the seven currently accepted *Hanseniaspora* and *Kloeckera* species are shown. Mutual DNA relatedness values were usually low, ranging from 1 to 40 %. However, several intermediate values were found among groups; 62 % between groups I and II, 66 % between groups IV and V and 51 and 53 %, respectively, between group III and groups IV and V. From these values, it can be concluded that the groups represent five novel species, and, from the intermediate similarity values in particular, it can be inferred that groups I and II and groups III, IV and V represent two groups of diverging species complexes.

Table 1. Strains studied

Culture collections are abbreviated as follows: CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; NCAIM, National Collection of Agricultural and Industrial Microorganisms, Budapest, Hungary; NRRL, ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA; UWO-PS, University of Western Ontario Plant Sciences Yeast Culture Collection, London, Ontario, Canada. Standard deviations of G+C content determinations were ±0·5 mol%. ND, Not determined; NK, not known.

Strain	Origin	G+C content (mol%)	D1/D2 accession no.
***Hanseniaspora meyeri* sp. nov. (group I)**			
CBS 8734^{T}* (=UWO-PS 91-661.1^{T}=NRRL Y-27513^{T})	Fruit of *Sapindus* sp., Hawaii, USA	37	AJ512454
CBS 8771* (=NCAIM Y.725)	Spoiled grape punch, Georgia, USA	37·2	
CBS 8773*	Flower from *Schotia* sp. tree, South Africa	37·4	
CBS 8774	Flower from *Schotia* sp. tree, South Africa	ND	
CBS 8775	Flower from *Schotia* sp. tree, South Africa	ND	
CBS 8815* (=UWO-PS 91-643.1)	Drosophilid from *Sapindus* sp. berries, Hawaii, USA	36·6	AJ512458
CBS 8823 (=UWO-PS 91-732.2)	Drosophilid from *Sapindus* sp. berries, Hawaii, USA	ND	
UWO-PS 87-2361.1	Stem rot, *Clermontia* sp., Hawaii, USA	ND	AJ512461
UWO-PS 91-637.1	Drosophilid from *Sapindus* sp. berries, Hawaii, USA	ND	AJ512460
***Hanseniaspora clermontiae* sp. nov. (group II)**			
CBS 8821^{T}* (=UWO-PS 87-2370.1^{T}=NRRL Y-27515^{T})	Stem rot, *Clermontia* sp., Hawaii, USA	35·7	AJ512452
CBS 8822* (=UWO-PS 87-2440.2)	Stem rot, *Clermontia* sp., Hawaii, USA	37·2	AJ512456
***Hanseniaspora lachancei* sp. nov. (group III)**			
CBS 8818^{T}* (=UWO-PS 92-218.1^{T}=NRRL Y-27514^{T})	Fermenting agave juice, Mexico	34·8	AJ512457
CBS 8819* (=UWO-PS 92-232.4)	*Drosophila* sp., fermenting agave juice, Mexico	35·6	
UWO-PS 92-221.1	Fermenting agave juice, Mexico	ND	AJ512459
***Hanseniaspora opuntiae* sp. nov. (group IV)**			
CBS 8733^{T}* (=UWO-PS 87-2121.3^{T}=NRRL Y-27512^{T})	*Opuntia ficus-indica* rot, Hawaii, USA	33·6	AJ512453
CBS 8820* (=UWO-PS 87-2120.3)	*Opuntia ficus-indica* rot, Hawaii, USA	35·3	AJ512451
***Hanseniaspora* sp. (group V)**			
CBS 8772* (NCAIM Y.741)	Orange juice concentrate, Georgia, USA	31·5	AJ512455
Hanseniaspora guilliermondii			
CBS 95	Fermenting bottled tomatoes, The Netherlands	33·4[a]†	
CBS 465^{T}*	Infected nail, South Africa	33·2[a]	
CBS 2591*	Trachea of bee, France	33·9[a]	

Table 1. cont.

Strain	Origin	G+C content (mol%)	D1/D2 accession no.
Hanseniaspora occidentalis			
CBS 282	Soil, Java, Indonesia	34·9[a]	
CBS 283	Soil, Java, Indonesia	35·6[a]	
CBS 2592^{T}*	Soil, West Indies (St Croix)	34·9[a]	
Hanseniaspora osmophila			
CBS 106*	Bark of tree, Denmark	40·3	
CBS 313^{T}*	Ripe Reisling grape, Germany	40·5[a]	
CBS 1999	Soil, West Indies (St Croix)	40·5[a]	
NCAIM Y.726	Pineapple juice concentrate, Georgia, USA	ND	
Hanseniaspora uvarum			
CBS 314^{T}*	Muscatel grape, Crimea, Ukraine	33·9[a]	
CBS 279	Institute of Brewing, Tokyo	34·4[a]	
CBS 2570*	*Drosophila* sp., Brazil	34·7	
CBS 2584	NK	34·4[a]	
CBS 2585	Sour dough, Portugal	32·9[a]	
Hanseniaspora valbyensis			
CBS 479^{T}*	Soil, Germany	30·0[a]	
CBS 281	Sap of tree, Japan	29·0[a]	
CBS 6558	Pulque, Mexico	29·0[a]	
Hanseniaspora vineae			
CBS 277*	Soil, Algeria	38·8[a]	
CBS 2171^{T}*	Soil of vineyard, South Africa	40·2[a]	
CBS 2568	*Drosophila persimilis*	40·5[a]	
CBS 8031	Black knot gall on *Prunus virginiana*, Canada	39·6[b]	
Kloeckera lindneri			
CBS 285^{T}*	Soil, Java, Indonesia	34·8	

*Strain used in DNA–DNA reassociation analysis.

†Data from Meyer *et al.* (1978) (*a*) and Smith & Poot (1985) (*b*).

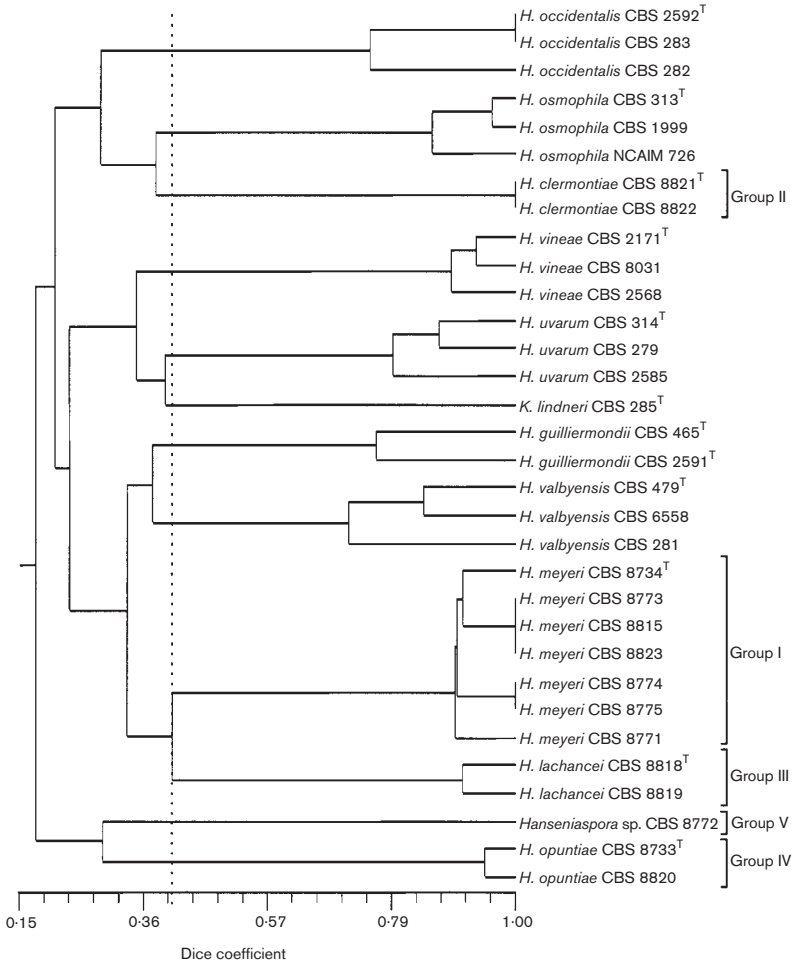

Fig. 1. UPGMA dendrogram of *Hanseniaspora–Kloeckera* strains based on combined RAPD-PCR fingerprints obtained with microsatellite primers $(ATG)_5$, $(GTG)_5$ and $(GACA)_4$. The distance between strains was calculated using the Dice coefficient. The dotted line indicates a similarity level of 40 %.

Sequence analysis and phylogenetic placement

The phylogenetic relationships of the four novel species and strain CBS 8772 to other *Hanseniaspora* and *Kloeckera* species were determined by sequence comparisons. The D1/D2 sequences of 26S rDNA of 11 selected strains were determined (GenBank accession numbers shown in Table 1) and aligned with those of the other *Hanseniaspora*

Table 2. DNA–DNA relatedness among taxa of *Hanseniaspora* and *Kloeckera*

Values are mean percentages of at least two determinations. The standard deviation for levels of reassociation within and among groups was $\leqslant 5$ %.

Source of DNA	1	2	3	4	5	6	7	8	9	10	11	12
1. *H. occidentalis*	–											
2. *H. vineae*	10	100										
3. *H. osmophila*	14	33	96									
4. *H. valbyensis*	11	28	24	100								
5. *K. lindneri*	21	24	14	37	–							
6. *H. uvarum*	7	9	12	20	28	99						
7. *H. guilliermondii*	8	12	13	15	33	22	100					
8. *H. meyeri* sp. nov. (group I)	25	22	26	22	36	23	18	98				
9. *H. clermontiae* sp. nov. (group II)	11	13	19	33	31	25	40	62	100			
10. *H. lachancei* sp. nov. (group III)	29	30	14	20	15	20	29	29	15	100		
11. *H. opuntiae* sp. nov. (group IV)	1	6	15	17	19	17	35	22	22	53	97	
12. *Hanseniaspora* sp. (group V)	30	16	7	21	21	31	19	24	34	51	66	–

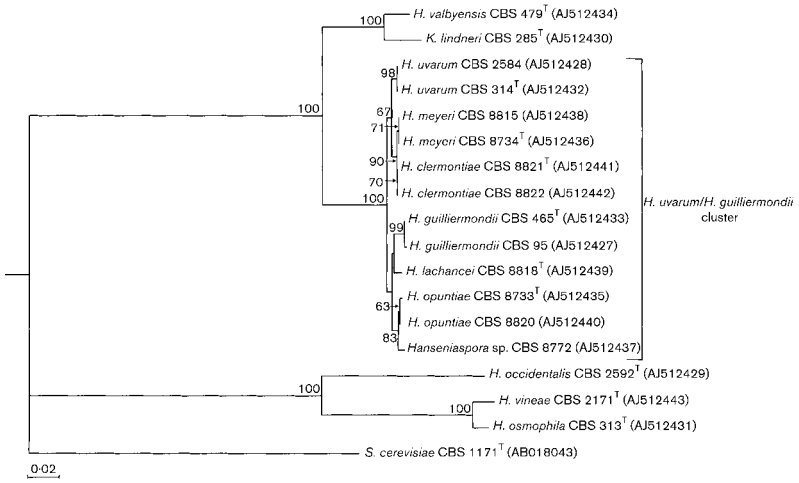

Fig. 2. Phylogenetic tree showing the relationships of *Hanseniaspora–Kloeckera* species inferred from ITS1–5·8S rDNA–ITS2 sequences. The tree was constructed by using the neighbour-joining analysis. Bootstrap percentages above 50 % from 1000 bootstrap replicates are shown. The outgroup species was *Saccharomyces cerevisiae*. Bar, 2 % sequence divergence.

and *Kloeckera* type strains. Pairwise comparisons revealed that sequence divergences among the six species and strain CBS 8772 belonging to the *H. guilliermondii–H. uvarum* cluster did not exceed 1 % (6 nucleotide substitutions), a value suggested by Kurtzman & Robnett (1998) to be the borderline of species separation. Nucleotide substitutions of more than 1 % are indicative of separate taxa, while differences of 0–3 nucleotides seem to be indicative of conspecificity or sister species. However, DNA–DNA reassociation may, as in the present case, detect genetic divergence that is not detectable by sequencing. The study of Boekhout *et al.* (1994) showed that *H. uvarum* and *H. guilliermondii*, species sharing low DNA relatedness [11–29 % (Meyer *et al.*, 1978); 22 %, this study], had sequence divergence in the D1/D2 region of only 1 % (6 base substitutions). Consequently, the rates for D1/D2 substitutions between the recently diverged species complexes *Hanseniaspora meyeri* sp. nov.–*Hanseniaspora clermontiae* sp. nov. and *Hanseniaspora lachancei* sp. nov.–*Hanseniaspora opuntiae* sp. nov. were proportionally lower, as *H. clermontiae* differed by only two base substitutions (0·35 % sequence divergence) from *H. meyeri* and *H. opuntiae* differed by three base substitutions (0·5 % sequence divergence) from *H. lachancei*. Strain CBS 8772, a sister taxon to the species complex *H. opuntiae–H. lachancei*, differed by four (0·7 % sequence divergence) and five (0·9 % sequence divergence) base substitutions, respectively, from *H. opuntiae* and *H. lachancei*. The D1/D2-based phylogenetic tree is available as Supplementary Fig. A in IJSEM Online.

The placement of the four novel species and CBS 8772 within the *H. uvarum–H. guilliermondii* cluster by D1/D2 sequence analysis was found to have a low level of statistical support (bootstrap values <70 %) and, therefore, the non-coding ITS regions, with higher rates of sequence substitution, were applied to resolve the phylogenetic position of these very closely related yeasts (James *et al.*, 1996; Oda *et al.*, 1997).

The ITS1–5·8S rDNA–ITS2 sequences of the type and other strains of *Hanseniaspora–Kloeckera* species were determined and used to construct a phylogenetic tree by the neighbour-joining method (Fig. 2). Our tree topology is consistent with that reported previously (Esteve-Zarzoso *et al.*, 2001). A high bootstrap value (100 %) supported the placement of *H. meyeri* and *H. clermontiae* in close relationship to *H. uvarum* and *H. lachancei*, *H. opuntiae* and strain CBS 8772 to *H. guilliermondii*, which is in agreement with phenotypic similarities and DNA reassociation results (Table 2). The short branches imply very close phylogenetic relationships between species of the *H. uvarum–H. guilliermondii* cluster. Detailed sequence analysis of the ITS1 and ITS2 regions of the genetically closer pairs revealed that *H. meyeri* differed from *H. clermontiae* (57–66 % DNA relatedness) by a single base substitution, *H. lachancei* differed from *H. opuntiae* (53–55 % DNA relatedness) by nine base substitutions and *Hanseniaspora vineae* differed from *Hanseniaspora osmophila* [38–60 % DNA relatedness (Meyer *et al.*, 1978); 33 %, this study] by 17 base substitutions. This lack of correspondence between the degree of sequence divergence and DNA reassociation data for *Hanseniaspora* species supports the observation of Seifert *et al.* (1995), that the rate of evolution in the ITS region varies from species to species.

Identification

The diagnostic key for identification of *Hanseniaspora* and *Kloeckera* species using standard yeast identification procedures was supplemented from Smith (1998b) and Barnett *et al.* (2000) as shown in Table 3.

H. lachancei can be distinguished from *H. guilliermondii*, *H. opuntiae* and strain CBS 8772 by its ability to assimilate 2-keto-D-gluconate as a sole carbon source and from the other *Hanseniaspora* and *Kloeckera* species by the presence of growth at 37 °C. *H. clermontiae* can be differentiated from its sister species *H. meyeri* and *H. uvarum* by the absence of growth at 30 °C and from *Hanseniaspora valbyensis* by its ability to assimilate 2-keto-D-gluconate. Conventional

Table 3. Diagnostic key for identification of *Hanseniaspora* and *Kloeckera* species

Key	
1. Growth at 37 °C	
a. Present	2
b. Absent	3
2. 2-Keto-D-gluconate assimilated	
a. Yes	*H. guilliermondii*/*H. opuntiae*/CBS 8772*
b. No	*H. lachancei*
3. Sucrose fermented	
a. Yes	*H. occidentalis*
b. No	4
4. 2-Keto-D-gluconate assimilated	
a. Yes	5
b. No	6
5. Growth at 30 °C	
a. Yes	*H. uvarum*/*H. meyeri**
b. No	*H. clermontiae*
6. Growth with 0·01 % cycloheximide	
a. No	*H. vineae*/*H. osmophila**
b. Yes	*H. valbyensis*/*K. lindneri**

*Species can be differentiated from one another either by rDNA sequencing or by PCR-RFLP of ITS regions (see Supplementary Fig. B in IJSEM Online).

physiological criteria cannot distinguish *H. meyeri* from *H. uvarum* or *H. opuntiae* and strain CBS 8772 from *H. guilliermondii*. For accurate identification of these species, sequencing of the ITS regions is needed. As an alternative to sequencing, a rapid identification method, PCR-RFLP of ITS regions, can be applied. Restriction of the ITS regions with enzyme *Mbo*II separates *H. meyeri* from *H. uvarum* or *H. opuntiae* from *H. guilliermondii* and *Hin*fI separates strain CBS 8772 from *H. guilliermondii* (Supplementary Fig. B in IJSEM Online).

Species descriptions

Of the five novel species, only four are described below. One taxon, represented by a single strain, will not be introduced until additional isolates are available. Nevertheless, it is included in the identification key.

Group I

Latin diagnosis of *Hanseniaspora meyeri* sp. nov.

In medio liquido post 48 *horas* 25 °C *cellulae apiculatae, ovoideae vel elongatae*, 2·5–12·5 × 1·5–6·0 μm, *singulae vel binae*; *gemmatione bipolari repruducentes. Post unum mensem annulus tenuis et sedimentum formantur. In agaro farina Zeae maydis confecto pseudomycelium rudimentarium. In quoque asco* 2–4 *ascosporae petasiformes. Glucosum et cellobiosum fermentantur. Glucosum, cellobiosum, salicinum, arbutinum, glucono-δ-lactonum,* 2-*ketogluconatum,* D-*glucitolum, ethylaminum, lysinum et cadaverinum assimilantur. Non assimilantur galactosum,* L-*sorbosum,* D-*glucosaminum,* D-*ribosum,* D-*xylosum,* L-*arabinosum,* D-*arabinosum,* L-*rhamnosum, sucrosum, maltosum, trehalosum, methyl* α-D-*glucosidum, melibiosum, lactosum, raffinosum, melezitosum, amylum solubile, glycerolum, erythritolum, ribitolum, xylitolum,* L-*arabinitolum,* D-*glucitolum,* D-*mannitolum, galactitolum, inositolum, acidum* D-*glucuronicum, acidum* D-*galacturonicum, acidum* DL-*lacticum, acidum succinicum, acidum citricum, methanolum et ethanolum. Maxima temperatura crescentiae* 30 °C. *Crescit in medio addito* 10 % NaCl, 50 % *glucoso et* 0·1 % *cycloheximido*. G+C *acidi deoxyribonucleati* 36·6–37·4 mol%. *Typus*: CBS 8734^T (=NRRL Y-27513^T) *ex pomo Sapindi* sp. *in* Hawaii, *isolatus. In collectione zymotica* Centraalbureau voor Schimmelcultures, *Trajectum ad Rhenum lyophilus depositus.*

Description of *Hanseniaspora meyeri* sp. nov.

Hanseniaspora meyeri (mey′er.i. N.L. gen. n. *meyeri* honouring Piet Meyer, a young, promising South African scientist interested in taxonomy, who died too young).

In YM liquid medium after 48 h at 25 °C, cells are apiculate, ovoid to elongate, 2·5–12·5 × 1·5–6·0 μm, single or in pairs. Budding is bipolar. A sediment is present. After 1 month, a very thin ring is formed. After 1 month at 25 °C, the streak culture on malt agar is cream-coloured, butyrous, smooth, glossy, flat to slightly raised at the centre, with an entire to slightly undulate margin. On cornmeal agar, a rudimentary pseudomycelium is formed. Asci containing two to four hat-shaped ascospores are observed after 2 weeks on 5 % Difco malt extract agar at 25 °C. Glucose and cellobiose are fermented. The following carbon compounds are assimilated: glucose, cellobiose, salicin, arbutin, glucono-δ-lactone, 2-ketogluconate and D-gluconate. The following are not assimilated: galactose, L-sorbose, glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, melibiose, lactose, raffinose, melezitose, starch, glycerol, erythritol, ribitol, xylitol, L-arabinitol, D-glucitol, D-mannitol, galactitol, *myo*-inositol, D-glucuronate, D-galacturonate, DL-lactate, succinate, citrate, methanol and ethanol. Assimilation of nitrogen compounds: positive for ethylamine, lysine and cadaverine; negative for sodium nitrate and sodium nitrite. Growth at 30 °C is positive; no growth at 35 °C. Growth on YM agar with 10 % NaCl is positive. Growth on 50 % glucose (w/w)/yeast extract agar is weak. Growth in the presence of 0·1 % cycloheximide is positive. Diazonium blue B reaction is negative. G+C content of nDNA (T_m) is 36·6–37·4 mol%.

The type, CBS 8734^T (=NRRL Y-27513^T), was isolated from fruit of *Sapindus* sp., Hawaii. It has been deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Group II

Latin diagnosis of *Hanseniaspora clermontiae* sp. nov.

In medio liquido post 48 *horas* 25 °C *cellulae apiculatae, ovoideae vel elongatae,* 3·5–18·0 × 2·5–5·0 μm, *singulae vel binae; gemmatione bipolari repruducentes. Post unum mensem annulus tenuis et sedimentum formantur. In agaro farina Zeae maydis confecto pseudomycelium rudimentarium. In quoque asco,* 2–4 *ascosporae petasiformes. Glucosum et cellobiosum fermentantur. Glucosum, cellobiosum, salicinum, arbutinum, glucono-δ-lactonum,* 2-*ketogluconatum,* D-*glucitolum, ethylaminum, lysinum et cadaverinum assimilantur. Non assimilantur galactosum,* L-*sorbosum,* D-*glucosaminum,* D-*ribosum,* D-*xylosum,* L-*arabinosum,* D-*arabinosum,* L-*rhamnosum, sucrosum, maltosum, trehalosum, methyl* α-D-*glucosidum, melibiosum, lactosum, raffinosum, melezitosum, amylum solubile, glycerolum, erythritolum, ribitolum, xylitolum,* L-*arabinitolum,* D-*glucitolum,* D-*mannitolum, galactitolum, inositolum, acidum* D-*glucuronicum, acidum* D-*galacturonicum, acidum* DL-*lacticum, acidum succinicum, acidum citricum, methanolum et ethanolum. Maxima temperatura crescentiae* 25 °C. *Crescit in medio addito* 10 % NaCl, 50 % *glucoso et* 0·1 % *cycloheximido.* G + C *acidi deoxyribonucleati* 35·7–37·2 mol%. *Typus*: CBS 8821^T (= NRRL Y-27515^T) *isolatus ex infecta Clermontia* sp. *in* Hawaii. *In collectione zymotica* Centraalbureau voor Schimmelcultures, *Trajectum ad Rhenum lyophilus depositus.*

Description of *Hanseniaspora clermontiae* sp. nov.

Hanseniaspora clermontiae (cler.mon′ti.ae. N.L. gen. n. *clermontiae* of *Clermontia*, the genus name of the host plant).

In YM liquid medium after 48 h at 25 °C, cells are apiculate, ovoid to elongate, 3·5–18·0 × 2·5–5·0 μm, single or in pairs. Budding is bipolar. A sediment is present. After 1 month, a very thin ring is formed. After 1 month at 25 °C, the streak culture on malt agar is cream-coloured, butyrous, smooth, glossy, flat to slightly raised at the centre, with an entire to slightly undulate margin. On cornmeal agar, a rudimentary pseudomycelium is formed. Asci containing two to four hat-shaped ascospores are observed after 2 weeks on 5 % Difco malt extract agar at 25 °C. Glucose and cellobiose are fermented. The following carbon compounds are assimilated: glucose, cellobiose, salicin, arbutin, glucono-δ-lactone, 2-ketogluconate and D-gluconate. The following are not assimilated: galactose, L-sorbose, glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, melibiose, lactose, raffinose, melezitose, starch, glycerol, erythritol, ribitol, xylitol, L-arabinitol, D-glucitol, D-mannitol, galactitol, *myo*-inositol, D-glucuronate, D-galacturonate, DL-lactate, succinate, citrate, methanol and ethanol. Assimilation of nitrogen compounds: positive for ethylamine, lysine and cadaverine; negative for sodium nitrate and sodium nitrite. Growth at 25 °C is positive; no growth at 30 °C. Growth on YM agar with 10 % NaCl is positive. Growth on 50 % glucose (w/w)/yeast extract agar is weak. Growth in the presence of 0·1 % cycloheximide is positive. Diazonium blue B reaction is negative. G + C content of nDNA (T_m) is 35·7–37·2 mol%.

The type, CBS 8821^T (= NRRL Y-27515^T), was isolated from *Clermontia* sp. rot, Hawaii. It has been deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Group III

Latin diagnosis of *Hanseniaspora lachancei* sp. nov.

In medio liquido post 48 *horas* 25 °C *cellulae apiculatae, ovoideae vel elongatae,* 2·5–18·5 × 1·0–5·5 μm, *singulae vel binae; gemmatione bipolari repruducentes. Post unum mensem annulus tenuis et sedimentum formantur. In agaro farina Zeae maydis confecto pseudomycelium rudimentarium. In quoque asco,* 4 *ascosporae petasiformes. Glucosum et cellobiosum fermentantur. Glucosum, cellobiosum, salicinum, arbutinum, glucono-δ-lactonum, acidum gluconicum, ethylaminum, lysinum et cadaverinum assimilantur. Non assimilantur galactosum,* L-*sorbosum,* D-*glucosaminum,* D-*ribosum,* D-*xylosum,* L-*arabinosum,* D-*arabinosum,* L-*rhamnosum, sucrosum, maltosum, trehalosum, methyl* α-D-*glucosidum, melibiosum, lactosum, raffinosum, melezitosum, amylum solubile, glycerolum, erythritolum, ribitolum, xylitolum,* L-*arabinitolum,* D-*glucitolum,* D-*mannitolum, galactitolum, inositolum,* 2-*ketogluconatum, acidum* D-*glucuronicum, acidum* D-*galacturonicum, acidum* DL-*lacticum, acidum succinicum, acidum citricum, methanolum et ethanolum. Maxima temperatura crescentiae* 37 °C. *Crescit in medio addito* 10 % NaCl, 50 % *glucoso et* 0·1 % *cycloheximido.* G + C *acidi deoxyribonucleati* 34·8–35·6 mol%. *Typus*: CBS 8818^T (= NRRL Y-27514^T) *isolatus e Agaves fermentatum in* Mexico. *In collectione zymotica* Centraalbureau voor Schimmelcultures, *Trajectum ad Rhenum lyophilus depositus.*

Description of *Hanseniaspora lachancei* sp. nov.

Hanseniaspora lachancei (la.chan′ce.i. N.L. gen. n. *lachancei* honouring M.-A. Lachance, for his enormous contributions to yeast taxonomy and ecology).

In YM liquid medium after 48 h at 25 °C, cells are apiculate, ovoid to elongate, 2·5–18·5 × 1·0–5·5 μm, single or in pairs. Budding is bipolar. Sediment is present. After 1 month, a very thin ring and a sediment are formed. After 1 month at 25 °C, the streak culture on malt agar is cream-coloured, butyrous, smooth, glossy, flat to slightly raised at the centre, with an entire to slightly undulate margin. On

cornmeal agar, a rudimentary pseudomycelium is formed. Asci containing four hat-shaped ascospores are observed on 5 % Difco malt extract agar at 25 °C. Glucose and cellobiose are fermented. The following carbon compounds are assimilated: glucose, cellobiose, salicin, arbutin, glucono-δ-lactone and D-gluconate. The following are not assimilated: galactose, L-sorbose, glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, melibiose, lactose, raffinose, melezitose, starch, glycerol, erythritol, ribitol, xylitol, L-arabinitol, D-glucitol, D-mannitol, galactitol, *myo*-inositol, 2-ketogluconate, D-glucuronate, D-galacturonate, DL-lactate, succinate, citrate, methanol and ethanol. Assimilation of nitrogen compounds: positive for ethylamine, lysine and cadaverine; negative for sodium nitrate and sodium nitrite. Growth at 37 °C is positive, growth at 40 °C is negative. Growth on YM agar with 10 % NaCl is positive. Growth on 50 % glucose (w/w)/yeast extract agar is weak. Growth in the presence of 0·1 % cycloheximide is positive. Diazonium blue B reaction is negative. G+C content of nDNA (T_m) is 34·8–35·6 mol%.

The type, CBS 8818^T (=NRRL Y-27514^T), was isolated from fermenting agave juice, Mexico. It has been deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Group IV

Latin diagnosis of *Hanseniaspora opuntiae* sp. nov.

In medio liquido post 48 *horas* 25 °C *cellulae apiculatae, ovoideae vel elongatae,* 3·0–16·0 × 1·5–5·0 μm, *singulae vel binae; gemmatione bipolari repruducentes. Post unum mensem annulus tenuis et sedimentum formantur. In agaro farina Zeae maydis confectopseudomycelium rudimentarium. In quoque asco,* 4 *ascosporae petasiformes. Glucosum et cellobiosum fermentantur. Glucosum, cellobiosum, salicinum, arbutinum, glucono-δ-lactonum,* 2-*ketogluconatum, acidum gluconicum, ethylaminum, lysinum et cadaverinum assimilantur. Non assimilantur galactosum,* L-*sorbosum,* D-*glucosaminum,* D-*ribosum,* D-*xylosum,* L-*arabinosum,* D-*arabinosum,* L-*rhamnosum,* s*ucrosum, maltosum, trehalosum, methyl* α-D-*glucosidum, melibiosum, lactosum, raffinosum, melezitosum, amylum solubile, glycerolum, erythritolum, ribitolum, xylitolum,* L-*arabinitolum,* D-*glucitolum,* D-*mannitolum, galactitolum, inositolum, acidum* D-*glucuronicum, acidum* D-*galacturonicum, acidum* DL-*lacticum, acidum succinicum, acidum citricum, methanolum et ethanolum. Maxima temperatura crescentiae* 37 °C. *Crescit in medio addito* 10 % NaCl, 50 % *glucoso et* 0·1 % *cycloheximido.* G+C *acidi deoxyribonucleati* 33·6–35·3 mol%. *Typus*: CBS 8733^T (=NRRL Y-27512^T) *isolatus ex Opuntia megacantha in* Hawaii. *In collectione zymotica* Centraalbureau voor Schimmelcultures, *Trajectum ad Rhenum lyophilus depositus.*

Description of *Hanseniaspora opuntiae* sp. nov.

Hanseniaspora opuntiae (op.un′ti.ae. N.L. gen. n. *opuntiae* of *Opuntia*, the genus name of the host plant).

In YM liquid medium after 48 h at 25 °C, cells are apiculate, ovoid to elongate, 3·0–16·0 × 1·5–5·0 μm, single or in pairs. Budding is bipolar. Sediment is present. After 1 month, a very thin ring and a sediment are formed. After 1 month at 25 °C, the streak culture on malt agar is cream-coloured, butyrous, smooth, glossy, flat to slightly raised at the centre, with an entire to slightly undulate margin. On cornmeal agar, a rudimentary pseudomycelium is formed. Asci containing four hat-shaped ascospores are observed on 5 % Difco malt extract agar at 25 °C. Glucose and cellobiose are fermented. The following carbon compounds are assimilated: glucose, cellobiose, salicin, arbutin, glucono-δ-lactone, 2-ketogluconate and D-gluconate. The following are not assimilated: galactose, L-sorbose, glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, melibiose, lactose, raffinose, melezitose, starch, glycerol, erythritol, ribitol, xylitol, L-arabinitol, D-glucitol, D-mannitol, galactitol, *myo*-inositol, D-glucuronate, D-galacturonate, DL-lactate, succinate, citrate, methanol and ethanol. Assimilation of nitrogen compounds: positive for ethylamine, lysine and cadaverine; negative for sodium nitrate and sodium nitrite. Growth at 37 °C is positive, growth at 40 °C is negative. Growth on YM agar with 10 % NaCl is positive. Growth on 50 % glucose (w/w)/yeast extract agar is weak. Growth in the presence of 0·1 % cycloheximide is positive. Diazonium blue B reaction is negative. G+C content of nDNA (T_m) is 33·6–35·3 mol%.

The type, CBS 8733^T (=NRRL Y-27512^T), was isolated from *Opuntia megacantha* in Hawaii. It has been deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

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