# Identification of Medically Important *Candida* and Non-*Candida* Yeast Species by an Oligonucleotide Array<sup>∇</sup>

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The incidence of yeast infections has increased in the recent decades, with *Candida albicans* still being the most common cause of infections. However, infections caused by less common yeasts have been widely reported in recent years. Based on the internal transcribed spacer 1 (ITS 1) and ITS 2 sequences of the rRNA genes, an oligonucleotide array was developed to identify 77 species of clinically relevant yeasts belonging to 16 genera. The ITS regions were amplified by PCR with a pair of fungus-specific primers, followed by hybridization of the digoxigenin-labeled PCR product to a panel of oligonucleotide probes immobilized on a nylon membrane for species identification. A collection of 452 yeast strains (419 target and 33 nontarget strains) was tested, and a sensitivity of 100% and a specificity of 97% were obtained by the array. The detection limit of the array was 10 pg of yeast genomic DNA per assay. In conclusion, yeast identification by the present method is highly reliable and can be used as an alternative to the conventional identification methods. The whole procedure can be finished within 24 h, starting from isolated colonies.

Fungal infections have increased in incidence in recent decades, often as a result of advanced medical treatments and the increase in the number of immunocompromised patients. Candida albicans is still the most frequent cause of fungal infections. However, the use of broad-spectrum antibiotics and antifungal agents for prophylaxis has led to a shift in the epidemiology and etiology of Candida and non-Candida yeast species infections (36, 37). Infections caused by non-Candida albicans and other less-common emerging yeasts, such as Cryptococcus, Pichia, Rhodotorula, Saccharomyces, and Trichosporon, have been widely reported in recent years (8, 12, 13, 19, 31, 33, 34, 45). The identification of yeast pathogens with this increasing diversity by conventional methods may be difficult and sometimes inconclusive (6). The introduction of reliable methods that have the potential to identify a wide and taxonomically diverse array of opportunistic yeasts is imperative, since some emerging or less common species may have quite different susceptibilities to antifungal agents (16, 41, 43).

Commercially available yeast identification systems, such as the Vitek Yeast Biochemical Card (bioMérieux Vitek, Taipei, Taiwan), API 20C (bioMérieux), and API ID32C (bio-Mérieux), are convenient to use. However, an incubation period of 24 to 48 h is normally required before biochemical reactions can be interpreted (14). In addition to the biochemical tests contained in these two kits, supplementary tests are occasionally required before a final identification can be obtained. While these commercial products are effective for the

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identification of commonly encountered yeasts, their application is somewhat more limited for the identification of less frequently recovered taxa (11, 40). These limitations are probably attributable, in part, to the databases currently employed in the profile indexes. Misidentifications of some species by commercial kits have been reported (7, 11, 28, 29, 40), and even the well-known and medically important yeast *Candida glabrata* has been misidentified by phenotypic methods (7).

DNA-based methods used to identify a variety of yeasts have been developed (4). These molecular methods include length polymorphism analysis of the internal transcribed spacer (ITS) regions of the rRNA gene (2, 26, 49), restriction fragment length polymorphism analysis (28, 47), probe hybridization (7, 10, 38), and DNA sequencing (6, 15, 17, 24, 39). Although these methods have been proved to be accurate, a common limitation of them is that only a limited number of species can be analyzed. Microarray platforms that can simultaneously analyze hundreds or thousands of targets may have the potential to identify a wide spectrum of yeasts with high sensitivity and specificity. In the past few years, DNA array technology has been used to identify a variety of yeasts and molds (20, 21, 25). However, less than 10 yeast species were included in the arrays in studies by Huang et al. (21) and Leinberger et al. (25).

In this study, an oligonucleotide array targeting the fungal ITS regions was developed to identify 77 yeast species (16 genera) of clinical importance. Instead of the detection of fluorescence intensity after hybridization, colorimetric detection was used in this study, and nylon membranes instead of glass slides were used as the solid supports for oligonucleotide probes.

#### MATERIALS AND METHODS

Yeast strains. A total of 419 target strains, representing 44 *Candida* species (275 strains) and 33 non-*Candida* species (144 strains), were used for identifi-

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TABLE 1. I	Reference	strains	and	clinical	isolates	used	in	this st	udy
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Candida spr.         Candida spr.         2005, CBS 2718, 2705, 9701, 641, 0599         431, 1000, 2514, 3321, 3434, 3623, 4623, 263         28           Candida conserves         2005, CBS 2718, 2705, 9701, 643, 1045, 2175, 2114, 0599         430, 0000, 214, 3321, 3434, 3125, 3434, 3623, 363         3           Candida conserves         Candida conserves         Candida conserves         430, 1000, 2514, 3321, 3434, 3423, 263, 425, 363         3           Candida conserves         Candida conserves         Candida conserves         Candida conserves         430, 1000, 2514, 3321, 3434, 3623, 463, 943         3           Candida conserves	Species (teleomorph) <sup>a</sup>	Reference strain(s) <sup><math>b</math></sup>	Clinical isolate(s)	Total no. of strains	
Candida ableons         ECK 2051, 12612, 2051, 20518, 2059, 2158, 34, 1009, 2158, 34, 1009, 2158, 34, 1009, 2164, 321, 344, 3023, 3653, 4252, 258         2001050, FB 1322, 1234, 1038, 2059, 205	Candida spp.				
Landah nabahi Candida carbanian Gandida carbanian Candida car	Candida albicans	BCRC 20511, 20512 <sup>T</sup> , 20513, 20518, 20519, 21538, 22063, CBS 2718, 2730, 5990, 6431, 6589	343, 1009, 2514, 3321, 3434, 3623, 3653, 4252, 4339, ATTC 66390, LMA 938838, 962507,	28	
Conditio commoniti         ECKC 21037 (2107 (2105 (2105 7, 2017 (2107	Candida boidinii Candida cacaoi (Yamadazyma farinosa)	BCRC 20464 <sup>+</sup> , 20472, 21432, 21483, 21757 BCRC 21368 <sup>T</sup> 21682 21881	499010350, RB 1325, 1326, 1331	3	
Candida catomidan         BCR C 2159, 2214         CBR 54, 55, 104         5           Candida catomidan         Condida catomidant (Transcryon diversity)         FCR C 2145, 22174         CBR 515, 691         5           Candida catomida control         CBR 2149, 22174         CBR 515, 691         5           Candida dattiti (Lachurea thermotherms)         CBR 2149, 22174         CBR 515, 691         5           Candida gibma         CCR 2145, 22174         CBR 504, 581, 591         7           Candida gibma         CCR 2155, CTR 586, 584, 691         7         7           Candida gibma         CCR 2155, CTR 586, 584, 691, 175, 700         7         75           Candida gibma         CCR 2155, CTR 586, 586, 1217, 700         7         75, 785, 785, 786, 786, 786, 1217, 700         7           Candida gibma         CCR 2155, 217, 1216, 175, 223         CR 1101, 152, 1167, 154, 1255, 1158, 2155, 2217         CR 1101, 152, 1167, 154, 1255, 1167, 154, 1255, 2155, 2217, 12177, 1217, 1217, 1217, 1217, 1217, 1217, 1217, 1217, 1217, 1217, 12	Candida cantarelli	BCRC 21613 <sup>T</sup> , CBS 5383, 5445, 5654		4	
Candid a collication (Terminaporo)         IRCR C 2149, 22774 (2B 518, 091)         4           Candid a duble (Lochnere Mernorekorm)         IRCR C 2149, 2717 (2B 77, 2018, 280, 2017         5           Candid a duble (Lochnere Mernorekorm)         IRCR C 2149, 2717 (2D 179, 1797 (2D 17	Candida catenulata Candida chodatii (Pichia hurtonii)	BCRC 21507, 22316 <sup>T</sup> , CBS 564, 565, 1904		5	
Candid adulti (Lachance) thermologram)         BCRC 22043, CBS 157, 2803, 2800, 2907         S           Candid adulticitumi         CBS 2747, 787, 789, 788, 800, 850         RB 1108, 1271, 1306         S           Candid adulticitumi         CBS 2747, 787, 789, 788, 789, 850, 850, 812, 173, 7307         1702, 9786, 9787, LMA 901085, 905756, 9457           Candid gibbra         CBR CC 2085, CBS 808, 801, 217, 7503         1702, 9786, 9787, LMA 901085, 905756, 14           Candid gibbra         CBR CC 2084, 21549, 21559         RB 1102, 1055, 126         7           Candid gibbra         CBR CC 2085, CBS 808, 801, 217, 7807         78         78           Candid gibbra         CBR CC 2085, CBS 99, 1735, 233         LMA 90288, RB 1226         6           Candid haremutoni         CBR CC 2085, 2159, 2160, 2155, 22097         LMA 901282, 98505, 98579, 98579, 94459, 11         11           Candid haremutoni         CBR CC 2084, 21541, 21542, 21596         LMA 901282, 945015, 948501, 13         11           Candid haremutoni         CBR CC 2084, 21541, 21542, 21596         LMA 901282, 945015, 948501, 14         14           Candid haremutoni         CBR CC 2085, 2157, 2107, 2127, 2208, 2209, 2204         223, 16462, LMA 91259, 945615, 948501, 14         14           Candid haremutoni         CBR CC 2085, 2157, 21741, 2158, 2159         LMA 90238, 40760, 947315, 948764, RB         11           Cand	Candida colliculosa (Torulaspora delbrueckii)	BCRC 22087, 22012 BCRC 21429, 22074 <sup>T</sup> , CBS 158, 6991		4	
Landad adamenting         CLS 2/47, 7987, 7987, 8201, 8201, 7997         KB 108, 127, 1306         8           Candida gabara         Candida gabara         CC 21355, 71218, 717, 7987, 7987, 7984, 7988, 7987, 7984, 7988, 7984, 7987, 7984, 7988, 7984, 7987, 7984, 7988, 7984, 7981, 7987, 7984, 7988, 7984, 7981, 7987, 7984, 7988, 7984, 7981, 7987, 7988, 7984, 7981, 7987, 7984, 798	Candida dattila (Lachancea thermotolerans)	BCRC 22043, CBS 1877, 2803, 2860, 2907		5	
Candid physichisal         DERC 21553         CIS 2161         2           Candid gibbua         DERC 21553         CIS 2161         1762,9796,9787, LMA 901085,90576,         4           Candid gibbua         CIRC 21553         CIS 2161         1762,9796,9787, LMA 901085,90576,         4           Candid gibbua         CIRC 21553         CIS 2167,2807,1810,2159         PB         1012,1055,1216         7           Candid gibbua         CIRC 21553         CIS 2167,2169,2159         PB         1012,1055,1216         7           Candid a homini (Kacacharia ariga)         CIRC 21557,2159,2160,22507         LMA 901285,9877,98770,94459,         4           Candida homini (Chela greentamanu)         CIRC 21547,2152,2159,2159         LMA 911225,945615,948501,         3           Candida homini (Chela greentamanu)         CIRC 21347,21677,21740,2155,2208,2109,2109         RB 1122         110           Candida membranifacion         CIRC 21347,21677,21742,2068,2109,2109         RB 1122         1283,1288,1294         11           Candida membranifacion         CIRC 21342,21677,21742,2159         CIRC 21345,22677,21742,2258,2159         LMA 91225,945615,948764, RB         11           Candida membranifacion         CIRC 21352,2174,21742,2159,21741,2159         CIRC 21352,2174,2174,2159,2174,21742,2159         CIRC 21352,21677,217412,2528,21047         1283,1289,2139,21741,25	Candida dubliniensis Candida famata (Debawomyces hansenii)	CBS 2747, 7987, 7988, 8500, 8501 BCRC 22304, 22712, CBS 1791, 1792, 1795 <sup>T</sup>	RB 1168, 1271, 1306	8	
Candida glabona         BCRC 20867, CBS 860, 60, 1275, 7007         1762, 9769, 9769, 716A 901085, 905766, 14           Candida glabona         BCRC 20867, CBS 860, 60, 1275, 7007         1762, 9769, 9765, 1524         2           Candida glabona         BCRC 20867, CBS 860, 60, 1275, 7007         1762, 9769, 9769, 1524         2           Candida functionical (Relovements)         BCRC 20867, 21649, 22567         104, 9028, 91256, 945615, 948501, 948	Candida freyschussii	BCRC 21555 <sup>T</sup> , CBS 2161		2	
Candida gibbosi (Letormyces matricess)         Usi 1a2, 1854         9453/4, 849, 1254, 1254, 1254         2           Candida gibbosi (Latormyces matricess)         Usi 1a2, 1854         9453/4, 849, 1255, 1216         3           Candida bolmi (Kazeristmia cigua)         Usi 1a2, 1854         1555, 1216         4           Candida bolmi (Kazeristmia cigua)         USI 1a2, 1859         11         3           Candida branei (Isateristika orientila)         USI 1a2, 1251, 2132, 2137, 2137, 1235, 2135, 2137         LMA 90289, RB 1226         6           Candida branei (Isateristika orientila)         USI 1a2, 1251, 2132, 2137, 2137, 12370, 2135, 2233         LMA 90289, RB 1226         6           Candida branei (Isateristika orientila)         USI 1a2, 1251, 2132, 2137, 2137, 1237, 2135, 21357         LMA 90289, RB 1226         6           Candida branei (Isateristika orientila)         USI 122, 1231, 2173, 2170, 2135, 2240         23         1646, 1441, 2151, 2154, 2159           Candida branei (Isateristika orientila)         USI 122, 2131, 2170, 2170, 2176, 2234, 2134, 124         23         11           Candida branei (Isateristika orientila)         USI 1254, 2159, 2154         LMA 932648, 947160, 947315, 948764, RB         11           Candida branei (Isateristika sentila)         USI 1255, 2239, 2154         LMA 932648, 947160, 947315, 948764, RB         11           Candida norienita         USI 1256,	Candida glabrata	BCRC 20586 <sup>T</sup> , CBS 860, 861, 2175, 7307	1762, 9796, 9787, LMA 901085, 905756,	14	
Candida homeniani         CRC 21572, 'CBS (597, 'N90, 'S90, 'S90	Candida globosa (Citeromyces matritensis) Candida guilliermondii (Pichia guilliermondii)	CBS $162^{\circ}$ , 864 BCRC 20862 21500 <sup>T</sup> 21549 21559	945574, RB 1284, 1295, 1324 RB 1012, 1055, 1216	2	
Candida holmin (Kazachsmia ergua)         BCR 2 (1537, 2199, 22000         3           Candida intermedia (Kayoromycs cellobiaroma)         BCR 2 (1537, 2199, 2104, 2157)         LMA 90289, RB 1226         6           Candida intermedia (Kayoromycs cellobiaroma)         BCR 2 (1537, 2199, 2114), 2153         LMA 90289, RB 1226         6           Candida krist (Kayoromycs cellobiaroma)         BCR 2 (1537, 2199, 2114), 2153         LMA 90289, RB 1226         6           Candida krist (Kayoromycs cellobiaroma)         BCR 2 (2017, 2108, 2107)         LMA 91232, 938657, 93879, 944459, 9473         11           Candida laghyfica (Varrowia lipobjeca)         BCR 2 (2014, 2159)         LMA 9028, RB 1226         6           Candida maloma         Candida maloma         CBS 514 <sup>1</sup> , 2132, 1217, 2179, 2179, 21796, 22242         2283, 14642, LMA 91256, 948501, 9401, 948501, 948501, 948501, 948501, 948501, 948501, 94	Candida haemulonii	BCRC $21572^{\text{T}}$ , CBS 6590, 7801, 7802	1012, 1055, 1210	4	
Control Constraint         BCK 2: 5105 7, 128 yr 41, 125, 233         EAN - 9125, 94561, 94857, 938779, 944459, 94764, 9481 227           Condida kerbyr (Kayreornycer marxiamb)         BCK 2: 5016, 2017, 721269, 1235, 22057         LMA - 911323, 93865, 938779, 944459, 94764, 818 227           Condida lambica (Pichi generatura)         BCK 2: 5016, 2017, 721269, 1235, 22042         2288, 16462, LMA 911256, 948561, 948501, 13           Condida mobiosica         BCK 2: 2017, 72126, 1270, 1270, 22342         2288, 16462, LMA 911256, 948674, RB         11           Condida mobiosica         BCK 2: 2017, 21249, 21014         CBK 2: 2017, 21249, 21014         1283, 1284, 1294         21           Condida mobiosica         BCK 2: 2017, 21249, 21014         CBK 2: 2017, 22097, CBS 1911         LMA 91325, 948674, RB         11           Condida mobiosica         BCK 2: 1281, 2: 1292, 1292, 1214         CBK 2: 1281, 2: 1292, 1237, 1377         LMA 91325, 948764, RB         11           Condida mobiosica         BCK 2: 1281, 2: 1292, 1292, 1214         CBK 2: 1281, 2: 2097, CBS 1911         LMA 91325, 948769, 947315, 948764, RB         11           Condida mobiosica         BCK 2: 1281, 2: 1292, 1237, 1377         LMA 91326, 948764, RB         11           Condida mobiosica         BCK 2: 1281, 2: 1292, 2: 1284, 2: 1294         24         240, 292, 2: 108, 4: 108, 9: 108           Condida mobiosica         BCK 2: 1281, 2: 1292, 2: 1284, 2: 1294         1	Candida holmii (Kazachstania exigua)	BCRC 21524 <sup>T</sup> , 21999, 22000	LNA 00200 DD 1226	3	
Candida kefyr (Kbyveromyces marianns)         BCRC 20316, 2135, 22037         ILMA 91325, 29367, 938779, 94449, 911           Candida kefyr (Kbyveromyces marianns)         BCRC 20317, 21320, 2170, 2136, 22342         2283, 11642, ILMA 911325, 095615, 948501, 13           Candida mbroid (Chartonia lipolytica)         BCRC 20317, 21321, 2170, 1270, 22302, 12017         S           Candida mabroid         BCRC 20317, 21321, 2170, 1270, 22302, 12047         S           Candida mabroid         BCRC 20347, 21321, 2170, 1270, 22302, 12047         S           Candida mabroid         BCRC 20347, 21321, 2170, 1270, 22302, 21047         S           Candida mabroid         BCRC 20347, 21342, 21506         LMA 9132648, 94706, 947315, 948764, RB         11           Candida mabroid         BCRC 20347, 21492, 21506         S         1233, 1238, 1234         240           Candida mobroificeiras         BCRC 20147, 21492, 21506         240, 282, 308, 413, 1905, 2985, 3080, 3851, 18         18           Candida mobroificeiras         BCRC 20147, 21249, 21206, 22037, 2237         240, 282, 308, 413, 1905, 2985, 3080, 3851, 183         18           Candida problesis, genotype II (Candida morogesis, genotype II (Candida	Candida inconspicua Candida intermedia (Kluvveromvces cellobiovorus)	BCRC 21658 <sup>-7</sup> , CBS 990, 1735, 2833 BCRC 20863, 21250 <sup>T</sup> , 21604, 22567	LMA 90289, KB 1226	6 4	
Candida kernsei (Issachenkia orientalis) Condida kernsei (Pichia fernetanis var. fernetanis)         BCRC 21547, 21321, 2170, 21706, 22242         2285, 16462, 1MA 911256, 948561, 948561, 143           Candida finitationa (Carrovia lipolytica)         BCRC 21347, 2137, 12740, 2209, 22091         RB 1222, 1237, 1317         4           Candida finitationa (Carrovia lipolytica)         BCRC 201547, 21321, 2137, 12740, CBS 7270         LMA 932648, 947060, 947315, 948564, RB         1           Candida morbanificarias         BCRC 201547, 21321, 21397, 21740, CBS 7270         LMA 932648, 947060, 947315, 948564, RB         1           Candida morbanificarias         BCRC 21563, 22397         LMA 932648, 947060, 947315, 948564, RB         1           Candida morbanificarias         BCRC 21562, 22096, 22097, CDS 1911         4         20         28, 1284, 2141, 21542, 21256           Candida norvegorias         BCRC 21667, CBS 5670, 4027, 4737         240, 282, 308, 403, 1905, 2985, 3080, 3851, 18         7410, 9300, 9672, LMA 932585, 961299, RB 1318, 1200         700, 8653         2           Candida parapsilosis, genotype II (Candida pintolopesii (Kacachstania telluris)         BCRC 2185, 21709         720, 8653, 21359, 21741, 22583 <sup>T</sup> 741, LMA 92971, RB 766, 778, 913         10           Candida pintolopesii (Kacachstania telluris)         BCRC 2185, 21707, 12745, 20205, 2204         731, LMA 48 92971, RB 766, 778, 913         10           Candida pintolopesii (Kacachstania telluris)	Candida kefyr (Kluyveromyces marxianus)	BCRC 20516, 20517, 21269, 21355, 22057 <sup>T</sup>	LMA 911323, 938657, 938779, 944459, 947644, RB 1227	11	
Conditial product (Carryonic lipoptica)         PCRC 20864, 21541, 21542, 2156         4           Condital mainine (Clarypora listianize)         PCRC 20852, 21387, 21740, CBS 270         LAA 932648, 947060, 947315, 948764, RB         11           Condital mainine (Clarypora listianize)         PCRC 2085, 21387, 21740, CBS 270         LAA 932648, 947060, 947315, 948764, RB         31           Candida morbinity         Cardida norvegits         PCRC 2085, 21387, 21740, CBS 270         LAA 932648, 947060, 947315, 948764, RB         31           Candida norvegits         PCRC 2085, 21387, 21740, CBS 270         LAA 932648, 947060, 947315, 948764, RB         31           Candida norvegits         PCRC 2085, 21387, 21740, CBS 270         LAA 932648, 947060, 947315, 948764, RB         32           Candida norvegits         PCRC 2085, 21252, 2124, 22007, CBS 1911         20         32         34           Candida protopesitos, genotype II (Candida ortolopesit (Kazachstania telliris)         BCRC 20857, 20858, 21559, 21741, 22583         701, 9803         2           Candida protopesitos, genotype III (Candida solutione (Casachstania telliris)         BCRC 2085, 7070         TAI, 1380, 1383, 130         30           Candida protopesito (Kazachstania telliris)         BCRC 21627, CBS 560, 5740         TR 158, 1358, 2049, 2049, 21447         RB 158, 1359         31           Candida protopesito (Kazachstani telliris)         BCRC 21627, C128, 5205, 2	Candida krusei (Issatchenkia orientalis) Candida lambica (Pichia fermentans var. fermentans)	BCRC 20514 <sup>T</sup> , 21321, 21720, 21796, 22342 BCRC 21347, 22067 <sup>T</sup> , 22068, 22090, 22091	2283, 16462, LMA 911256, 945615, 948501, RB 1222, 1237, 1317	13 5	
Condida haistaniae (Clavisport lasitaniae)         BCRC 2035, 21387, 21740, CBS 7270         LIA 932648, 947060, 947315, 948764, RB         11           Condida melhiosica         Candida melhiosica         CBS 8147, 6211         1283, 1288, 1294         3           Candida melhiosica         CBS 8147, 6211         CBS 8147, 6211         24         3           Candida norvegensis         BCRC 21365, 22387, 22399         2390         CBS 911         4           Candida norvegensis         BCRC 2165, 22387, 22399         CBS 911         4           Candida norvegensis         BCRC 2165, 22387, 22399         240, 282, 348, 403, 1905, 2985, 3480, 3851, 18         18           Candida participacita         BCRC 2165, 21253, 2154         770, 8053         2           Candida participacita         BCRC 2085, 2085, 2159, 21741, 22583 <sup>T</sup> 731, LMA 892971, RB 766, 778, 913         10           Candida robusta (Saccharomyces cervisiae)         BCRC 2085, 2109, 700, 2071, 2040, 2049, 21447         781         731, LMA 892971, RB 766, 778, 913         10           Candida robusta (Saccharomyces cervisiae)         BCRC 2178, 2053, 20270, 2027, 2049, 2049, 2249         731, LMA 892971, RB 766, 778, 913         10           Candida viscontine elloris in control         BCRC 2178, 2199, 2030, 2223         731, LMA 892971, RB 766, 778, 913         10           Candida viscontine elloris in co	Candida lipolytica (Yarrowia lipolytica)	BCRC 20864, 21541, 21542, 21596		4	
Cardida mitissi         Ds. RC 2157, 2144, 2194         125, 1284, 1294         3           Candida mitissica         Ds. RC 2157, 2299         2           Candida momparization         Ds. RC 2157, 2299, 2294, 2295, 2299, 2299, 2294, 2295, 2299, 2299, 2299, 2299, 2294, 2295, 2299, 2294, 2295, 2294, 2295, 2294, 2295, 2294, 2295, 2204, 2295, 22	Candida lusitaniae (Clavispora lusitaniae)	BCRC 20326, 21387 <sup>T</sup> , 21740, CBS 7270	LMA 932648, 947060, 947315, 948764, RB	11	
Candida membranificiens         BCRC 21543, 223987, 22399         3           Candida norvegnisi (Pichia norvegnisi)         BCRC 21543, 22397, 22397, C28 191         4           Candida norvegnisi (Pichia norvegnisi)         BCRC 21543, 22397, 22397, C28 191         4           Candida parapsilosis, genotype I         BCRC 2151 <sup>2</sup> , 21253, 2154         240, 282, 308, 403, 1905, 2985, 3080, 3851, 1         4           C parapsilosis, genotype II (Candida         BCRC 2065         43, 1833, 2304, C4-2         5           Candida parapsilosis, genotype III (Candida         BCRC 20857, 20858, 21359, 21741, 22583 <sup>T</sup> 4731, LMA 892971, RB 766, 778, 913         10           Candida policypeii (Kazachiania telluris)         BCRC 20263, 20270, 20271, 20405, 20409, 2144 <sup>TT</sup> RB 1254, 1299         8           Candida solucaria         BCRC 20263, 20270, 20271, 20405, 20409, 2144 <sup>TT</sup> RB 158         3           Candida solucaria (Kluris)         BCRC 2162, 2161 <sup>T</sup> , C17, C185, 504, 514         3         3           Candida solucaria (Kluris)         BCRC 2162, 2161 <sup>T</sup> , C17, 2252, C165         4         3           Candida solucaria (Kluris)         BCRC 21749, 2232, C185 562, 2160 <sup>T</sup> 104, 1075, 2785, 4996, 8023, 8173, 8327, LIAA         17           Candida solucaria (Kluris)         BCRC 2174, 2252, C185 76, C185, 2604         5         3           Candida solucolerus (Kluri	Candida maliosa Candida melibiosica	CBS $5814^{T}$ 6211	1283, 1288, 1294	3	
Condida norvegensis (Pchia norvegensis)         BCRC 21851, 22097, CES 1911         4           Condida norvegia         4           Condida paragsilosis, genotype I         BCRC 21617, CES 2570, du 207, 4737         240, 282, 308, 403, 1905, 2985, 3080, 3851, 1741, 12533, 2154, 226, 226, 308, 403, 1905, 2985, 3080, 3851, 1741, 09360, 9692, LMA 93588, 961299, TR, B 1318, 1320         18           C. paragsilosis, genotype II (Candida orthopsilosis)         BCRC 20857, 20858, 2159, 21741, 22583 <sup>T</sup> 4731, LMA 892971, RB 766, 778, 913         10           Candida principesii (Kacachannia telluris)         BCRC 20857, 20858, 2159, 21741, 22583 <sup>T</sup> 4731, LMA 892971, RB 766, 778, 913         10           Candida principesii (Kacachannia telluris)         BCRC 2163, 21077, 20271, 20248, 20208, 22263         78         88           Candida subicesia (Vichia nonmala)         BCRC 2163, 21077, 20271, 20248, 20208, 226         88         81158         3           Candida subicesia (Vichia hoshii)         CBS 4069, 9140 <sup>II</sup> , 4141         4         3         3           Candida subice (Vichia hoshiii)         CBS 4069, 9140 <sup>II</sup> , 21452, 21051, 22054, 2105         5         3           Candida subice (Vichia hoshiii)         CBS 4069, 9140 <sup>II</sup> , 21433, 2150         5         3           Candida subice (Vichia hoshiii)         CBS 4069, 9140 <sup>II</sup> , 21452, 21051, 21456, 21457         4         3           Candida subice (Vichia hos	Candida membranifaciens	BCRC 21563, 22398 <sup>T</sup> , 22399		3	
Candida prospilosis, genotype I         DKC 21001, '023, '473'         240, 282, 308, 403, 1905, 2985, 3080, 3851, 18           C. parapsilosis, genotype II (Candida orthopsilosis)         C. parapsilosis, genotype II (Candida orthopsilosis)         710, 8053         2           C. andida parapsilosis, genotype II (Candida orthopsilosis)         BCRC 20865         43, 1833, 2304, C4-2         5           Condida parapsilosis, genotype II (Candida parapsilosis, genotype II (Candida parapsilosis, genotype II (Candida parapsilosis)         BCRC 20857, 20858, 21359, 21741, 22583 <sup>T</sup> 4731, LMA 892971, RB 766, 778, 913         10           Condida problema Control (Candida parapsilosis)         BCRC 21637, C158, 5690, 5740         RB 158         3           Condida softward (Candida softward)         BCRC 2161 <sup>T</sup> , CI58, 5690, 5740         RB 158         3           Condida softward (Candida softward)         BCRC 2161 <sup>T</sup> , CI58, 5690, 5740         RB 158         3           Condida softward (Candida softward)         BCRC 2161 <sup>T</sup> , CI58, 5690, 5740         RB 158         3           Condida softward (Candivard)         BCRC 21747, 7213, 22154, 22053, 22204         3         3           Candida viskoal (Candivard)         BCRC 21747, 2232, CI85, 7624, 42143         3         3         3           Candida viskoal (Candivard)         BCRC 2130 <sup>T</sup> , 22534, 22537, 22544, 22875, 22564         3         3         3	Candida norvegensis (Pichia norvegensis)	BCRC 21851, 22096 <sup>T</sup> , 22097, CBS 1911 BCRC 21616 <sup>T</sup> , CBS 2670, 4027, 4727		4	
C. parapsilosis, genotype II (Candida orthopsilosis)         TRV 1318, 1520           Candida parapsilosis, genotype III (Candida orthopsilosis)         BCRC 20865         43, 1833, 2304, C4-2         5           Candida parapsilosis, genotype III (Candida metapsilosis)         BCRC 20857, 20858, 21359, 21741, 22583 <sup>T</sup> 4731, LMA 892971, RB 766, 778, 913         10           Candida princlopesti (Kazachstania telluris)         BCRC 20143, 22003, 22239         4731, LMA 892971, RB 766, 778, 913         10           Candida substa (Sacchstamia telluris)         BCRC 20156, 2077, 20271, 20405, 20490, 21447 <sup>T</sup> RB 1254, 1299         8           Candida substa (Saccharomyces cerevisiae)         BCRC 21621 <sup>T</sup> , CBS 5690, 5740         RB 1158         3           Candida substa (Saccharomyces tellosi)         BCRC 21147, 2252, 21617 <sup>T</sup> , CBS 464, 5421         RB 1158         3           Candida substa (Saccharomyces tellosi)         BCRC 21147, 22522, CBS 7624         5         3           Candida substa (Saccharomyces tellosi)         BCRC 21147, 22522, CBS 7624         5         3           Candida substa (Saccharomyces tellosi)         BCRC 20147, 22522, CBS 7625, 22604         5         3           Candida substa (Pichia adomii)         BCRC 20147, 22523, 21445, 21437, 21560         104, 1075, 2785, 4996, 8023, 8173, 8327, LMA         17           Candida substa (Pichia adobia)         BCRC 20149, 2199, 91241	Candida norvegica Candida parapsilosis, genotype I	BCRC 21010, CBS 2070, $4027$ , $4757$ BCRC 20515 <sup>T</sup> , 21253, 21544	240, 282, 308, 403, 1905, 2985, 3080, 3851, 7410, 9360, 9692, LMA 938558, 961299,	4 18	
Constitution         Description         S         S           Condida parapsilosis, genotype III (Candida metapsilosis)         BCRC 20857, 20858, 21359, 21741, 22583 <sup>T</sup> 4731, LMA 892971, RB 766, 778, 913         10           Candida prinolopesii (Kacachstania telluris)         BCRC 2005, 2003, 22239         4731, LMA 892971, RB 766, 778, 913         10           Candida robusta (Sacchromyces cerevisiae)         BCRC 2003, 20271, 20071, 20071, 20071, 20071, 20073, 20074, 20090, 21447 <sup>T</sup> RB 1254, 1299         8           Candida solutia (Sacchromyces cerevisiae)         BCRC 21621 <sup>T</sup> , CBS 5690, 5740         3         8         3           Candida solutiocal (Pichia holstii)         CBS 4009, 4140 <sup>T</sup> , 441         3         3         3           Candida solution (Klupveromyces learks)         BCRC 2176 <sup>T</sup> , 2232, CBS 7652         3         3           Candida tild (Pichia membranifaciens)         BCRC 2200 <sup>T</sup> , 2139, 2144, 2147, 2154, 2105, 22161         104, 1075, 2785, 4996, 8023, 8173, 8327, LMA         4           Candida tild (Pichia membranifaciens)         BCRC 2130 <sup>T</sup> , 22554, 2205, 22604         104, 1075, 2785, 4996, 8023, 8173, 8327, LMA         5           Candida sequanoides (Pichia dubia)         BCRC 2100 <sup>T</sup> , 2139, 21441, 21447, 21420         104, 1075, 2785, 4996, 8023, 8173, 8327, LMA         5           Candida visuanathii         BCRC 2100 <sup>T</sup> , 2139, 21417 <sup>T</sup> , 2149, 22396, CBS 947         LMA 91304	C. parapsilosis, genotype II (Candida		RB 1318, 1320 770, 8053	2	
	Candida parapsilosis, genotype III (Candida metapsilosis)	BCRC 20865	43, 1833, 2304, C4–2	5	
Candida robusta (Saccharomyces cerevisiae)       BCRC 20263, 20270, 20271, 20405, 20490, 21447 <sup>T</sup> RB 1254, 1299       8         Candida sufamariae       BCRC 21356, 21709 <sup>T</sup> RB 1158       3         Candida sufamariae       BCRC 21352, 21617 <sup>T</sup> , CBS 5690, 5740       3         Candida sufamariae       BCRC 21352, 21617 <sup>T</sup> , CBS 4261, 4515 <sup>T</sup> 4         Candida sufamariae       BCRC 21562, 21617 <sup>T</sup> , CBS 4261, 4515 <sup>T</sup> 4         Candida sufamariae       BCRC 21766, 22133, 2154, 22055, 22604       5         Candida traporcentic (Zygoascus hellenicus)       BCRC 21767, 2232, CBS 564, 8242       3         Candida tropicalis       BCRC 20200, 20352, 20334, 20860, 20928 <sup>T</sup> , 21357       9077, 921810, 945762, RB 1298, 1330       6         Candida tropicalis       BCRC 2130 <sup>T</sup> , 2259, 20334, 20860, 20928 <sup>T</sup> , 21357       9077, 921810, 945762, RB 1298, 1330       3         Candida viswanathii       BCRC 2130 <sup>T</sup> , 2259, 21347, 21441       9077, 921810, 945762, RB 1298, 1330       3         Candida viswanathii       BCRC 2130 <sup>T</sup> , 22594, CBS 570, 21441       9077, 921810, 945762, RB 1298, 1330       3         Candida viswanathii       BCRC 21401, 22503 <sup>T</sup> , 22504, CBS 2556       4       4         Candida viswanathii       BCRC 21401, 22503 <sup>T</sup> , 22504, CBS 256       4       4         Candida spp.       Arthroascus schoenii       BC	Candida pelliculosa (Pichia anomala) Candida pintolopesii (Kazachstania telluris)	BCRC 20857, 20858, 21359, 21741, 22583 <sup>T</sup> BCRC 21439, 22003, 22239	4731, LMA 892971, RB 766, 778, 913	10 3	
Candida skeBCRC 2162, 12,02KB 11383Candida skeBCRC 2162, 17, CBS 5690, 57403Candida skeBCRC 2162, 21617, CBS 4261, 4515 <sup>T</sup> 4Candida skvicola (Pichia holstii)CBS 4069, 4140 <sup>T</sup> , 41413Candida shvicola (Vichia holstii)BCR 21716, 22153, 22154, 22055, 226045Candida strutorerans (Vanderwaltozyma yarrowii)BCRC 2174, 2232, CBS 76523Candida tunnotolerans (Vanderwaltozyma yarrowii)BCR 21747, 2282, CBS 2684, 82424Candida tuilis (Pichia jadinii)BCRC 20260, 20325, 20334, 20860, 20928 <sup>T</sup> , 213579077, 921810, 945762, RB 1298, 13306Candida tuilis (Pichia jadinii)BCRC 21743 <sup>T</sup> , 21749, 22396, CBS 947LMA 913045Candida stype,Arthroascus schoeniiBCRC 21401, 22503 <sup>T</sup> , 22504, CBS 25564Arthroascus schoeniiBCRC 21735, CBS 570, 2744, 87704Brettanomyces bruxellensis (Dekkera bruxellensis)BCRC 20527 <sup>T</sup> , 21860, CBS 969LMA 9354795Candida stype,BCRC 20527 <sup>T</sup> , 21860, CBS 56942Candida stype,BCRC 20527 <sup>T</sup> , 21860, CBS 56942Candida stype, Carobita BarcellensisBCRC 21735, CBS 570, 2744, 87704Cryptococcus laurentiiBCRC 21209 <sup>T</sup> , 2270, 274, 87703Cryptococcus laurentiiBCRC 21529, 2208 <sup>T</sup> , 2059, 2083, 22874, 22875, 24439, 4889, 7241, LMA 94277, 925461, 202Cryptococcus laurentiiBCRC 21737, 1730, 2770, 294, 4257935479, 957786, 959159, 498001235Cryptococcus laurentiiBCRC 2129, 2208 <sup>T</sup> , 2059, 2051, 56634	Candida robusta (Saccharomyces cerevisiae)	BCRC 20263, 20270, 20271, 20405, 20490, 21447 <sup>T</sup>	RB 1254, 1299	8	
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Candida sake	BCRC 21530, 21709 BCRC 21621 <sup>T</sup> , CBS 5690, 5740	KB 1136	3	
Candida silvicola (Pichia holstii)CBS 4069, 4140', 41413Candida silvicola (Pichia holstii)BCR 21746 <sup>7</sup> , 2232, CBS 7552, 226045Candida steatolytica (Zygoascus hellenicus)BCR 21746 <sup>7</sup> , 2232, CBS 7552, 226043Candida tumotolerans (Vanderwaltozyma yarrowii)BCR 21747, 22822, CBS 2684, 82424Candida turpoicalisBCR 21747, 22822, CBS 2684, 82424Candida turpoicalisBCR 21747, 22822, CBS 2684, 82424Candida valla (Pichia iadinii)BCR 21747, 22822, CBS 2684, 82429077, 921810, 945762, RB 1298, 1330Candida viswanathiiBCR 2106 <sup>9</sup> , 21399, 214419077, 921810, 945762, RB 1298, 1330Candida sylanoides (Pichia dubia)BCR 21743 <sup>7</sup> , 21749, 22396, CBS 947LMA 91304Non-Candida spp.Arthroascus schoeniiBCR 2 1743 <sup>7</sup> , 21749, 22396, CBS 947LMA 91304Candida neoformars (Filobasidiella neoformans)BCR 2 0252, 2033 <sup>7</sup> , 22504, CBS 25564Cryptococcus laurentiiBCR 2 0255, 6172 <sup>7</sup> , 21800, CBS 969LMA 9354795Cryptococcus laurentiiBCR 2 0252 <sup>7</sup> , 129722Candida neoformans (FilobasidiumFilobasidiumCBS 823, 919, 1622, 6955, 6977935479, 957786, 959159, 498001232Cryptococcus laurentiiBCR 2 1259, 2209 <sup>8</sup> , CBS 4409, 525442Debaryomyces chellsiiBCR 2 1259, 2209 <sup>8</sup> , CBS 4409, 52544Debaryomyces chellsiiBCR 2 1259, 22108, CBS 917, 314, 25825Klocckera apis (Hanseniaspora valyensi)BCR 2 2159, 50034Klocckera apis (Hanseniaspora valyensi)BCR 2 2159, 22112 <sup>7</sup> , CB	Candida santamariae	BCRC 21562, 21617 <sup>T</sup> , CBS 4261, 4515 <sup>T</sup>		4	
$ \begin{array}{c} Candida steatolytica (Z)goascus hellenicus \\ Candida steatolytica (Z)goascus hellenicus \\ Candida tannotolerans (Vandervaltozyma yarrowii) \\ BCR C 21747, 22822, CBS 7652 \\ Candida tannotolerans (Vandervaltozyma yarrowii) \\ BCR C 21747, 22822, CBS 7652 \\ Candida tropicalis \\ Candida viiis (Pichia jadinii) \\ Candida viiis (Pichia membranifaciens) \\ Candida viiis (Pichia dubia) \\ BCR C 210260, 20325, 20334, 20380, 20928T, 21357 \\ Candida viiswanathii \\ BCR C 21030T, 22554 \\ Candida syswanathii \\ BCR C 21401, 22503T, 22504, CBS 256 \\ Candida abidus \\ Cryptococcus curvatus \\ Cryptococcus unigutultatus (Filobasidium BCR 21529, 22097, CBS 702, 724, 8770 \\ Cryptococcus unigutultatus (Filobasidium BCR 21529, 22098T, CBS 4409, 5254 \\ Debaryomyces carsonii \\ Debaryomyces carsonii \\ Checkera apicultat (Hanseniaspora uvarum) \\ Kloeckera apicultat (Hanseniaspora uvarum) \\ Kloeckera apic (Hanseniaspora valbyensis) \\ Kloexera apic (Hanseniaspora valb$	Candida silvicola (Pichia holstii) Candida sphaerica (Kluvveromyces lactis)	CBS 4069, 4140 <sup>+</sup> , 4141 BCRC 21716, 22153, 22154, 22055, 22604		3	
	Candida steatolytica (Zygoascus hellenicus)	BCRC 21746 <sup>T</sup> , 22232, CBS 7652		3	
Candida tropicalisBCRC 20207, 20521, 21437, 21500 $104, 1075, 2785, 4996, 8025, 8173, 8527, LMA17Candida vilis (Pichia jadinii)BCRC 20206, 20235, 20334, 20860, 20928+, 213579077, 921810, 945762, RB 1298, 13306Candida vilia (Pichia membranifaciens)BCRC 20206, 20235, 20334, 20860, 20928+, 213579077, 921810, 945762, RB 1298, 13303Candida zeylanoides (Pichia dubia)BCRC 21307+, 22542Non-Candida spp.BCRC 21401, 22503+, 22504, CBS 25564Arthroascus schoeniiBCRC 20322, 21414+, 21440, 21518, 215195Candida neoformans (Filobasidiella neoformans)BCRC 20527, 129972Candida neoformans (FilobasidiumBCRC 20527, 21997935479, 957786, 959159, 49800123Cryptococcus uniguttulatus (FilobasidiumCBS 1272, 1730, 2770, 2994, 4257935479, 957786, 959159, 49800123Cryptococcus uniguttulatus (FilobasidiumCBS 2152, 22098+, CBS 312, 314, 25824Debaryomyces etarbelisiBCRC 21529, 22098+, CBS 2012, 5519, 56034Debaryomyces etarbelisiBCRC 21529, 22098+, CBS 312, 314, 25822Kloeckera apic (Hanseniaspora valuyenxi)BCRC 21529, 2105, CBS 12, 314, 25822Kloeckera iaponica (Hanseniaspora valuyenxi)BCRC 21349, CBS 21703Kloeckera iaponica (Makaseomycesdelphensis)BCRC 21349, 21592, 22178+, 22556, 225573Kloeckera iaponica (Hanseniaspora valuyenxi)BCRC 21349, 21592, 22178+, 2256, 225573Kloeckera iaponica (Hanseniaspora valuyenxi)BCRC 21349, 21592, 22178+, 22556, 225573Kloeckera iaponica (Ha$	Candida tannotolerans (Vanderwaltozyma yarrowii)	BCRC 21747, 22822, CBS 2684, 8242	104 1075 2705 4007 0022 0172 0227 I.N.A	4	
Candida valida (Pichia membranifaciens) Candida viswanathiBCRC 22069 <sup>T</sup> , 21399, 21441Softy Pictor, 1000,	Candida tropicaus Candida utilis (Pichia jadinii)	BCRC 20520 <sup>-7</sup> , 20521, 21436, 21437, 21500 BCRC 20260 20325 20334 20860 20928 <sup>T</sup> 21357	9077 921810 945762 RB 1298 1330	17	
Candida viswanahii       BCRC 21330 <sup>1</sup> , 2254       2         Candida zeylanoides (Pichia dubia)       BCRC 21743 <sup>T</sup> , 21749, 22396, CBS 947       LMA 91304       5         Non-Candida spp.       Arthroascus schoenii       BCRC 21401, 22503 <sup>T</sup> , 22504, CBS 2556       4         Berttanomyces bruxellensis (Dekkera bruxellensis)       BCRC 20932, 21414 <sup>T</sup> , 21440, 21518, 21519       5         Candida albidus       BCRC 20526, 21672 <sup>T</sup> , 21860, CBS 969       LMA 935479       5         Cryptococcus curvatus       BCRC 20527, 7, 21997       2       2         Candida neoformans (Filobasidiella neoformans)       BCRC 20528 <sup>T</sup> , 20532, 22241, 22873, 22874, 22875, 4439, 4889, 7241, LMA 94277, 925461, 20       20         Cyptococcus uniguttulatus (Filobasidium       CBS 883, 919, 1622, 6955, 6997       935479, 957786, 959159, 49800123       5         Cryptococcus uniguttulatus (Filobasidium       CBS 1727, 1730, 2770, 2994, 4257       935479, 957786, 959159, 49800123       5         Debaryomyces cthellsii       BCRC 21529, 22098 <sup>T</sup> , CBS 409, 5254       4       4         Debaryomyces dramuus       BCRC 2052, 216, CBS 1958, 131, 314, 2582       5         Kloeckera apis (Hanseniaspora uvarum)       BCRC 20152, 2106, 22112 <sup>T</sup> , CBS 4378       4         Kloeckera ipaonica (Hanseniaspora valbyensis)       BCRC 21738, CBS 2950, 22577       3         Kloeckera ipaonica (Hanseniaspor	Candida valida (Pichia membranifaciens)	BCRC 22069 <sup>T</sup> , 21399, 21441	<i>56777, 521610, 516762, 162 1250, 1000</i>	3	
Canadad zeylanoldes (Pichul aluola)       BCRC 21/45 <sup>+</sup> , 21/49, 22390, CBS 947       LMA 91504       5         Non-Candida spp.       Arthroascus schoenii       BCRC 21401, 22503 <sup>T</sup> , 22504, CBS 2556       4         Brettanomyces bruxellensis (Dekkera bruxellensis)       BCRC 20932, 21414 <sup>T</sup> , 21440, 21518, 21519       5         Candida albidus       BCRC 2175, CBS 570, 2744, 8770       4         Cryptococcus laurentii       BCRC 20527, 21997       4         Candida neoformans (Filobasidiella neoformans)       BCRC 2052 <sup>T</sup> , 20532, 20241, 22873, 22874, 22875, 4439, 4889, 7241, LMA 94277, 925461, 20       20         Cyptococcus uniguttulatus (Filobasidium       CBS 1727, 1730, 2770, 2994, 4257       935479, 957786, 959159, 49800123       5         Debaryomyces carsonii       BCRC 21479 <sup>+</sup> , CBS 4009, 5254       4       4         Debaryomyces techellsii       BCRC 21479 <sup>+</sup> , CBS 4009, 5254       4       2         Kloeckera apiculata (Hanseniaspora uvarum)       BCRC 20539, 21362, CBS 312, 314, 2582       5         Kloeckera apis (Hanseniaspora valbyensis)       BCRC 22105, 22106, 22112 <sup>+</sup> , CBS 4378       4         Klopeckera apis (Hanseniaspora valbyensis)       BCRC 22139, 21592, 2217 <sup>*</sup> , 2556, 22557       5         Klopeckera apis (Hanseniaspora valbyensis)       BCRC 21479 <sup>+</sup> , CBS 2950, 2951, 5666       4         Klephensis)       BCRC 21479 <sup>+</sup> , CBS 2950, 2951, 5666 <td>Candida viswanathii Candida andanaidan (Bishin duhin)</td> <td>BCRC 21330<sup>T</sup>, 22554</td> <td>T.M.A. 01204</td> <td>2</td>	Candida viswanathii Candida andanaidan (Bishin duhin)	BCRC 21330 <sup>T</sup> , 22554	T.M.A. 01204	2	
Non-Candida spp.       Arthroascus schoenii       BCRC 21401, 22503 <sup>T</sup> , 22504, CBS 2556       4         Brettanomyces bruxellensis (Dekkera bruxellensis)       BCRC 20932, 21414 <sup>T</sup> , 21440, 21518, 21519       5         Candida albidus       BCRC 20526, 21672 <sup>T</sup> , 21860, CBS 969       LMA 935479       5         Cryptococcus curvatus       BCRC 20527 <sup>T</sup> , 21997       2         Candida neoformans (Filobasidiella neoformans)       BCRC 20528 <sup>T</sup> , 20532, 22241, 22873, 22874, 22875, 4439, 4889, 7241, LMA 94277, 925461, 20       20         Cryptococcus uniguttulatus (Filobasidium       CBS 1727, 1730, 2770, 2994, 4257       5         uniguttulatum       CBS 1229, 2098 <sup>T</sup> , CBS 4409, 5254       4         Debaryomyces carsonii       BCRC 21479 <sup>T</sup> , CBS 2012, 5519, 5603       4         Debaryomyces etchellsii       BCRC 21053, 21362, CBS 312, 314, 2582       5         Kloeckera apiculata (Hanseniaspora guillermondii)       BCRC 2105, 22106, 22112 <sup>T</sup> , CBS 4378       4         Kloeckera apis (Hanseniaspora valbyensis)       CBS 281, 479 <sup>T</sup> , 2590       3         Kloyeromyces delphensis (Nakaseomyces       BCRC 2017, CBS 2170       2         Kodamaea ohmeri       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557	Canalaa Zeylanolaes (Fichia aubia)	BCRC 21/45, 21/49, 22590, CBS 947	LMA 91504	5	
Artnroascus schoenu       BCRC 21401, 22503, 22503, 22504, CBS 2556       4         Brettanomyces bruxellensis (Dekkera bruxellensis)       BCRC 20932, 21414 <sup>T</sup> , 21440, 21518, 21519       5         Candida albidus       BCRC 20526, 21672 <sup>T</sup> , 21860, CBS 969       LMA 935479       5         Cryptococcus laurentii       BCRC 20527, 21997       2         Candida neoformans (Filobasidiella neoformans)       BCRC 2052 <sup>T</sup> , 20532, 22241, 22873, 22874, 22875, 4439, 4889, 7241, LMA 94277, 925461, 20       2         Cryptococcus uniguttulatus (Filobasidium       CBS 883, 919, 1622, 6955, 6997       935479, 957786, 959159, 49800123       5         Cryptococcus uniguttulatus (Filobasidium       CBS 1727, 1730, 2770, 2994, 4257       5       4         Debaryomyces carsonii       BCRC 21529, 22098 <sup>T</sup> , CBS 4409, 5254       4       4         Debaryomyces maramus       BCRC 21520, CBS 1958 <sup>T</sup> 2       2         Kloeckera apiculata (Hanseniaspora uvarum)       BCRC 20139, 21106, 22112 <sup>T</sup> , CBS 4378       4         Kloeckera apiculata (Hanseniaspora valbyensis)       BCRC 22017, CBS 2170       3         Kloeckera apaonica (Hanseniaspora valbyensis)       BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666       4         Klopekera apiculata (Hanseniaspora valbyensis)       BCRC 2179 <sup>T</sup> , 2246, 22557       5         Klopekera apiculata (Hanseniaspora valbyensis)       BCRC 2107, CBS 2170	Non-Candida spp.	DODG 21 (01 22502T 22501 CDC 2555			
Candida albidus       DECC 20526, 21672, 21474, 21470, 21479       LMA 935479       5         Candida albidus       BCRC 20526, 21672, 21860, CBS 969       LMA 935479       5         Cryptococcus laurentii       BCRC 20526, 21672, 21807       4         Cryptococcus laurentii       BCRC 20527, 21997       2         Candida neoformans (Filobasidiella neoformans)       BCRC 20527, 20532, 22241, 22873, 22874, 22875, 4439, 4889, 7241, LMA 94277, 925461, 20       20         Cryptococcus uniguttulatus (Filobasidium       CBS 883, 919, 1622, 6955, 6997       935479, 957786, 959159, 49800123       5         Cryptococcus anguttulatus (Filobasidium       CBS 1727, 1730, 2770, 2994, 4257       5       4         Debaryomyces carsonii       BCRC 21529, 22098 <sup>T</sup> , CBS 409, 5254       4         Debaryomyces maramus       BCRC 21526, CBS 1958 <sup>T</sup> 2         Kloeckera apiculata (Hanseniaspora uvarum)       BCRC 22015, 22106, 22112 <sup>T</sup> , CBS 4378       4         Kloeckera apiculata (Hanseniaspora vulyensis)       CBS 281, 479 <sup>T</sup> , 2590       3         Kluyveromyces delphensis (Nakaseomyces       BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666       4         Kodamaea ohmeri       BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21439, 21592, 22178 <sup>T</sup>	Arthroascus schoenu Brettanomyces bruvellensis (Dekkera bruvellensis)	BCRC 21401, 22503 <sup>+</sup> , 22504, CBS 2556 BCRC 20932, 21414 <sup>T</sup> , 21440, 21518, 21519		4	
Cryptococcus curvatus       BCRC 21735, CBS 570, 2744, 8770       4         Cryptococcus laurentii       BCRC 20527 <sup>T</sup> , 21997       22         Candida neoformans (Filobasidiella neoformans)       BCRC 20528 <sup>T</sup> , 20532, 22241, 22873, 22874, 22875, 4439, 4889, 7241, LMA 94277, 925461, CBS 883, 919, 1622, 6955, 6997       935479, 957786, 959159, 49800123         Cryptococcus uniguttulatus (Filobasidium       CBS 1727, 1730, 2770, 2994, 4257       5         uniguttulatum)       BCRC 21529, 22098 <sup>T</sup> , CBS 4409, 5254       4         Debaryomyces carsonii       BCRC 21526, CBS 1958 <sup>T</sup> 2         Lobckera apiculata (Hanseniaspora guillermondii)       BCRC 21526, CBS 1958 <sup>T</sup> 2         Kloeckera apiculata (Hanseniaspora guillermondii)       BCRC 22105, 22106, 22112 <sup>T</sup> , CBS 4378       4         Kloeckera apiculata (Hanseniaspora valbyensis)       CBS 281, 479 <sup>T</sup> , 2590       3         Kluyveromyces delphensis (Nakaseomyces       BCRC 21749, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666       4         Locderence growthere       BCRC 21132, 2170 <sup>T</sup> , 2256, 22557       5         Lachancea cidri       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21439, 21592, 22178 <sup>T</sup> , 2256, 22577       5 </td <td>Candida albidus</td> <td>BCRC 20526, 21672<sup>T</sup>, 21860, CBS 969</td> <td>LMA 935479</td> <td>5</td>	Candida albidus	BCRC 20526, 21672 <sup>T</sup> , 21860, CBS 969	LMA 935479	5	
Cyptococcus taturentitBCRC 2052*, 2199721997Candida neoformans (Filobasidiella neoformans)BCRC 2052*, 20532, 22241, 22873, 22874, 22875, 4439, 4889, 7241, LMA 94277, 925461, CBS 883, 919, 1622, 6955, 6997935479, 957786, 959159, 49800123Cryptococcus uniguttulatus (Filobasidium uniguttulatum)CBS 1727, 1730, 2770, 2994, 42575Debaryomyces carsoniiBCRC 21529, 22098 <sup>T</sup> , CBS 4409, 52544Debaryomyces maramusBCRC 21526, CBS 1958 <sup>T</sup> 2Kloeckera apiculata (Hanseniaspora guillermondii) Kloeckera apiculata (Hanseniaspora guillermondii)BCRC 22105, 22106, 22112 <sup>T</sup> , CBS 43784Kloeckera apiculata (Hanseniaspora valbyensis) Klupveromyces delphensis (Nakaseomyces delphensis)BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 225573Kodamaea ohmeriBCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 225575Lachancea cidriBCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 225575Lachancea cidriBCRC 21728 <sup>T</sup> , CBS 2950, 2951, 56664LoddrewnyeesBCRC 2139, 20190, 22132 <sup>T</sup> , CBS 2666, 59123	Cryptococcus curvatus	BCRC 21735, CBS 570, 2744, 8770		4	
Candidat neojonnans (r nosizidatella heojonnans)       Delse 2832, 919, 1622, 6955, 6997       935479, 957786, 959159, 49800123         Cryptococcus uniguttulatus (Filobasidium       CBS 1727, 1730, 2770, 2994, 4257       935479, 957786, 959159, 49800123         Debaryomyces carsonii       BCRC 21529, 22098 <sup>T</sup> , CBS 4409, 5254       4         Debaryomyces etchellsii       BCRC 21529, 22098 <sup>T</sup> , CBS 2012, 5519, 5603       4         Debaryomyces maramus       BCRC 21526, CBS 1958 <sup>T</sup> 2         Kloeckera apis (Hanseniaspora guillermondii)       BCRC 22105, 22106, 22112 <sup>T</sup> , CBS 4378       4         Kloeckera apis (Hanseniaspora valbyensis)       CBS 281, 479 <sup>T</sup> , 2590       3         Kluyveromyces delphensis (Nakaseomyces delphensis (Nakaseomyces delphensis (Nakaseomyces delphensis)       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557       5       5         Lachancea cidri       BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666       4         Loddroremyces delpinenti       BCRC 21389, 21960 <sup>T</sup> , 22453       3         Kodanaea ohmeri       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21349, 21592, 22178 <sup>T</sup>	Cryptococcus laurentu Candida neoformans (Filobasidiella neoformans)	BCRC 2052/ <sup>1</sup> , 2199/ BCRC 20528 <sup>T</sup> 20532 22241 22873 22874 22875	4439 4889 7241 IMA 94277 925461	20	
Cryptococcus uniguttulatus (Filobasidium         CBS 1727, 1730, 2770, 2994, 4257         5           uniguttulatum)         Debaryomyces carsonii         BCRC 21529, 22098 <sup>T</sup> , CBS 4409, 5254         4           Debaryomyces carsonii         BCRC 21529, 22098 <sup>T</sup> , CBS 2012, 5519, 5603         4           Debaryomyces maramus         BCRC 21526, CBS 1958 <sup>T</sup> 2           Kloeckera apiculata (Hanseniaspora uvarum)         BCRC 201529, 22016, 22112 <sup>T</sup> , CBS 312, 314, 2582         5           Kloeckera apis (Hanseniaspora guillermondii)         BCRC 22105, 22106, 22112 <sup>T</sup> , CBS 4378         4           Kloeckera apis (Hanseniaspora valbyensis)         CBS 281, 479 <sup>T</sup> , 2590         3           Kluyveromyces delphensis (Nakaseomyces         BCRC 2017, CBS 2170         2           delphensis)         BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557         5           Lachancea cidri         BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666         4           Lachancea fermentati         BCRC 21390 <sup>T</sup> , CBS 206, 5912         3	Cunada neojonnans (Phobasalena neojonnans)	CBS 883, 919, 1622, 6955, 6997	935479, 957786, 959159, 49800123	20	
uniguitulatum)       BCRC 21529, 22098 <sup>T</sup> , CBS 4409, 5254       4         Debaryomyces carsonii       BCRC 21479 <sup>T</sup> , CBS 2012, 5519, 5603       4         Debaryomyces maramus       BCRC 21526, CBS 1958 <sup>T</sup> 2         Kloeckera apis (Hanseniaspora guillermondii)       BCRC 22105, 22106, 22112 <sup>T</sup> , CBS 312, 314, 2582       5         Kloeckera apis (Hanseniaspora guillermondii)       BCRC 22105, 22106, 22112 <sup>T</sup> , CBS 4378       4         Kloeckera apis (Hanseniaspora guillermondii)       BCRC 22017, CBS 2170       3         Kluyveromyces delphensis (Nakaseomyces       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21433, 21760 <sup>T</sup> , 2453       4         Lachancea fermentati       BCRC 2138, 21760 <sup>T</sup> , 22453       3         Lachancea regress       BCRC 21390 <sup>T</sup> , CBS 2666, 5912       3	Cryptococcus uniguttulatus (Filobasidium	CBS 1727, 1730, 2770, 2994, 4257		5	
Debaryomyces etchellsii         DBCRC 21479 <sup>†</sup> , CBS 2012, 5519, 5603         4           Debaryomyces aramus         BCRC 21526, CBS 1958 <sup>†</sup> 2           Kloeckera apiculata (Hanseniaspora uvarum)         BCRC 21526, CBS 1958 <sup>†</sup> 2           Kloeckera apiculata (Hanseniaspora guillermondii)         BCRC 22105, 22106, 22112 <sup>†</sup> , CBS 4378         4           Kloeckera apis (Hanseniaspora valbyensis)         CBS 281, 479 <sup>†</sup> , 2590         3           Kluyveromyces delphensis (Nakaseomyces         BCRC 22107, CBS 2170         2           delphensis)         BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557         5           Lachancea cidri         BCRC 21433, 21760 <sup>†</sup> , 24433         4           Lodderswerse domignoreus         BCRC 21390, 21592, 22178 <sup>T</sup> , 22556, 22557         5	uniguttulatum) Debarvomvces carsonii	BCRC 21529 22098 <sup>T</sup> CBS 4409 5254		4	
Debaryomyces maramus         BCRC 21526, CBS 1958 <sup>T</sup> 2           Kloeckera apiculata (Hanseniaspora guillermondii)         BCRC 20539, 21362, CBS 312, 314, 2582         5           Kloeckera apis (Hanseniaspora guillermondii)         BCRC 22105, 22106, 22112 <sup>T</sup> , CBS 4378         4           Kloeckera apis (Hanseniaspora valbyensis)         BCRC 22005, 22106, 22112 <sup>T</sup> , CBS 4378         3           Kluyveromyces delphensis (Nakaseomyces         BCRC 22017, CBS 2170         2           delphensis)         BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557         5           Lachancea cidri         BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557         5           Lachancea cidri         BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557         5           Lachancea cidri         BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557         5           Lachancea cidri         BCRC 21439, 21592, 22178 <sup>T</sup> , 22556, 22557         5           Lachancea cidri         BCRC 21439, 2150 <sup>T</sup> , 22453         3           Loddersweese alongingnerus         BCRC 21390 <sup>T</sup> CBS 2666 5912         3	Debaryomyces etchellsii	BCRC 21479 <sup>T</sup> , CBS 2012, 5519, 5603		4	
Kloeckera apis (Hanseniaspora tivarum)       BCRC 2053, 21562, CBS 312, 314, 2582       5         Kloeckera apis (Hanseniaspora guillermondii)       BCRC 20152, 22106, 22112 <sup>T</sup> , CBS 4378       4         Kloeckera apis (Hanseniaspora valbyensis)       BCRC 22105, 22106, 22112 <sup>T</sup> , CBS 4378       3         Kluyveromyces delphensis (Nakaseomyces       BCRC 22017, CBS 2170       2         delphensis)       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21439, 21502, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea fermentati       BCRC 21439, 2170 <sup>T</sup> , CBS 2950, 2951, 5666       4         Lodderswees domigneers       BCRC 21390 <sup>T</sup> CBS 2666, 5912       3	Debaryomyces maramus	BCRC 21526, CBS 1958 <sup>T</sup>		2	
Klockera japonica (Hanseniaspora valbyensis)       CBS 281, 479 <sup>T</sup> , 2590       3         Klockera japonica (Hanseniaspora valbyensis)       CBS 281, 479 <sup>T</sup> , 2590       3         Kluyveromyces delphensis (Nakaseomyces delphensis)       BCRC 22017, CBS 2170       2         delphensis)       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666       4         Lachancea fermentati       BCRC 21439, 2170 <sup>T</sup> , 22453       3         Loddersurves dominarcus       BCRC 21390 <sup>T</sup> CBS 2666, 5912       3	Kloeckera apiculata (Hanseniaspora uvarum) Kloeckera apis (Hanseniaspora guilliermondii)	BCRC 20539, 21362, CBS 312, 314, 2582 BCRC 22105, 22106, 22112 <sup>T</sup> CBS 4378		5	
Kluyveromyces delphensis (Nakaseomyces delphensis)BCRC 22017, CBS 21702Kodamaea ohmeriBCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 225575Lachancea cidriBCRC 21728 <sup>T</sup> , CBS 2950, 2951, 56664Lachancea fermentatiBCRC 21433, 21760 <sup>T</sup> , 224533Loddersomvers elongiaporusBCRC 21349, C18, 20103	Kloeckera japonica (Hanseniaspora galacemonali)	CBS 281, 479 <sup>T</sup> , 2590		3	
aeipnensis)       Kodamaea ohmeri       BCRC 21349, 21592, 22178 <sup>T</sup> , 22556, 22557       5         Lachancea cidri       BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666       4         Lachancea fermentati       BCRC 21433, 21760 <sup>T</sup> , 22453       3         Loddrownees elongioperus       BCRC 21300 <sup>T</sup> CBS 2666, 5912       3	Kluyveromyces delphensis (Nakaseomyces	BCRC 22017, CBS 2170		2	
Lachancea cidri         BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666         4           Lachancea fermentati         BCRC 21433, 21760 <sup>T</sup> , 22453         3           Loddrownses clongingerus         BCRC 21390 <sup>T</sup> CBS 2666, 5912         3	aeipnensis) Kodamaea ohmeri	BCRC 21349, 21592, 22178 <sup>T</sup> 22556, 22557		5	
Lachancea fermentati BCRC 21433, 21760 <sup>T</sup> , 22453 3 Loddrammers dominants BCRC 21390 <sup>T</sup> CBS 2606 5912 3	Lachancea cidri	BCRC 21728 <sup>T</sup> , CBS 2950, 2951, 5666		4	
	Lachancea fermentati	BCRC 21433, 21760 <sup>T</sup> , 22453 BCRC 21300 <sup>T</sup> , CRS 2606, 5012		3	
Dotation/yets congisportas         Decke 21590         CBS 2000, 5912         5           Pichia spartinae         BCRC 22766 <sup>T</sup> , CBS 6059, 6077, 6661         4	Pichia spartinae	BCRC 22766 <sup>T</sup> , CBS 2000, 3912 BCRC 22766 <sup>T</sup> , CBS 6059, 6077, 6661		3 4	

Continued on following page

TABLE	1—Continued
IADLE	1-Commuea

Species (teleomorph) <sup>a</sup>	Reference strain(s) <sup>b</sup>	Clinical isolate(s)	Total no. of strains
Rhodotorula glutinis (Rhodosporidium	BCRC 20576, 21418 <sup>T</sup>		2
diobovatum)			
Rhodotorula minuta	BCRC 22482, 22483, 22484, 22485		4
Rhodotorula mucilaginosa	BCRC 21442, 21667 <sup>T</sup> , 21712, 21713, 21770		5
Saccharomyces kluyveri	BCRC 21498 <sup>T</sup> , 21977, 22001, CBS 2861		4
Saccharomycopsis fibuligera	BCRC 20455, 21379, 21380, 21449, 21465, 21511 <sup>T</sup>		6
Sporobolomyces salmonicolor (Sporidiobolus	CBS 490, 4474		2
salmonicolor)			
Trichosporon aquatile	BCRC 22271 <sup>T</sup> , 22272, CBS 5973, 5988		4
Trichosporon asahii	CBS 2479, 2936, 4829, 7631		4
Trichosporon cutaneum	BCRC 21675 <sup>T</sup> , 22273	LMA 94117, 94256, 931422, 931440	6
Trichosporon inkin	BCRC 21503 <sup>T</sup> , CBS 7613, 7629, 7655	, , , , , , , , , , , , , , , , , ,	4
Trichosporon pullulans	BCRC 22275 22313 CBS 2543		3
Williopsis saturnus var saturnus	BCRC 20463 21360 21659 21692 21765		5
Zvaosaccharomyces bisporus	BCRC 21505 <sup>T</sup> 21725 21726		3
Zygotorulaspora florentinus	BCRC 21648 <sup>T</sup> , CBS 748, 6078, 6703		4

<sup>a</sup> Anamorphic names are used in this study, and teleomorphic species names are given in parentheses. Some species were only given the names of teleomorphs since they have no anamorphic names.

<sup>b</sup> A superscript "T" indicates type strains.

cation by the array (Table 1). Among these strains, 309 were reference (or type) strains and 110 were clinical isolates. Reference strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA), the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan), and Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). Clinical isolates were obtained from the Mycology Reference Centre, Department of Microbiology, Leeds Teaching Hospitals Trust (Leeds, United Kingdom), the Laboratory of Parasitology and Mycology of Angers University Hospital (Angers, France), and the National Cheng Kung University Medical Center (Tainan, Taiwan). Isolates were identified to the species level based on traditional criteria (18) or with the API ID 32C system. In addition, a total of 33 nontarget strains (33 species) were used for specificity testing of the array (data not shown).

**DNA extraction.** Yeasts were subcultured on Sabouraud dextrose agar and incubated at 28°C for 24 to 48 h. Colonies of yeast were suspended in saline, and the genomic DNA was extracted by using the blood and tissue genomic DNA extraction Miniprep system (Viogene, Taipei, Taiwan), as described previously (24), except that the lyticase digestion step was omitted.

**Amplification of the ITS regions for hybridization.** Digoxigenin (DIG)-labeled amplicons for array hybridization were obtained by PCR using the fungus-specific universal primers ITS1 (5'-DIG-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-DIG-TCCTCCGCTTATTGATATGC-3') (50). Each primer was labeled with a digoxigenin molecule at its 5' end and was obtained from MdBio, Inc. (Taipei, Taiwan). The PCR products encompassed ITS 1, the 5.8S rRNA gene, ITS 2, and partial regions of the 18S and 28S rRNA genes. The reaction mixtures and thermocycling conditions used for PCR were described previously (24).

Design of oligonucleotide probes. The oligonucleotide probes (16- to 30-mers) used for the identification of 77 yeast species are listed in Table 2. Probes were designed from either the ITS 1 or the ITS 2 region. The corresponding ITS sequences used for probe design were available in the GenBank database or determined in our previous studies (2, 24). Sequences extracted from GenBank (Table 2) were confirmed by at least one sequence from another reference strain of the same species in public databases. The alignment of multiple ITS sequences was made by using Discovery Studio Gene software (DS Gene, version 1.5; Accelrys, Inc., San Diego, CA). Based on the aligned sequences, areas displaying high intraspecies similarities and low interspecies similarities were used for probe synthesis. The melting temperature, GC content, and possible secondary structure of a designed probe were examined by using Vector NTI software (Invitrogen Corporation, Carlsbad, CA). In addition, the designed probes were checked for partial sequence homology with other microorganisms in public databases using the basic local alignment search tool (BLAST) algorithm. A total of 79 species-specific probes and one group-specific probe were used for fabrication of the oligonucleotide array (Table 2). In addition, a probe designed from a conserved sequence of the fungal 5.8S rRNA gene was used as a positive control probe (probe code, PC) (Table 2). Five to 17 bases of thymine were added to the 3' or 5' ends of probes that displayed weak hybridization signals after preliminary testing (1). Moreover, a digoxigenin-labeled bacterial universal primer 6R (5-D IG-GGGTTYCCCCRTTCRGAAAT-3, where Y is C or T and R is A or G) (probe code, M) was spotted on the array and used as a position marker (Fig. 1 and 2) (48). All probes were synthesized by MdBio, Inc. (Taipei, Taiwan).

**Fabrication of oligonucleotide arrays.** The oligonucleotide probes were diluted 1:1 (final concentration, 10  $\mu$ M) with a tracking dye solution composed of glycerol, dimethyl sulfoxide, sodium EDTA, and bromophenol blue (3). The probes were spotted onto a positively charged nylon membrane (Roche, Mannheim, Germany) by an Ezspot arrayer (model no. SR-A300; EZlife Technology, Taipei, Taiwan) using a 400- $\mu$ m-diameter solid pin as described previously (48). The array (0.75 by 0.9 cm) contained 108 dots, including 80 dots for the identification of 77 yeast species (16 genera), 2 for positive controls (probe code, PC; final concentration, 5  $\mu$ M), 7 for negative controls (probe code, NC; tracking dye only), and 19 for position markers (probe code, M; final concentration, 0.4  $\mu$ M) (Fig. 1). The position markers formed a cross on the array after hybridization and helped to locate the hybridized probes (Fig. 1 and 2). Once all probes had been applied, the membrane was exposed to shortwave UV (Stratalinker 1800; Stratagene, La Jolla, CA) for 30 s.

Hybridization procedures. Except where otherwise indicated, the hybridization procedures were carried out at room temperature (approximately 27°C) with a shaking speed of 60 rpm. Most reagents except buffers were obtained from the DIG nucleic acid detection kit (Roche). The hybridization procedures were the same as those described previously (20), except that the hybridization step was conducted at 50°C for 90 min. Unbound oligonucleotides on the array were removed by three washes (3 min each) in 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate (SDS). Each array was prehybridized at room temperature for 2 h with hybridization solution (5' SSC, 1% [wt/vol] blocking reagent, 0.1% N-laurylsarcosine, and 0.02% SDS) in an individual well of a 24-well cell culture plate. The DIG-labeled PCR product amplified from an isolate was denatured at 95°C and immediately cooled on an ice bath. Ten microliters of denatured PCR products were diluted with 0.3 ml of hybridization solution and added to each well. Hybridization was carried out at 50°C for 90 min. After removal of the nonhybridized PCR products, the array was washed four times (5 min each) with 0.25× SSC-0.1% SDS, followed by incubation for 1 h with blocking solution (1% [wt/vol] blocking reagent dissolved in maleic acid buffer [0.1 M maleic acid, 0.15 M NaCl, pH 7.5]). After removal of the blocking solution, 0.3 ml of alkaline phosphatase-conjugated anti-DIG antibodies (diluted 1:2,500) was added to each well and incubated for 1 h. The array was washed three times (each 15 min) with washing solution (0.3% [vol/vol] Tween 20 in maleic acid buffer), followed by washing with detection buffer (0.1 M Tris-HCl, 0.15 M NaCl, pH 9.5) for 5 min. Finally, 0.3 ml of alkaline phosphatase substrates (nitroblue tetrazolium chloride-5-bromo-4-chloro-3-indolylphosphate) was added to each array and incubated at 37°C for 30 to 60 min without shaking. The hybridized array was washed three times with distilled water and air dried. The image of the hybridization pattern was captured and processed by a scanner (PowerLook 3000; UMAX, Taipei, Taiwan).

**Definition of sensitivity and specificity.** A yeast strain was identified as one of the 77 target yeasts when the probe designed for the species and the positive control probes (Fig. 2) were hybridized. Sensitivity was defined as the number of

		TABLE 2. Ongoindereotide probes used in this stud	1y			
Species (teleomorph) or control	Probe code <sup>a</sup>	Sequence $(5' \text{ to } 3')^b$	Length (bp)	$T_m$ (°C) <sup>c</sup>	Location <sup>d</sup>	GenBank accession no.
Candida albicans	CAB5	TTATCAACTTGTCACACCAGATTATTACT(tttttt)	29	53	102-130(1)	AY207067
Candida boidinii	CB1	TAACTCTTTGGGAAAACTCTATACACTTTG	30	56	81–110 (1)	AY936499
Candida cacaoi	PF1	TTTACAGTAGATAAATGCCGTTTGACTCTT	30	58	80–109 (2)	AF218989
Candida cantarelli	CCA1	AGACTTCTCCCATACACTTGTGAACTTT	28	56	26-53 (1)	AY936503
Candida catenulata	CCT1	AAAGTGATTGGTGTAGTATTACAGTTTACT	30	52	44-73 (2)	AY493436
Candida chodatii	CCH3	AGCTCTTAGTTCAGTCCATTCGAAAAGT	28	58	37-64 (2)	AY936510
Candida colliculosa	CCO5b	ATTTT <u>T</u> CTGGCTTGGATGACTTTGT(ttttttt)	25	56	83-107 (2)	AJ229075
Candida dattila	CDAT2a	CAA <u>T</u> TCGTAGTGGCGTTAGTA(ttttttttttt)	21	48	130–150 (2)	AY046207
Candida dubliniensis	CDU1a	AAACITGTCACGAGATTATT(tttttttttttt)	20	41	108-127(1)	AB049124
Candida famata	CFAMIa	GGCCAGAGGTTTACGAA(ttttttttttttttttt)	18	45	10/-124(1)	AF336834
Canalaa freyschussil	CFRE2		30	33	8/-110(2)	AF218905
Candida glabrala	CGLO1a		30	08 55	1/7 - 200(1) 12, 25(2)	A Y 207008
Candida guilliarmondii	CGU2	$\frac{11001111A0010110000A01A(1111111)}{CTCCTCTCAATCT(1111111)}$	25	55	13-33(2) 02 112(2)	A 1950514
Candida haemulonii	CHAE12	$\Delta \Delta TC \Delta \Delta CC \Delta CC GTT \Delta \Delta GTT C \Delta \Delta (ttttttttt))$	21	52	92-112(2) 32(53(1))	A I 207070 A I606467
Candida holmii	CHO2	GGTTGTTGCAGCTTATAGTTTTTGTGTAAT	30	58	32-33(1) 78 107(2)	D80804
Candida inconspicua	CINC3		23	56	73-107(2) 71-93(2)	ΔV036516
Candida intermedia	CIT2	GTTGTCGCAATACGTTACTTCAACTTTATT	30	58	45-74(2)	AF218968
Candida kefvr	CKEF2	AGTTTTCTATTTCTCATCC(ttttttttttt)	19	37	$\frac{43}{88-106}$ (1)	A I401700
Candida krusei	CK3	TGTGGAATATAGCATATAGTCGACAAGAGA	30	57	54-83(1)	AY207070
Candida lambica	CLAM3a	TTCTTGGAGCGGWGCTCCAGA(tttttttttttt)	21	59	3-23(2)	AF218969
Candida lipolytica	CLI1a	CTCAATGATTACGTCATTTCACC(ttttttttttt)	23	51	44-66(2)	AF218983
Candida lusitaniae	CLUS1	TCAAACACGTTTACAGCACGACATTTC	27	60	49-75 (2)	AY139788
Candida maltosa	CML4	TAGTAATGTACCGACGTAAACGACTTAGGT	30	57	59-88 (2)	AY936522
Candida melibiosica	CMEL1	ATATCGCTCGCACTGTTTCTAAGCTAACA	29	61	34-62 (2)	AY936524
Candida membranifaciens	CMB1	AACTGGGGCAGTAAATTTCTAGTAATTGG	29	59	136–164 (2)	AJ606465
Candida norvegensis	CNO2b	TGTCACCCAGAGAAAATCTCAAACGAG(ttttttt)	27	61	65-91 (1)	AY139789
Candida norvegica	CNOR1a	(ttttttt)TAGCCGGAGACTACAACCAAACTAATTT	28	58	80-107(1)	AY936525
Candida parapsilosis	CP6	TTCCACTCATTGGTACAAACTCCAAAACTT	30	61	87-116 (2)	AY207079
	CP8	TTTGGTAGGCCTTCTATATGGGGGCCT(tttttt)	26	62	67–92 (1)	AY207072
	CP10	TTAACTGCGACCCTTTTCTTTCTACACA	28	60	21-48 (1)	AY391849
Candida pelliculosa	CPEL3	ATATTGACTTAGCAAGAGT(ttttttttttttt)	19	35	58-76 (2)	AF218991
Candida pintolopesii	CPI2	ACGTCTTCGTAGTAGGTTCTGCCAATT	27	59	131–157 (2)	AJ223029
Candida robusta	SC2	TGTAAGTTTCTTTCTTGCTATTCCAAACGG	30	61	135–164 (1)	Z95935
Candida rugosa	CRUG2	CGCGACCGTCTAAAACAGTTAAGCTTG	27	62	49-75 (2)	AF218971
Candida sake	CSAK3	ACTIGCTIGCAAGAACACTAATAATTTA(tttttt)	28	54	39-66 (1)	AJ549822
Canalaa santamarlae	CSANI		1/	30	133-149(2)	DQ000054
Canalaa suvicola Canalaa anhaariaa	CSIL2		29	02	33-01(2)	A 1930330
Candida steatobtica	CSF II Sa		20	40 50	55-52(2)	A 1 040215
Candida tannotolarans	KVAD1		25	59	201 225 (1)	A 1950552
Candida tropicalis	CT3c		20	41	201-223(1) 69-88(2)	A V207080
Candida utilis	CUT5	CAACTCGTTATTTTCCAGACAGACT(	20	53	119-143(2)	AY936536
Candida valida	CVAL 2a	AAGAAACGTTGCGGACGAAGCG(tttttttttt)	23	62	$72_{-93}(2)$	DO104722
Candida viswanathii	CVIS4a	CTTGTGCAGTCGGCTCACCA(ttttttttt)	20	57	99-118(2)	AY139792
Candida zevlanoides	CZEY2c	GAGCAGTATAGTATTTG(ttttttttttt)	17	27	133-149(2)	AF218976
Arthroascus schoenii	AS1	ATGCTTCCCTTACCTTGTTAAGTAGCTTTA	30	58	80-109 (2)	AY936498
Brettanomyces bruxellensis	DB3	CGAGGGTGTTTTCTTCAAAGGGAAG	25	60	33-57 (2)	AF043503
Cryptococcus albidus	CAL5	CTAAAGACCGCTTTCTAATCCATTGATCT	29	59	172 - 200(2)	AY382336
Cryptococcus curvatus	CCUR2	AGTGAATTTAACATTTGTCTTCTGGCG	27	58	77–103 (2)	AF410467
Cryptococcus laurentii	CLR4	ACCTCTGTGAACTGTGGACC(ttttttttttttt)	20	48	36-55 (1)	AF335935
Cryptococcus neoformans	CN4b	TTTTATTACCTGTTGGACTTGGATT(ttttttttt)	25	53	11-35 (2)	AY207082
Cryptococcus uniguttulatus	CUNI2	CTGGACTTGTCTATATGACTGGTTTGA	27	55	94–120 (2)	AY382334
Debaryomyces carsonii	PCAR1b	TTGCTTTGGCTTGTCTCTAGA(tttttttttttt)	21	50	79–99 (1)	AB054097
Debaryomyces etchellsii	PETC2	TACTGGATAGTACTGTTATGGCTTCTTCA	29	55	83-111 (2)	AJ586528
Debaryomyces maramus	DMAR1c	GGCTAGAGACTTACTGAA(tttttttttt)	18	35	107–124 (1)	AB053102
Kloeckera apiculata	KAP2	ATTGGAGACTGTTTCAG(tttttttttttttt)	17	35	61-77 (2)	AY046200
Kloeckera apis	KAPIS2b	GTATTTATGAATTTATTC(ttttttttttttttt)	18	28	130–147 (2)	AJ512427
Kloeckera japonica	KJAP1	CAGTCAACTACTACACACAG(tttttttttttt)	20	36	138–157 (1)	AJ512434
Kluyveromyces delphensis	KDEL3	TAAGITTGTTGTGGGGATGCTAATTCCTTT	29	60	168–196 (2)	AY198400
Kodamaea ohmeri	PO2	GACGACAGTACTCTACAAAACGGTACC	27	56	44-70 (2)	AF219004
Lachancea cidri	ZCI3	CAACTCGTAGGGGGCTTA(ttttttttt)	17	43	131–147 (2)	AY046205
Lachancea fermentati	ZF6a	TGAG <u>T</u> GGACGCTACAAAG(tttttttttttttt)	18	44	146-163(2)	AY046206
Lodderomyces elongisporus	LEL2	AAUCACIUCATIGIGUITAATAAAAAGC	28	58	89–116 (2)	AY391848
Picnia spartinae	PSPA3	AATACAGUGUAUTUGAUAATUA(ttttttt)	22	22	86 - 107 (2)	AF423028
Knoaotorula glutinis	KGLUI DM4		21	50	23-43(2)	AF444539
Knoaotorula minuta	KIM4	GATTAIGGIIGICIGICGGCGIAAII	26	59	45-70(2)	AF444620

TABLE 2. Oligonucleotide probes used in this study

Continued on following page

Species (teleomorph) or control	Probe code <sup>a</sup>	Sequence $(5' \text{ to } 3')^b$	Length (bp)	$T_m$ (°C) <sup>c</sup>	Location <sup>d</sup>	GenBank accession no.
Rhodotorula mucilaginosa	RRUB1	TAATGATTGAAGAGGTGTTTTGG(tttttttt)	22	49	23-44 (2)	AF444541
Saccharomyces kluyveri	SKLU1	TGTTAACGGTTGTCTCTT(ttttttttttt)	18	39	73–90 (1)	AB037405
Saccharomycopsis fibuligera	EF4	GATTGAGTTTTCCATATATTTGCTTAAGGA	30	57	76-105(2)	AF218988
Sporobolomyces salmonicolor	SSAL3	GCCTTCGGGTTACTGAGC(ttttttttttttttt)	18	50	153–170 (2)	AF387784
Trichosporon aquatile/ Trichosporon asahii/ Trichosporon inkin	TAQAS1 <sup>e</sup>	TTGACATTAATGTCTGGTG(ttttttttttttttt)	19	40	85-103 (2)	AF410475
Trichosporon aquatile	TAQU3	TTGGGCGTTGCGATCT(ttttttttt)	16	50	38-53 (2)	AF410475
Trichosporon asahii	TASA1b	ATATCCACTTACACCTGT(tttttttttttttt)	18	35	27-44 (1)	AY055381
Trichosporon cutaneum	TCUT3b	TCGGTCAATTGATTTTACAA(ttttttttttttt)	20	46	60-79 (1)	AF335957
Trichosporon inkin	TINK2	TTGACATTCATGTCTGG(tttttttttttttt)	17	37	84-100(2)	AF218981
Trichosporon pullulans	TP1a	TCCAGGCTATCATTTCATACAAACT(ttttttttt)	25	53	92–116 (1)	AF444418
Williopsis saturnus var. saturnus	HS2	AGCCCAĀACCTTACACACTGTGATTAGTTT	30	61	40-69 (1)	Z93875
Zygosaccharomyces bisporus	ZB5	AACTGAGGTGGGTGATAGAAATATCGAAC	29	59	186–214 (2)	AJ229176
Zygotorulaspora florentinus	ZFLO1a	CTCTGTAACATGGGAGTTAGC(tttttttttt)	21	46	36-56 (2)	AY046168
Positive control	$\mathbf{PC}^{f}$	GCATCGATGAAGAACGCAGC(tttttttt)	20	56	5.8S rRNA gene	EF134625

TABLE 2—Continued

<sup>a</sup> Oligonucleotide probes are arranged on the array as indicated in Fig. 1.

<sup>b</sup> Five to 17 additional bases of thymine, indicated by "(t)," were added to the 5' or 3' end of the probe. Underlined nucleotides indicate a single mismatch base that was incorporated into the probe.

 $^{c}T_{m}$ , melting temperature.

<sup>d</sup> The location of probe is indicated by the nucleotide number of either ITS 1 or ITS 2; the numbers (1 or 2) in parentheses indicate the ITS region from which the probe was designed.

<sup>e</sup> A group-specific probe shared by three species (Trichosporon aquatile, Trichosporon asahii, and Trichosporon inkin).

<sup>f</sup> The positive control probe was designed from a conserved region of the 5.8S rRNA gene.

target strains correctly identified (true positives) divided by the total number of target strains tested (30). Specificity was defined as the number of nontarget strains producing negative hybridization reactions (true negatives) divided by the total number of nontarget strains tested (30).

Analysis of discrepant strains. For strains producing discrepant identification between the methods based on phenotypic characteristics and array hybridization, the D1-D2 region of the large-subunit RNA gene, ITS 1, and ITS 2 were amplified by PCR, sequenced, and then compared with sequences in public databases using the BLAST algorithm for species clarification. The fungusspecific, universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify the ITS 1 region, while the primer pair ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') was used to amplify the ITS 2 region (50). The D1-D2 region was amplified by primers NL1 (5'-GCATATCA ATAAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') (23). The conditions used for amplification of the ITS 1, ITS 2, and D1-D2 regions were the same as those for amplifying the whole ITS segment as described previously (24). PCR products were purified with a PCR-M cleanup kit (Viogene, Taipei, Taiwan) and sequenced on a model 377 sequencing system (Applied Biosystems, Taipei, Taiwan).

Limit of detection of the array. Two strains (*C. albicans* BCRC 20512 and *Kloeckera apis* BCRC 22112) were used to determine the limit of detection of the oligonucleotide array. The DNAs of both strains were serially diluted 10-fold (10 ng/ $\mu$ l) to 1 fg/ $\mu$ l) with a carrier DNA (1 ng/ $\mu$ l) extracted from a bacterium

22	1	2	3	4	5	6	7	8	9	10	11	12
Α	CAB5	CB1	CCA1	CCT1	CCH3	CCO5b	М	AS1	CAL5	CCUR2	CLR4	CN4b
В	CDAT2a	CDU1a	CFAM1a	CFRE2	CGL1	CGLO1a	М	CUNI2	DMAR1c	DB3	EF4	HS2
С	CGU2	CHAE1a	CHO2	CINC3	CIT2	CKEF2	М	KAP2	KAPIS2b	KJAP1	KDEL3	KYAR1
D	CK3	CLAM3a	CLI1a	CLUS1	CML4	CMEL1	М	LEL2	PCAR1b	PETC2	PF1	PC
E	М	М	М	М	М	М	NC	М	М	М	М	М
F	CMB1	CNO2b	CNOR1a	CP6	CP8	CP10	М	PO2	PSPA3	RGLU1	RM4	RRUB1
G	CPEL3	CPI2	CRUG2	CSAK3	CSAN1	CSIL2	М	SC2	SKLU1	SSAL3	ZB5	ZCI3
Η	CSPH3a	CST3	CT3c	CUT5	CVAL2a	CVIS4a	М	ZF6a	ZFLO1a	TAQAS1	TAQU3	TASA1b
I	PC	NC	NC	NC	NC	CZEY2c	М	TCUT3b	TINK2	TP1a	NC	NC

FIG. 1. Layout of oligonucleotide probes on the array (0.75 by 0.9 cm). The positive control probe PC (located at D12 and I1) was designed from a conserved region of the fungal 5.8S rRNA gene. Probe NC (located at E7, I2 to I5, I11, and I12) was a negative control (tracking dye only). Probe M (located at E1 to E12 and at A7 to I7, except E7) was a DIG-labeled bacterial universal primer and was used as a position marker. The group-specific probe TAQAS1 (located at H10) is underlined. The corresponding sequences of all probes are listed in Table 2.

(*Xanthobacter flavus* BCRC 12271) by the boiling method (32). After PCR amplification of the diluted DNA, the amplicon was hybridized to the oligonucleotide array.

## RESULTS

**Oligonucleotide probe development.** Initially, one to five probes were designed for each of the 77 target species. Through a preliminary hybridization test, probes cross-reacting with heterologous species or producing weak hybridization signals with their homologous species were discarded. Finally, 80 probes (Table 2) were selected for the fabrication of the array. Under most conditions, a single probe was used to identify a species (Table 2). Among these probes, a group-specific probe (probe code, TAQAS1) was designed; the probe could hybridize with species of *Trichosporon aquatile*, *Trichosporon asahii*, and *Trichosporon inkin*. However, each of the three *Trichosporon species* had its own specific probe (Table 2).

Due to the presence of three genotypes among isolates of *Candida parapsilosis* (27, 42, 46), three probes (probe codes, CP6, CP8, and CP10) were designed to identify this frequently isolated species. Probe CP6 was shared by all three genotypes of *C. parapsilosis*; however, strains of genotypes II or III were able to hybridize with, in addition to probe CP6, an additional probe (CP8 or CP10). For example, *C. parapsilosis* BCRC 20515 (genotype I) hybridized only to probe CP6 (Fig. 2A). However *C. parapsilosis* BCRC 20865 (genotype III) hybridized to probes CP6 and CP10 (Fig. 2A), while clinical isolate *C. parapsilosis* 770 (genotype II) hybridized to probes CP6 and CP10 were relatively weaker compared to that of probe CP6.

Twelve probes were intentionally designed to incorporate one single base mismatch with their respective complementary target sequences (Table 2) to eliminate weak cross-hybridizations produced by nonhomologous species. These 12 probes were used to identify *Candida colliculosa*, *Candida dattila*, *Candida globosa*, *Candida haemulonii*, *Candida lambica*, *Candida lipolytica*, *Candida tropicalis*, *Candida viswanathii*, *Candida zeylanoides*, *Debaryomyces maramus*, *Lachancea fermentati*, and *Trichosporon pullulans* (Table 2). The 12 modified probes had no cross-hybridization with other species, but still displayed good hybridization signals with their respective target yeasts.

**Identification of reference strains by the array.** A total of 342 reference strains, including 309 target strains (Table 1) and 33 nontarget strains (Table 3), were tested. Figure 2 shows the hybridization results of reference strains of different target species. All 309 target strains were correctly identified by the oligonucleotide array, producing a test sensitivity of 100%. Of the 33 nontarget reference strains (33 species), only one strain, *Trichosporon ovoides* CBS 7556, cross-hybridized to the group-specific probe (probe code, TAQAS1) shared by *Trichosporon aquatile, Trichosporon asahii*, and *Trichosporon inkin*. The remaining 32 strains did not produce any hybridization signals with probes on the array except the positive control probe. Therefore, the test specificity of the array was 97% (32/33).

**Identification of clinical isolates by the array.** A total of 110 clinical isolates, including 14 *Candida* species (96 strains) and 3 non-*Candida* species (15 strains), were analyzed by the array (Table 1). Of the 110 isolates, 98 were correctly identified and

12 produced discrepant identifications by phenotypic characteristics and array hybridization. Among the 12 isolates, 5 (Candida pelliculosa LMA 892971 and Trichosporon cutaneum LMA 94117, 94256, 931422, and 931440) were not identified by the oligonucleotide array. By sequencing the ITS 1, ITS 2, and D1-D2 regions of the five isolates, our previous study demonstrated that Candida pelliculosa LMA 892971 (Pichia anomala, teleomorph) was a misidentification of Pichia fabianii and that the four isolates of Trichosporon cutaneum were misidentifications of Trichosporon dermatis (24). Both ITS 2 and D1-D2 regions of Candida pelliculosa LMA 892971 had a sequence similarity of 100% with reference sequences (accession numbers AF335967 and AF335971) of Pichia fabianii in GenBank. Likewise, the four discrepant isolates of Trichosporon cutaneum (LMA 94117, 94256, 931422, and 931440) displayed a sequence similarity of 100% with reference sequences (accession number AF143557 in the ITS 1 and ITS 2 regions and accession number AF143555 in the D1-D2 region) of Trichosporon dermatis in GenBank (24). Since Pichia fabianii and Trichosporon dermatis were nontarget species in this study, their PCR products did not hybridize with any probes on the array except the positive control probe.

Of the remaining seven discrepant isolates, Candida dubliniensis RB 1168, Candida guilliermondii RB 1055, Candida inconspicua LMA 90289, Candida inconspicua RB 1226, Candida krusei RB 1237, Candida rugosa RB 1158, and Cryptococcus albidus LMA 935479 were identified as Candida albicans, Candida parapsilosis, Candida krusei, Candida glabrata, Pichia norvegensis, Saccharomyces cerevisiae, and Cryptococcus neoformans, respectively, by the array. The accuracy of identification of the seven discrepant isolates by hybridization was confirmed by sequence analysis of the ITS 1, ITS 2, and D1-D2 regions in our previous study (24). As the 12 discrepant clinical isolates (5 unidentified isolates and 7 misidentified isolates) were proved to be misidentified by phenotypic methods, the test sensitivity of clinical isolates by the array was 100% (105/105). If reference strains and clinical isolates were taken together, the overall test sensitivity of the array was also 100% (414/414).

Limit of detection of the array. Serial 10-fold dilutions of DNA extracted from *Candida albicans* BCRC 20512 and *Kloeckera apis* BCRC 22112 were used to determine the limit of detection of the array. The present method was able to detect genomic DNA at a level of 10 pg per assay of both strains (data not shown).

## DISCUSSION

In this study, an oligonucleotide array was developed to identify 77 species (16 genera) of medically relevant yeasts, including some less-common emerging species belonging to *Cryptococcus, Pichia, Rhodotorula, Saccharomyces*, and *Trichosporon* species. A test sensitivity of 100% (414/414) and a specificity of 97% (32/33) were obtained by the array. The prominent feature of the present method is that it replaces the various morphological and metabolic characteristics for yeast identification with a single standardized protocol encompassing DNA extraction, PCR amplification of the ITS regions, and the hybridization of PCR product to the array. The hybridized spot (blue color on a white background), having a diameter of 400  $\mu$ m, could be recognized easily by the naked eye. The

А								
1 :	2	3	<sup>4</sup> •:	5 • :	<sup>6</sup> • :	7	8	9
Candida albicans BCRC 20512	C. boidinii BCRC 20464	C. cacaoi BCRC 21368	C. cantarellii BCRC 21613	C. catenulata BCRC 21507	C. chodatii BCRC 22087	C. colliculosa BCRC 22074	C. dattila BCRC 22043	C. dubliniensis CBS 7987
10 C. famata CBS 1795	11 C. freyschussii BCRC 21555	12 C. glabrata BCRC 20586	13 C. globosa CBS 162	14 C. guilliermondii BCRC 21500	15 C. haemulonii BCRC 21572	16 C. holmii BCRC 21524	17 C. inconspicua BCRC 21658	18 C. intermedia BCRC 21250
19 C. kefyr BCRC 22057	20 C. krusei BCRC 20514	21 C. lambica BCRC 22067	22 C. lipolytica BCRC 20864	23 C. lusitaniae BCRC 21387	24 C. maltosa BCRC 21614	25 C. melibiosica CBS 5814	26 C. membranifaciens BCRC 22398	27 C. norvegensis BCRC 22096
28 C. norvegica BCRC 21616	29 C. parapsilosis (genotype I) BCRC 20515	30 C. parapsilosis (genotype III) BCRC 20865	31 C. pelliculosa BCRC 22583	32 C. pintolopesii BCRC 21439	33 C. robusta BCRC 21447	34 C. rugosa BCRC 21709	35 C. sake BCRC 21621	36 C. santamariae BCRC 21617
37 C. silvicola CBS 4140	38 C. sphaerica BCRC 21716	39 C. steatolytica BCRC 21746	40 C. tannotolerans BCRC 21747	41 C. tropicalis BCRC 20520	42 C. utilis BCRC 20928	43 C. valida BCRC 22069	44 C. viswanathii BCRC 21330	45 C. zeylanoides BCRC 21743
B	47	49	40	50	54	53	52	54
Arthroascus schoenii BCRC 22503	Brettanomyces bruxellensis BCRC 21414	40 Cryptococcus albidus BCRC 21672	Cryp. curvatus BCRC 21735	Cryp. laurentii BCRC 20527	Cryp. neoformans BCRC 20528	Cryp. uniguttulatus CBS 1727	Debaryomyces carsonii BCRC 22098	D. etchellsii BCRC 21479
55	56	57	58	59	60	61	62	63
<i>D. maramus</i> BCRC 21526	Kloeckera apiculata BCRC 20539	K. apis BCRC 22112	K. japonica CBS 479	Kluyveromyces delphensis BCRC 22017	Kodamaea ohmeri BCRC 22178	Lachancea cidri BCRC 21728	L. fermentati BCRC 21760	Lodderomyces elongisporus BCRC 21390
64 Pichia spartinae BCRC 22766	65 Rhodotorula glutinis BCRC 21418	66 R. minuta BCRC 22482	67 R. mucilaginosa BCRC 21667	68 Saccharomyces kluyveri BCRC 21498	69 Saccharomycopsis fibuligera BCRC 21511	70 Sporobolomyces salmonicolor CBS 490	71 Trichosporon aquatile BCRC 22271	72 T. asahii CBS 2479
73 T. cutaneum BCRC 21675	74 T. inkin BCRC 21503	75 T. pullulans BCRC 22275	76 Williopsis saturnus var. saturnus BCRC 21463	77 Zygosaccharomyces bisporus BCRC 21505	78 Zygotorulaspora florentinus BCRC 21648			

present method will be useful when the identification of yeast pathogens to the species level is necessary, and the current array has the potential to be extended by adding further oligonucleotides to it without significantly increasing cost or complexity.

The divergence in the ITS length and sequence among different species has been used for fungal identification (5, 9, 26, 49). Based on the length polymorphism of the ITS 2 region (237 to 429 bp), Chen et al. (5) were able to identify 92% of the clinical yeast isolates (34 species) by using the capillary electrophoresis technique. Recently, an oligonucleotide microarray based on the ITS 2 sequence was developed to identify 20 species of pathogenic fungi, including Aspergillus, Candida, Cryptococcus, Microsporum, Mucor, Trichophyton, and other genera (21). Our previous study also demonstrated that the ITS 2 region is a more discriminative target than the ITS 1 region for yeast identification (24). However, considering the factors (specificity, melting temperature, GC content, and secondary structure) that can influence the array performance (35), probes were designed from either the ITS 1 or the ITS 2 region in this study. Among the 80 probes listed in Table 2, 58 were designed from the ITS 2 region.

C. parapsilosis is a frequently isolated yeast pathogen. Strains of C. parapsilosis could be divided into three genotypes (27, 42). Recently, Tavanti et al. (46) proposed two new species (Candida orthopsilosis and Candida metapsilosis) to replace Candida parapsilosis genotypes II and III, respectively, and the species Candida parapsilosis is retained for genotype I isolates only. However, the new names are not widely used yet, as reflected in the culture lists of some prestigious culture collection centers, such as the ATCC and CBS. In this study, a common probe (CP6) was used to identify all genotypes of Candida parapsilosis, and two additional probes (CP8 and CP10) that can hybridize with genotypes II and III were constructed. For this reason, if only probe CP6 was hybridized, the test organism was identified as Candida parapsilosis (genotype I). If probe CP8 or CP10 was also hybridized, the strain would be Candida parapsilosis genotype II (Candida orthopsilosis) or III (Candida metapsilosis).

Trichosporon asahii, Trichosporon aquatile, and Trichosporon inkin are closely related species (44). Molecular phylogenetic trees based on both ITS sequences revealed that the three species and several other species form a distinct cluster among other Trichosporon species (44). A common probe (probe code, TAQAS1) was designed for the three Trichosporon species; however, each individual species can be differentiated from the other two species by its own specific probe (Table 2). Recently, a fatal case of sternal wound infection caused by Trichosporon inkin following aortic root surgery was reported (8). Trichosporon asahii can cause white piedra and onychomycosis in immunocompetent patients as well as various localized or disseminated infections in immunodeficient individuals.

TABLE 3. Nontarget species used for specificity test in this study

Species (teleomorph) <sup>a</sup>	Strain <sup>b</sup>
Acremonium strictum	BCRC 32239 <sup>T</sup>
Aspergillus fumigatus (Sartorya fumigata)	BCRC 30099
Aspergillus nidulans (Emericella nidulans)	BCRC 30100 <sup>T</sup>
Candida kruisii	BCRC 21573 <sup>T</sup>
Candida methanolovescens (Pichia minuta var.	DCDC 2047(
minuta)	BCRC 20476
Candida mogu (Zygosaccharomyces rouxii)	BCRC 21506*
Cryptococcus daszewskae	CBS 5123
Cryptococcus humicola	BCRC 21639 <sup>1</sup>
Cryptococcus luteolus	BCRC 22372
Debaryomyces polymorphus	BCRC 214781
Exophiala jeanselmei	CBS 835.95
Fusarium solani (Nectria haematococca)	ATCC 36031
Geotrichum capitatum	CBS 327.86
Microsporum canis (Arthroderma otae)	ATCC 10214
Microsporum gypseum (Arthroderma gypseum)	CBS 161.69
Mucor flavus	CBS 673.66
Penicillium marneffei	CBS 334.59 <sup>1</sup>
Pichia ciferrii	BCRC 22168 <sup>1</sup>
Pichia henricii	BCRC 22170
Pichia pastoris	BCRC 21531
Rhizopus oryzae	BCRC 31107
Saccharomycopsis capsularis	NRRL Y-17639
Saccharomycopsis crataegensis	BCRC 22563
Scedosporium prolificans	CBS 100390
Sporobolomyces roseus var. roseus	BCRC 22375
Torulopsis methanothermo (Pichia angusta)	BCRC 20467
Trichosporon debeurmannianum	CBS 1896
Trichosporon dermatis	CBS 2043
Trichosporon jirovecii	CBS 6864 <sup>T</sup>
Trichosporon mucoides	CBS 7625 <sup>T</sup>
Trichosporon ovoides	CBS 7556 <sup>T</sup>
Trichophyton rubrum	BCRC 32805
Trichophyton verrucosum	ATCC 28203

<sup>*a*</sup> Anamorphic names are used in this study, and teleomorphic species names are given in parentheses. Some species were only given the names of teleomorphs since they have no anamorphic names.

<sup>b</sup> A superscript "T" indicates a type strain.

Fungemia caused by *Trichosporon asahii* was recently reported for a very low-birth-weight neonate (33).

It has been found that the addition of multiple thymine (or adenosine) bases to the 3' (or 5') ends of probes can improve the hybridization signal of oligonucleotide probes (1, 35). Although the mechanisms of adding low numbers (5 to 20) of thymine bases to a probe is not clear, it was proposed that this might decrease the steric hindrance between the probe and target DNA during hybridization or might increase the binding of probes to the nylon membrane (1, 35). In this study, five to 17 additional bases of thymine were added to the 3' (or 5') ends of some probes that displayed weak hybridization signals (Table 2). In our experience, the benefit of adding thymine bases to a probe is especially obvious for relatively short probes (16- to 20-mers).

Although the designed probes were carefully screened to

FIG. 2. Hybridization results for 77 yeast species. Chips 1 to 45 (A) and 46 to 78 (B) are the hybridization patterns of *Candida* spp. (44 species) and non-*Candida* spp. (33 species), respectively. The chips are alphabetically arranged according to the species names. The corresponding probes hybridized on the arrays are indicated in Fig. 1, and the corresponding sequences of the hybridized probes are shown in Table 2. The positive control probe was located at D12 and I1 on each array.

avoid sequence homology with other microorganisms, many probes still cross-hybridized to nonhomologous species. To avoid cross-hybridization, 12 probes were intentionally designed to incorporate a mismatch base in each of them (Table 2). This strategy successfully eliminated nonspecific reactions, although the hybridization signals produced by the modified probes towards their target species decreased slightly. The incorporation of a mismatched base into a probe was based on the observations made previously by Ikuta et al. (22). Their results indicated that the G-T and G-A mismatches slightly destabilize a duplex, while the A-A, T-T, C-T, and C-A mismatches have significant destabilization effects. It was hoped that the incorporation of a mismatch in each of the 12 probes could eliminate nonspecific reaction, but at the same time still retain good hybridization signals toward their target yeasts with the result that sensitivity would not be sacrificed in the process of increasing specificity. This was successfully achieved in this study.

Commercially available identification kits, such as the API ID32C strip or Vitek YBC card, are commonly used for yeast identification in clinical laboratory. A recent study indicated that only 87% of clinical isolates were identified correctly to the species or genus level by the ID32C kit, with the remaining 13% isolates being either unidentified or misidentified (6). The most problematic species were *Candida rugosa* and *Candida utilis*; however, the two species were well distinguished by the present array (Fig. 2A). *Candida rugosa* is an emerging fungal pathogen and, along with *Candida glabrata* and *Candida krusei*, is a species of *Candida* with reduced susceptibility to the azole antifungals (37). In addition, strains of *Candida inconspicua* tend to be misidentified as *Candida norvegensis* by the ID32C panel (28), but both species were well differentiated by array hybridization (Fig. 2A).

In conclusion, the identification of clinically relevant yeasts by the present method is highly reliable and can be used as an accurate alternative to conventional identification methods. The method follows a common protocol that can be completed for isolated colonies within 24 h.

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