DISTRIBUTION OF PYROPHOSPHATIDIC ACID IN YEAST SPECIES

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The presence of pyrophosphatidic acid (pyro-PA) was investigated in the lipid extracts of yeasts (241 strains belonging to 167 species of 43 genera). Pyro-PA can be clearly separated from other polar lipids by two-dimensional thin layer chromatography, and it can be distinguished from other phospholipids by its specific staining (purple blue) with Dittmer reagent. Pyro-PA was found exclusively in basidiomycetous species (127 strains belonging to 78 species of 14 genera). In contrast, no detectable amount of pyro-PA was found in the cellular lipids of ascomycetous species (114 strains belonging to 89 species of 31 genera). These results demonstrate that the determination of the presence of pyro-PA in the cellular lipids is useful in distinguishing ascomycetous from basidiomycetous yeasts.

Ascomycetous and basidiomycetous yeasts have been discriminated by characteristics of cell wall structure (1–4), Diazonium Blue B (DBB) staining (4–6), urease activity (7, 8) and the GC content of DNA (9–12). Ascomycetous yeasts have two-layered cell walls, are not stained by DBB, usually lack extracellular urease, and have about 30 to 50 mol% GC content. Basidiomycetous yeasts have laminar cell walls, stain with DBB, usually produce extracellular urease, and have about 50 to 70 mol% GC content.

ITOH and KANEKO (13) reported that pyrophosphatidic acid [*P,P*-bis-(1,2-diacyl-*sn*-glycero-3)-pyrophosphate] (pyro-PA), was first found in the cellular lipid of *Cryptococcus neoformans* (13), and was also found in 2 strains of *Cryptococcus*, 2 of *Rhodotorula*, and 1 of *Trichosporon* (14, 15). This paper describes the distribution of pyro-PA in ascomycetous and basidiomycetous yeast species (241 strains

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belonging to 167 species of 43 genera), and suggests that the presence of pyro-PA in yeast species is significant in yeast taxonomy.

MATERIALS AND METHODS

Yeast strains used. The strains used in this study (167 species of 43 genera) are listed in Table 1.

Growth and harvest of yeast cells. Yeasts were inoculated on YM agar plates (10 g of glucose, 3 g of yeast extract, 3 g of malt extract, 10 g of peptone and 20 g of agar in 1,000 ml of tap water) and grown for 48 to 96 hr at 18 to 22°C. The cells were harvested after they spread over all of the surface of the plate (14).

Lipid extraction. Lipids were extracted from fresh yeast cells according to ITOH and KANEKO (16). Five to 10 g of the wet cells was suspended in 15 ml of a chloroform : methanol (1 : 2 v/v) mixture containing 10 g of glass beads (1.0 mm diameter). The mixture was homogenized for 30 min in a cell homogenizer (mix-tower A-14, Taiyou). The disrupted cell suspension was filtered through a glass filter (4G). The residual cell debris was suspended in 50 ml of a chloroform : methanol (2 : 1 v/v) mixture and stirred with a magnetic stirrer for 30 min at room temperature under an atmosphere of nitrogen, then filtered. The stirring and filtering processes were repeated three more times. The combined chloroform-methanol filtrate was washed with 0.9% NaCl solution to remove the non-lipid contaminants according to FOLCH's method (17).

Thin layer chromatography (TLC). The TLC methods described by ITOH and KANEKO (16) were used with Silica gel precoated plates (Merck Art. 5721, 10 × 10 cm). The following solvent mixtures were used: first dimension, chloroform : methanol : water (65 : 25 : 4); second dimension, chloroform : methanol : 7 N NH₄OH (65 : 35 : 5 v/v). The following detection reagents were used; the polar lipids were detected by spraying Dittmer reagent and charring at 150°C for 30 min. Pyro-PA was clearly separated from other polar lipids on the two-dimensional TLC, and distinguished from other phospholipids by specific staining purple blue with Dittmer reagent. Therefore, the presence of pyro-PA in a lipid sample was determined primarily by two-dimensional TLC. When a trace amount of pyro-PA was detected, the sample was concentrated for further chromatography as follows. The lipid-fraction was extracted with a chloroform : methanol (2 : 1 v/v) mixture from the spot corresponding to pyro-PA on the chromatograms of the crude total lipids. After this the lipid fraction was dried and the occurrence of pyro-PA was confirmed by two-dimensional TLC as described above.

DBB test. The Diazonium Blue B (DBB) color test was performed as described by VAN DER WALT and HOPU-HAVU (5). Cultures were plated on YM agar for 2 weeks at 24°C, or 20°C in the case of psychrophilic strains. The reagent was prepared immediately before use by dissolving the DBB salt (1 mg/ml, Fast Blue Salt B, Tokyo Chemical Ind. Co., Tokyo) in chilled 0.1 M Tris-HCl buffer (pH 7.0). The chilled DBB (1 to 2 drops) was applied directly to the surface of colonies,

and the color reaction was recorded. The alkaline-ethanol procedure was applied to the red yeast strains according to the method described by HAGLER and AHEARN (6). The strains were grown in 5.0 ml of Yeast Nitrogen Base with 0.5% glucose for 5 days at 24°C. The psychrophilic strains were cultured at 20°C.

Urease test. Production of urease was determined using Christensen urea agar (Difco), and the results were recorded after 2 and 5 days.

Fermentation test. Fermentation of glucose was observed by the Durham tube method after 1, 2, and 3 weeks at 25°C or 20°C for psychrophilic strains, Nitrogen Base (Difco) supplemented with 2% glucose was used.

RESULTS AND DISCUSSION

Presence of pyro-PA in yeasts other than Candida and Trichosporon

Pyro-PA can be clearly separated from other polar lipids on two-dimensional TLC (Fig. 1), and this can be confirmed by its specific staining—purple blue—with Dittmer reagent. The presence of pyro-PA, DBB coloring, and urease activity in the 150 strains used, other than *Candida* and *Trichosporon*, are shown in Tables 2, 3, and 4.

Pyro-PA was found in 33 strains belonging to 18 species in the 6 genera of basidiomycetous yeast species (Strain No. B 01–B 33) *Cystofilobasidium*, *Filobasidiella*, *Filobasidium*, *Leucosporidium*, *Rhodosporidium*, and *Sporidiobolus*, and in 55 strains belonging to 35 species of the 6 genera of basidiomycetous anamorphs *Bullera*, *Cryptococcus*, *Phaffia*, *Rhodotorula*, *Sporobolomyces*, and *Sterigmatomyces*. In *Rhodosp. malvinellum* (B 23 and B 24), *B. alba* (I 31), *B. tsugae* (I 38), and *Sp. roseus* (I 79) the presence of pyro-PA was confirmed by re-chromatography. In contrast, pyro-PA was not detected in ascosporogenous yeast species (34 strains belonging to 28 species of 23 genera (A 01–A 35)) nor in 28 strains belonging to 18 species of the 6 genera of ascomycetous anamorphs (I 01–I 28) *Brettanomyces*, *Kloeckera*, *Myxozyma*, *Oosporidium*, *Saitoella*, and *Trigonopsis*. ITOH and KANEKO (14) reported that pyro-PA was detected in *Kl. apiculata*, but this phospholipid was not found in the same strain in the following study (15). In this study, pyro-PA was not found in 3 strains of *Kl. apiculata*, 10 strains belonging to 4 species of *Kloeckera*, and 6 strains belonging to 4 species of its teleomorph, *Hanseniaspora*. Two strains of *Myxozyma* (I 23 and I 24) were formerly identified as *Torulopsis melibiosum* and *Candida mucilagina* (18, 19). They were established (20) on the basis of their production of extracellular polysaccharide, having a two-layered structure of cell walls (20, 21) and carbohydrate composition of intact cells (mannan and glucan as dominant components) (20). From those characteristics, this genus was considered to be ascomycetous. Pyro-PA was not detected in 2 strains of *Myxozyma melibiosi* (I 23) and *Myxozyma mucilagina* (I 24). *Saitoella complicata* (22) was formerly identified as *Rhodotorula glutinis* (23). This yeast was identified as ascomycetous anamorph on the basis of having two-layered of cell walls and a negative reaction to DBB. Pyro-PA was not found in the two strains of

Table 1. The yeast strains used in this study.

Strain No.	Yeasts	Sources
A 01	<i>Ambrosiozyma platypodis</i> (Baker et Kreger-van Rij) van der Walt	IFO 1471
A 02	<i>Citeromyces matritensis</i> (Santa-Maria) Santa Maria	IFO 0954
A 03	<i>Debaryomyces hansenii</i> (Zopf) Lodder et Kreger-van Rij	IAM 4978
A 04	<i>Dekkera bruxellensis</i> van der Walt	CBS 74 ^T
A 05	<i>Hanseniaspora osmophila</i> (Niehaus) Phaff, Miller et Shifrine	CBS 313 ^T
A 06		AJ 5186
A 07	<i>uvarum</i> (Niehaus) Shehata, Mrak et Phaff	AJ 5184
A 08	<i>valbyensis</i> Kloecker	CBS 479 ^T
A 09		AJ 5181
A 10	<i>vineae</i> van der Walt et Tschenschner	CBS 2171
A 11	<i>Hansenula anomala</i> (Hansen) H. et Sydow	RY I-2-1
A 12	<i>Issatchenkia terricola</i> (van der Walt) Kurtzman, Smiley et Johnson	RY 4024
A 13	<i>Kluyveromyces phaffi</i> (van der Walt) van der Walt	CBS 4417
A 14	<i>Lipomyces starkeyi</i> Lodder et Kreger-van Rij	CBS 1807
A 15	<i>Lodderomyces elongisporus</i> (Recca et Mrak) van der Walt	AJ 5470
A 16	<i>Metschnikowia pulcherrima</i> (Metschnikoff) Kamienski	RY K-427
A 17	<i>Nadsonia elongata</i> Konokotina	IFO 0665
A 18	<i>fluvescens</i> (Nadson et Konokotina) Sydow	IFO 0666
A 19	<i>Pachysolen tannophilus</i> Boidin et Adzet	AJ 4282
A 20	<i>Pichia membranaefaciens</i> Hansen	IAM 4987
A 21		RY IV-5-1
A 22	<i>Saccharomyces cerevisiae</i> Meyen et Hansen	CBS 1171 ^T
A 23		RY W-3
A 24	<i>Saccharomycodes ludwigii</i> Hansen	IFO 1043
A 25		RY K-511
A 26	<i>Saccharomycopsis capsularis</i> Schionning	IAM 4307
A 27	<i>Schizosaccharomyces malidevorans</i> Rankine et Fornachon	AWRI 442
A 28	<i>Schizosaccharomyces pombe</i> Lindner	RY No. 1011
A 29		RY No. 1012
A 30	<i>Sporopachthermia lactativola</i> Rodrigues de Miranda	CCY 17-12-1
A 31	<i>Torulaspora delbrueckii</i> (Lindner) Lindner	IAM 12236
A 32	<i>Zygosaccharomyces rouxii</i> (Boutroux) Yarrow	NRRL 2547
A 33	<i>Wickerhamia fluorescens</i> Soneda	AJ 4285
A 34	<i>Yarrowia lipolytica</i> (Wickerham et al.) van der Walt et von Arx	CBS 6303
B 01	<i>Cystofilobasidium capitatum</i> (Fell, Hunter et Tallman) Oberw. et Bandoni	ATCC 24507 ^T
B 02		ATCC 24508
B 03	<i>Filobasidiella neoformans</i> Kown-Chung var. <i>neoformans</i>	NIH 12 ^T
B 04	var. <i>bacillispora</i>	NIH 191
B 05	<i>Filobasidium capsuligenum</i> (Fell, Statzell, Hunter et Phaff)	
	Rodrigues de Miranda	IFO 1119
B 06	<i>floriforme</i> Olive	CBS 6241 ^T
B 07	<i>uniguttulatum</i> Kown-Chung	CU-1
B 08	<i>Leucosporidium frigidum</i> Fell, Statzell, Hunter et Phaff	IFO 1851
B 09	<i>navalis</i> Fell, Hunter et Phaff	IFO 1852
B 10	<i>scottii</i> Fell, Statzell, Hunter et Phaff	IFO 1287
B 11	<i>Rhodosporidium dacryoidum</i> Fell, Hunter et Tallman	CBS 6353 ^T
B 12	<i>diobovatum</i> Newell et Hunter	IFO 0667
B 13		RY FC 16

Table 1. (continued)

Strain No.	Yeasts	Sources
B 14		RY No. 231
B 15		RY K-1-1
B 16		IAM 12259
B 17		IFO 1828
B 18		IAM 12260 ^T
B 19	<i>Rhodospiridium infirmominiatum</i> Fell, Hunter et Tallman	CBS 6962
B 20		IFO 1057
B 21		CBS 6350
B 22		CBS 6352
B 23	<i>malvinellum</i> Fell et Hunter	CCY 62-4-1 ^T
B 24		CCY 62-4-2
B 25	<i>sphaerocarpum</i> Newell et Fell	IAM 12262
B 26		CCY 62-1-1
B 27	<i>toruloides</i> Banno	IFO 0559 ^T
B 28		CCY 62-3-1
B 29		CCY 62-3-2
B 30	<i>Sporidiobolus johansonii</i> Nyland	IFO 6903
B 31	<i>pararoseus</i> Fell et Tallman	IFO 0376
B 32	<i>ruinenii</i> Holzschu, Tredick et Phaff	IFO 1689
B 33	<i>salmonicolor</i> Fell et Tallman	CBS 6832
I 01	<i>Brettanomyces abstinentis</i> Yarrow et Ahearn	CBS 6055 ^T
I 02	<i>anomalous</i> Custers	CBS 77 ^T
I 03	<i>bruxellensis</i> Kufferath et van Laer	CBS 72 ^T
I 04	<i>claussenii</i> Custers	CBS 76 ^T
I 05	<i>custersianus</i> van der Walt	CBS 4805 ^T
I 06	<i>intermedius</i> (Krumbholz et Tauschanoff) van der Walt et van Kerken	CBS 73 ^T
I 07	<i>lambicus</i> Kufferath et van Laer	CBS 75 ^T
I 08	<i>naardenensis</i> Kalfschoten et Yarrow	CBS 6042 ^T
I 09	<i>Kloeckera africana</i> (Klocker) Janke	CBS 277 ^T
I 10		AJ 5173
I 11		RY K-415
I 12	<i>apiculata</i> (Reess emend. Klocker) Janke	CBS 104 ^T
I 13		AJ 4806
I 14		RY 00-1
I 15	<i>corticis</i> (Klocker) Janke	AJ 5171
I 16		AJ 5198
I 17		CBS 106 ^T
I 18		RY 983
I 19		RY 991
I 20	<i>japonica</i> Saito et Ohtani	CBS 281 ^T
I 21		AJ 5469
I 22	<i>javanica</i> (Klocker) Janke	RY 29B
I 23	<i>Myxozyma melibiosi</i> (Shifrine et Phaff) van der Walt, Weijman et von Arx	CBS 2102
I 24	<i>mucilagina</i> (Ohaff, Starmer, Mirand et Miller) van der Walt, Weijman et von Arx	UCD 76-236C
I 25	<i>Oosporidium margariferum</i> Stautz	IFO 1208

Table 1. (continued)

Strain No.	Yeasts	Sources
I 26	<i>Saitoella complicata</i> Goto, Sugiyama, Hamamoto et Komagata	RY H-3-9-1
I 27		RY H-3-9-2
I 28	<i>Trigonopsis variabilis</i> Schachner	IAM 4994
I 31	<i>Bullera alba</i> (Hanner) Derx	*K No. 85
I 32		*K No. 92
I 33		*K No. 101
I 34		*K No. 105
I 35	<i>dendrophila</i> van der Walt et Scott	CBS 6074 ^T
I 36	<i>piricola</i> Stadelmann	CCY 12-3-2
I 37	<i>singularis</i> (Phaff et do Carmo-Sousa) Rodrigues de Miranda	CBS 5109 ^T
I 38	<i>tsugae</i> Phaff et do Carmo-Sousa	CCY 12-2-1
I 39	<i>Cryptococcus albidus</i> (Saito) var. <i>albidus</i>	IFO 1320
I 40	var. <i>aerius</i> (Saito) Phaff et Fell	IAM 4317
I 41	<i>ater</i> Castellani et Cooke	CBS 4685 ^T
I 42	<i>bhutanensis</i> Goto et Sugiyama	AJ 5129
I 43	<i>gastricus</i> Reiersol et di Menna	CCY 17-5-1
I 44	<i>heveanensis</i> (Groenewege) Baptist et Kurtzman	CBS 569
I 45	<i>himalayensis</i> Goto et Sugiyama	RY H-3-11-6
I 46	<i>kuetzingii</i> Fell et Phaff	CCY 17-11-1
I 47	<i>laurentii</i> (Kufferath) Skinner	RY EC 2-3
I 48	<i>luteolus</i> (Saito) Skinner	IAM 12207
I 49	<i>macerans</i> (Frederiksen) Phaff et Fell	CCY 17-19-1
I 51	<i>neoformans</i> (Sanfelice) Vuillemin	IAM 12253
I 52	<i>skinneri</i> Phaff et do Carmo-Sousa	CCY 17-17-1
I 53	<i>terreus</i> di Menna	CCY 17-8-1
I 54	<i>Phaffia rhogozyma</i> Miller, Yoneyama et Soneda	CBS 5905
I 55	<i>Rhodotorula araucariae</i> Grinbergs et Yarrow	CBS 6031 ^T
I 56	<i>aurantiaca</i> (Saito) Lodder	RY EC 7-4
I 57	<i>fujisanensis</i> (Soneda) Johnson et Phaff	CBS 4551 ^T
I 58		RY No. 870
I 59	<i>glutinis</i> (Fresenis) Harrison	IAM 4988
I 60		IAM 12263
I 61		IFO 0697
I 62		IFO 1099
I 63		RY KC 4-4
I 64		RY KC 9
I 65		IAM 12228
I 66		CBS 20 ^T
I 67	<i>glutinis</i> (Fres.) Harrison var. <i>salinaria</i> Hirosawa et Takada	IFO 1438
I 68	<i>graminis</i> di Menna	IFO 0190
I 69		CCY 20-14-1 ^T
I 70	<i>lactosa</i> Hasegawa	IAM 12230 ^T
I 71	<i>minuta</i> (Saito) Harrison	IFO 0387 ^T
I 72		CBS 5475
I 73	<i>rubra</i> (Demme) Lodder	ATCC 9449 ^T
I 74	<i>Sporobolomyces albo-rubescens</i> Derx	CBS 482 ^T
I 75	<i>gracilis</i> Derx	IFO 1227 ^T

Table 1. (continued)

Strain No.	Yeasts	Sources
I 76	<i>Sporobolomyces hispanicus</i> Pelaez et Ramirez	CBS 497
I 77	<i>odorus</i> Derx	*K No. 19
I 78	<i>roseus</i> Kluyver et van Niel	*K No. 69
I 79		*K No. 70
I 80		*K No. 71
I 81		*K No. 72
I 82		*K No. 73
I 84	<i>salmonicolor</i> (Fisher et Brebeck) Kluyver et van Niel	*K No. 7
I 85	<i>shibatanus</i> (Okunuki) Verona et Ciferri	*K No. 56
I 86		*K No. 75
I 87	<i>Sterigmatomyces elviae</i> Sonk et Yarrow	IFO 1843 ^T
C 01	<i>Candida acutus</i> Goto	RY C-96-2
C 02	<i>albicans</i> (Robin) Berkhout	IAM 4966
C 03	<i>amyolenta</i> vander Walt, Scott et van der Klift	CBS 6039 ^T
C 04	<i>antarctica</i> (Goto, Sugiyama et Iizuka) Kurtzman, Smiley, Johnson et Hoffman	CBS 6821
C 05	<i>aquatica</i> Jones et Slooff	CBS 5443 ^T
C 06	<i>auriculariae</i> (Nakase) Meyer et Yarrow	IFO 1580
C 07	<i>auringiensis</i> Santa Maria	CBS 6913
C 08	<i>bacarum</i> (Buhagiar) Meyer et Yarrow	CBS 6526 ^T
C 09	<i>blankii</i> Buckley et van der Uden	CBS 1898 ^T
C 10	<i>boidinii</i> Ramirez	AJ 4779
C 11	<i>bombicola</i> (Spencer, Gorin et Tulloch) Meyer et Yarrow	ATCC 22214
C 12	<i>buffonii</i> (Ramirez) van Uden et Buckley	CBS 2838 ^T
C 13	<i>catenulata</i> Diddens et Lodder	IAM 12182
C 14	<i>chiropterorum</i> Grose et Marinkelle	CBS 6064 ^T
C 15	<i>colliculosa</i> (Hartmann) Meyer et Yarrow	IAM 4426
C 16	<i>curiosa</i> Komagata et Nakase	IFO 1336
C 17	<i>curvata</i> (Diddens et Lodder) Lodder et Kreger-van Rij	IFO 1159
C 18	<i>diffluens</i> Ruinen	CBS 5233 ^T
C 19		IFO 1521
C 20	<i>edax</i> van der Walt et Nel	CBS 5657 ^T
C 21	<i>famata</i> (Harrison) Meyer et Yarrow	IFO 0623
C 22		IFO 0380
C 23		AJ 4385
C 24	<i>foliorum</i> Ruinen	CBS 6370
C 25	<i>fusiformata</i> Buhagiar	CBS 6951
C 26	<i>guilliermondii</i> (Castellani) Langeron et Guerra	IAM 4974
C 27	<i>hellenica</i> (Verona et Picci) King et Jong	CBS 4099 ^T
C 28	<i>humicola</i> (Daszewska) Diddens et Lodder	IFO 0760
C 29		IFO 1527
C 30		AJ 4385
C 31	<i>hydrocarbofumaria</i> Yamada et al. ex Ramirez	IFO 1973
C 32	<i>hylophila</i> van der Walt, van der Klift et Scott	CBS 6226 ^T
C 33	<i>ingeniosa</i> (di Menna) Meyer et Yarrow	AJ 5002
C 34	<i>inositophila</i> Nakase	IFO 1575
C 35	<i>intermedia</i> (Ciferri et Ashford) Lahgeron et Guerra	IAM 12185
C 36	<i>javanica</i> Ruinen	CBS 5236

Table 1. (continued)

Strain No.	Yeasts	Sources
C 37	<i>Candida krissii</i> Goto, Yamasato et Iizuka	CBS 6519 ^T
C 38	<i>kruisii</i> (Kockova-Kratochvilova et Ondrusova) Meyer et Yarrow	ATCC 52828
C 39	<i>krusei</i> (Castellani) Berkhout	RY WF 16
C 40	<i>lambica</i> (Lindner et Genoud) van Uden et Buckley	AJ 4461
C 41	<i>lusitaniae</i> van Uden et do Carmo-Sousa	IAM 12189
C 42	<i>magnoliae</i> (Lodder et Kreger-van Rij) Meyer et Yarrow	IFO 0661
C 43	<i>maltosa</i> Komagata, Nakase et Katsuya	IAM 12247
C 44	<i>mamillae</i> Goto	RY PJ 819
C 45	<i>mucilagina</i> Phaff, Starmer, Miranda et Miller	CBS 7071 ^T
C 46	<i>muscorum</i> di Menna	CBS 6921 ^T
C 47	<i>parapsilosis</i> (Ashford) Langeron et Talice	IAM 12270
C 48		AJ 4575
C 49	<i>philyla</i> (van der Walt, van der Klift et Scott) Meyer et Yarrow	CBS 6272 ^T
C 50	<i>podzolica</i> Babjeva et Reshetova	CBS 6819 ^T
C 51	<i>psychrophila</i> (Goto, Sugiyama et Iizuka) Meyer et Yarrow	CBS 5956 ^T
C 52	<i>pustula</i> (Buhagiar) Meyer et Yarrow	CBS 6527 ^T
C 53	<i>rugosa</i> (Anderson) Diddens et Lodder	IAM 12198
C 54	<i>sake</i> (Saito et Ota) van Uden et Buckley	IAM 12200
C 55	<i>sonckii</i> Hopsu-Haru, Tunnela et Yarrow	CBS 8115
C 55	<i>sonckii</i> Hopsu-Havu, Tunnela et Yarrow	CBS 8115
C 57	<i>stellata</i> (Kroemer et Krumbholz) Meyer et Yarrow	AJ 4394
C 58	<i>terebra</i> Sugiyama et Goto	RY KC-1-2
C 59	<i>tropicalis</i> (Castellani) Berkhout	IAM 4185
C 60		RY E-16
C 61	<i>tsukubaensis</i> Onishi	RY No. 668
C 62	<i>utilis</i> (Henneberg) Lodder et Kreger-van Rij	IAM 4961
C 63	<i>valdiviana</i> Grinberg et Yarrow	CBS 5721
C 64	<i>valida</i> (Leberle) van Uden et Buckley	IAM 12190
C 65		RY K-504
C 66	<i>versatilis</i> (Ettchells et Bell) Meyer et Yarrow	IAM 4993
C 67	<i>zeylanoides</i> (Castellani) Langeron et Guerra	IAM 12204
T 01	<i>Trichosporon aculeatum</i> Phaff, Miller et Shifrine	ATCC 15540
T 02		ATCC 28689
T 03	<i>aquatile</i> Hedrick et Dupont	ATCC 22310 ^T
T 04	<i>brassicae</i> Nakase	**SH 280
T 05	<i>capitatum</i> Diddens et Lodder	**SH 233
T 06		**SH 281
T 07	<i>cutaneum</i> (de Beurm., Gougerot et Vaucher) Ota	CBS 4829 ^T
T 08		CBS 7065
T 09		IFO 1200
T 10		**SH 285
T 11		**SH 287
T 12		**SH 418
T 13		**SH 422
T 14		ATCC 24659
T 15		ATCC 34396

Table 1. (continued)

Strain No.	Yeasts	Sources
T 16	<i>Trichosporon fennicum</i> Sonck et Yarrow	CBS 5928
T 17	<i>fermentans</i> Diddens	**SH 292
T 18		**SH 428
T 19		**SH 429
T 20	<i>melibiosaceum</i> Scott et van der Walt	CBS 6087
T 21	<i>pullulans</i> (Lindner) Diddens et Lodder	**SH 293
T 22		**SH 316
T 23		**SH 317
T 24		**SH 318

Abbreviations: IFO, Institute for Fermentation, Osaka, Japan; IAM, Institute of Applied Microbiology, The University of Tokyo, Japan; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; AJ, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan; RY, Laboratory of Applied Microbiology, Institute of Enology and Viticulture, Yamanashi University, Kofu, Japan; AWRI, Australian Wine Research Institute, Adelaide, Australia; CCY, Czechoslovak Collection of Yeasts, Bratislava, Czechoslovakia; ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; NRRL, ARS Culture Collection, Northern Regional Research Center, Peoria, U.S.A.; NIH, National Institutes of Health, Bethesda, Md., U.S.A.

*K strains accepted an offer from Prof. Dr. K. Komagata, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan and **SH strains accepted an offer from Prof. Dr. T. Sutoh, Nippon Veterinary and Zootechnical College, Musashino, Tokyo, Japan.

T: type strain.

Fig. 1. Thin layer chromatograms: (A) the polar lipids of *Rhodosp. toruloides* IFO 0559^T (B 27); (B) the polar lipids of *R. glutinis* CBS 20^T (I 66).

Plate: silica gel precoated plate, 10 × 10 cm (Merck Art. 5715). Solvent system: first dimension, chloroform:methanol:water (65:24:4 v/v); second dimension, chloroform:methanol:7N ammonium hydroxide (56:35:5 v/v). Detection: by spraying the chromatograms with Dittmer reagent and charring at 160°C for 60 min. Pyro-PA = pyrophosphatidic acid.

Table 2. The occurrence of pyrophosphatidic acid, DBB reaction and urease activity in ascosporeogenous yeasts.

Strain No.	Yeasts	Pyro-PA	DBB	Urease
A 01	<i>A. platypodis</i>	—	—	—
A 02	<i>Cit. matritensis</i>	—	—	+
A 03	<i>Deb. hansenii</i>	—	—	—
A 04	<i>D. bruxellensis</i>	—	—	—
A 05	<i>H'spora osmophila</i>	—	—	—
A 06		—	—	—
A 07	<i>uvarum</i>	—	—	—
A 08	<i>valbyensis</i>	—	—	—
A 09		—	—	—
A 10	<i>vineae</i>	—	—	—
A 11	<i>H. anomala</i>	—	—	—
A 12	<i>I. terricola</i>	—	—	+
A 13	<i>K. phaffi</i>	—	—	—
A 14	<i>L. starkeyi</i>	—	—	+
A 15	<i>Lod. elongisporus</i>	—	—	—
A 16	<i>M. pulcherrima</i>	—	—	—
A 17	<i>N. elongata</i>	—	—	+
A 18	<i>fluvescens</i>	—	—	+
A 19	<i>Pa. tannophilus</i>	—	—	—
A 20	<i>P. membranaefaciens</i>	—	—	—
A 21		—	—	—
A 22	<i>Sacch. cerevisiae</i>	—	—	—
A 23		—	—	—
A 24	<i>S'codes ludwigii</i>	—	—	—
A 25		—	—	—
A 26	<i>S. capsularis</i>	—	—	—
A 27	<i>Schiz. malidevorans</i>	—	—	+
A 28	<i>pombe</i>	—	—	+
A 29	<i>pombe</i>	—	—	+
A 30	<i>Sporop. lactativola</i>	—	—	—
A 31	<i>T'spora delbrueckii</i>	—	—	—
A 32	<i>Zygosacch. rouxii</i>	—	—	—
A 33	<i>W. fluorescens</i>	—	—	—
A 34	<i>Y. lipolytica</i>	—	—	+

+, positive; —, negative.

Saitoella complicata (I 26 and I 27).

Generally, for the yeast species in which pyro-PA was detected, the DBB reaction and urease activity were positive. The species having those characteristics belong to the basidiomycetous yeasts.

The DBB test (1, 3–6, 12), in conjunction with the cell wall structure, was a practical procedure for discrimination between ascomycetous and basidiomycetous yeasts in which a sexual stage was not demonstrated. However, the simple procedure on solid medium often gave indecisive results, particularly in the coloration of red yeasts such as *Rhodosporidium*, *Rhodotorula*, and *Sporobolomyces*.

Table 3. The occurrence of pyrophosphatidic acid, DBB reaction and urease activity in basidiosporogenous yeasts.

Strain No.	Yeasts	Pyro-PA	DBB	Urease
B 01	<i>Cystof. capitatum</i>	+	v	w
B 02		+	v	w
B 03	<i>Fil. neoformans</i> var. <i>neoformans</i>	+	+	+
B 04	var. <i>bacillispora</i>	+	+	+
B 05	<i>F. capsuligenum</i>	+	+	+
B 06	<i>floriforme</i>	+	+	+
B 07	<i>uniguttulatum</i>	+	+	+
B 08	<i>Leu. frigidum</i>	+	+	+
B 09	<i>nivalis</i>	+	+	+
B 10	<i>scottii</i>	+	+	+
B 11	<i>Rhodosp. dacryoidum</i>	+	+	+
B 12	<i>diobovatum</i>	+	+	+
B 13		+	v	+
B 14		+	+	+
B 15		+	+	+
B 16		+	v	+
B 17		+	+	+
B 18		+	+	w
B 19	<i>infirmominiatum</i>	+	+	+
B 20		+	+	w
B 21		+	+	+
B 22		+	v	+
B 23	<i>malvinellum</i>	+	v	w
B 24		+	v	—
B 25	<i>sphaerocarpum</i>	+	v	+
B 26		+	+	+
B 27	<i>toruloides</i>	+	v	+
B 28		+	v	+
B 29		+	v	+
B 30	<i>Sporid. johansonii</i>	+	v	+
B 31	<i>pararoseus</i>	+	v	+
B 32	<i>ruinenii</i>	+	+	+
B 33	<i>salmonicolor</i>	+	v	+

+, positive; w, weak; v, less definite; —, negative.

On the other hand, the alkaline-ethanol-DBB test described by HAGLER and AHEARN (6) required the exact preparation of DBB reagent and culture conditions and its procedures are very complicated. As for the results of a simple procedure on solid medium and alkaline-ethanol procedure, they often gave less definitive results. In this study, we observed indecisive coloration in 35 strains belonging to 21 species of *Cystofilobasidium*, *Rhodosporidium*, *Sporidiobolus*, *Bullera*, *Rhodotorula*, and *Sporobolomyces*. Generally, extracellular urease production is common in basidiomycetous yeasts. However, it has been reported that some ascomycetous yeasts also produce urease. In this study, urease activities were observed in several

Table 4. The occurrence of pyrophosphatidic acid, DBB reaction and urease activity in imperfect yeasts.

Strain No.	Yeasts	Pyro-PA	DBB	Urease
I 01	<i>Br. abstinens</i>	—	—	—
I 02	<i>anomalus</i>	—	—	—
I 03	<i>bruxellensis</i>	—	—	—
I 04	<i>clausenii</i>	—	—	—
I 05	<i>custersianus</i>	—	—	—
I 06	<i>intermedius</i>	—	—	—
I 07	<i>lambicus</i>	—	—	—
I 08	<i>naardenensis</i>	—	—	—
I 09	<i>Kl. africana</i>	—	—	—
I 10		—	—	—
I 11		—	—	—
I 12	<i>apiculata</i>	—	—	—
I 13		—	—	—
I 14		—	—	—
I 15	<i>corticis</i>	—	—	—
I 16		—	—	—
I 17		—	—	—
I 18		—	—	—
I 19		—	—	—
I 20	<i>japonica</i>	—	—	—
I 21		—	—	—
I 22	<i>javanica</i>	—	—	—
I 23	<i>My. melibiosi</i>	—	—	—
I 24	<i>mucilagina</i>	—	—	—
I 25	<i>O. margaritifera</i>	—	—	—
I 26	<i>Sait. complicata</i>	—	—	+
I 27		—	—	+
I 28	<i>Trig. variabilis</i>	—	—	—
I 31	<i>B. alba</i>	+	—	—
I 32		+	—	—
I 33		+	—	—
I 34		+	—	—
I 35	<i>dendrophila</i>	+	+	+
I 36	<i>piricola</i>	+	+	+
I 37	<i>singularis</i>	+	+	+
I 38	<i>tsugae</i>	+w	v	v
I 39	<i>Cr. albidus</i> var. <i>albidus</i>	+	+	+
I 40	var. <i>aerius</i>	+	+	+
I 41	<i>ater</i>	+	+	+
I 42	<i>bhutanensis</i>	+	+	v
I 43	<i>gastricus</i>	+	+	+
I 44	<i>heveanensis</i>	+	+	+
I 45	<i>himalayensis</i>	+	+	+
I 46	<i>kuetzingii</i>	+	+	+
I 47	<i>laurentii</i>	+	+	+
I 48	<i>luteolus</i>	+	+	+
I 49	<i>macerans</i>	+	+	+
I 51	<i>neoformans</i>	+	+	+

Table 4. (continued)

Strain No.	Yeasts	Pyro-PA	DBB	Urease
I 52	<i>Cr. skinneri</i>	+	+	+
I 53	<i>terreus</i>	+	+	+
I 54	<i>Ph. rhodozyma</i>	+	+	+
I 55	<i>Rh. araucariae</i>	+	v	+
I 56	<i>aurantiaca</i>	+	v	+
I 57	<i>fujisanensis</i>	+	+	+
I 58		+	+	+
I 59	<i>glutinis</i>	+	v	+
I 60		+	v	+
I 61		+	v	+
I 62		+	v	+
I 63		+	v	+
I 64		+	v	+
I 65		+	v	+
I 66		+	v	+
I 67	<i>glutinis</i> var. <i>salinaria</i>	+	+	+
I 68	<i>graminis</i>	+	v	+
I 69		+	v	+
I 70	<i>lactosa</i>	+	+	+
I 71	<i>minuta</i>	+	+	+
I 72		+	v	+
I 73	<i>rubra</i>	+	v	+
I 74	<i>Sp. albo-rubescens</i>	+	+	+
I 75	<i>gracilis</i>	+	+	+
I 76	<i>hispanicus</i>	+	v	+
I 77	<i>odorus</i>	+	v	+
I 78	<i>roseus</i>	+	+	+
I 79		+	v	+
I 80		+	+	+
I 81		+	+	+
I 82		+	v	+
I 84	<i>salmonicolor</i>	+	v	+
I 85	<i>shibatani</i>	+	+	+
I 86		+	v	+
I 87	<i>St. elviae</i>	+	+	+

+, positive; w, weak; v, less definite; —, negative.

ascomycetous species: *Cit. matritensis* (A 02), *I. terricola* (A 12), *L. starkeyi* (A 14), 2 strains (A 17 and A 18) of *Nadsonia*, 3 (A 27, A 28 and A 29) of *Schizosaccharomyces*, 2 (I 26 and I 27) of *Saitoella complicata*, and *Yarrowia lipolytica* (A 34). In contrast, urease activity was indicated very faintly in several basidiomycetous yeasts: 2 strains (B 01 and B 02) of *Cystof. capitatum*, 1 (B 18) of *Rhodosp. diobovatum*, 1 (B 20) of *Rhodosp. infirmominiatum*, 2 (B 23 and B 24) of *Rhodosp. malvinellum*, 1 (I 38) of *B. tsugae*, and 1 (I 42) of *Cr. bhutanensis*. Therefore, the urease test does not reliably discriminate between ascomycetous and basidiomycetous species.

Table 5. The occurrence of pyrophosphatidic acid and DBB reaction, urease activity, and fermentation in *Candida*.

Strain No.	Species	Pyro-PA	DBB	Urease	Fermentation
C 01	<i>C. acutus</i>	+	+	+	—
C 03	<i>amyolenta</i>	+	+	+	—
C 04	<i>antarctica</i>	+	+	+	—
C 05	<i>aquatica</i>	+	+	+	—
C 06	<i>auriculariae</i>	+	+	+	—
C 08	<i>bacarum</i>	+	+	+	—
C 16	<i>curiosa</i>	+	+	+	+
C 17	<i>curvata</i>	+	+	+	—
C 18	<i>diffloens</i>	+	+	+	—
C 19		+	+	+	—
C 24	<i>foliorum</i>	+	v	+	—
C 25	<i>fusiformata</i>	+	v	+	—
C 28	<i>humicola</i>	+	+	+	—
C 29		+	+	+	—
C 30		w	+	+	—
C 32	<i>hylophila</i>	+	+	+	—
C 33	<i>ingeniosa</i>	+	+	+	—
C 36	<i>javanica</i>	+	w	+	—
C 46	<i>muscorum</i>	+	+	+	—
C 49	<i>philyla</i>	+	+	+	—
C 50	<i>podzolica</i>	+	+	+	—
C 52	<i>pustula</i>	+	+	+	—
C 55	<i>sonkii</i>	+	+	v	—
C 61	<i>tsukubaensis</i>	+	+	+	—
C 02	<i>albicans</i>	+	—	—	—
C 07	<i>auriculariae</i>	—	—	—	+
C 09	<i>blankii</i>	—	—	—	w
C 10	<i>boidinii</i>	—	—	—	+
C 11	<i>bombicola</i>	—	—	—	+
C 12	<i>buffonii</i>	—	—	v	+
C 13	<i>catenulata</i>	—	—	—	w
C 14	<i>chiropterorum</i>	—	—	—	—
C 15	<i>colliculosa</i>	—	—	—	+
C 20	<i>edax</i>	—	—	—	—
C 21	<i>famata</i>	—	—	—	+
C 22		—	—	—	+
C 23		—	—	—	+
C 26	<i>guilliermondii</i>	—	—	—	+
C 27	<i>hellenica</i>	—	—	—	+
C 31	<i>hydrocarbofumaria</i>	—	—	—	—
C 34	<i>inositophila</i>	—	—	—	+
C 35	<i>intermedia</i>	—	—	—	+
C 37	<i>krissii</i>	—	—	—	—/+
C 38	<i>kruisii</i>	—	—	—	+
C 39	<i>krusei</i>	—	—	w	+
C 40	<i>lambica</i>	—	—	—	+
C 41	<i>lusitaniae</i>	—	—	—	+
C 42	<i>magnoliae</i>	—	—	—	+

Table 5. (continued)

Strain No.	Species	Pyro-PA	DBB	Urease	Fermentation
C 43	<i>C. maltosa</i>	—	—	—	+
C 44	<i>mamillae</i>	—	—	—	+
C 45	<i>mucilagina</i>	—	—	—	—
C 47	<i>parapsilosis</i>	—	—	—	+
C 48		—	—	—	+
C 51	<i>psychrophila</i>	—	—	—	—
C 53	<i>rugosa</i>	—	—	—	—
C 54	<i>sake</i>	—	—	—	+
C 56	<i>steatolytica</i>	—	—	—	+
C 57	<i>stellata</i>	—	—	—	+
C 58	<i>terebra</i>	—	—	—	+
C 59	<i>tropicalis</i>	—	—	—	+
C 60		—	—	—	+
C 62	<i>utilis</i>	—	—	—	+
C 63	<i>valdiviana</i>	—	—	—	+
C 64	<i>valida</i>	—	—	—	+
C 65		—	—	—	+
C 66	<i>versatilis</i>	—	—	—	+
C 67	<i>zeylanoides</i>	—	—	—	+

+, positive; w, weak; v, less definite; —, negative.

Presence of pyro-PA in genus Candida

For the genus *Candida*, the results of pyro-PA, DBB, urease, and fermentation tests are shown in Table 5. Pyro-PA was detected in 19 species (22 strains) that were assumed to be basidiomycetous by MEYER et al. (24), and in *C. acutus* (C 01) and *C. fusiformata* (C 25). *C. acutus* was described as a hemibasidiomycetous species by GOTO (25) and *C. fusiformata* was reported as being a species related to *Rhodotorula* and *Cryptococcus* (26). In contrast, no pyro-PA was detected in 43 strains of 38 ascomycetous species. Exceptionally, pyro-PA was found in *C. albicans* (C 02) which was considered to be an ascomycetous species. The DBB test in 19 basidiomycetous species (22 strains) in which pyro-PA was found were positive, although coloration was less definitive in *C. foliorum* (C 24) and *C. fusiformata* (C 25). Twenty basidiomycetous yeasts (23 strains) in which pyro-PA was detected, produced urease, although urease activity was indefinite in *C. sonckii* (C 55). Twenty basidiomycetous species (23 strains), except *C. curiosa* (C 16), did not ferment glucose. As in the above results, we observed that the DBB and urease tests gave less definitive results for several species of *Candida*. Therefore, basidiomycetous species in the genus *Candida* can be discriminated correctly by the pyro-PA test, in addition to the DBB, urease, and fermentation tests as a practical procedure. *C. albicans* (C 02), the ascomycetous species in which pyro-PA was found may require additional studies to elucidate this presence of pyro-PA.

Table 6. The occurrence of pyrophosphatidic acid and DBB reaction, urease activity, and fermentation in *Trichosporon*.

Strain No.	Species	Pyro-PA	DBB	Urease	Fermentation
T 01	<i>Tr. aculeatum</i>	—	—	—	—/w
T 02		—	—	—	—/w
T 05	<i>capitatum</i>	—	—	—	—
T 06		—	—	—	—
T 16	<i>fennicum</i>	—	—	—	+
T 17	<i>fermentans</i>	—	—	—	+
T 18		—	—	—	+
T 19		—	—	—	+
T 20	<i>melibiosaceum</i>	—	—	—	+
T 03	<i>aquatile</i>	+	+	+	—
T 04	<i>brassicae</i>	+	+	+	—
T 07	<i>cutaneum</i>	+	+	+	—
T 08		+	+	+	—
T 09		+	+	+	—
T 10		+	+	+	—
T 11		+	+	+	—
T 12		+	+	+	—
T 13		+	+	+	—
T 14		+	+	+	—
T 15		+	+	+	—
T 21	<i>pullulans</i>	+	+	+	—
T 22		+	+	+	—
T 23		+	+	+	—
T 24		+	+	+	—

+, positive; w, weak; —, negative.

Presence of pyro-PA in the genus Trichosporon

For the genus *Trichosporon*, the results of the pyro-PA, DBB, urease and fermentation tests are shown in Table 6. Pyro-PA was found in a total of 15 strains of 4 species (*Tr. aquatile*, *Tr. brassicae*, *Tr. cutaneum*, and *Tr. pullulans*) that were assumed to be basidiomycetous by KREGER-VAN RIJ (27). On the other hand, pyro-PA was not detected in 9 strains of 5 species (2 strains of *Tr. aculeatum*, 2 of *Tr. capitatum*, 1 of *Tr. fennicum*, 3 of *Tr. fermentans*, and 1 of *Tr. melibiosaceum*) that were classified as ascomycetous species by KREGER-VAN RIJ (27). For basidiomycetous species in which pyro-PA was found, the DBB and urease tests were positive and the fermentation test was negative, without exception. On the contrary, for ascomycetous species in which pyro-PA was not detected, the results of DBB and urease tests were negative without exception. Some authors have proposed to split the genus *Trichosporon* (28–30). According to these authors *Trichosporon* can be separated into the basidiomycetous and ascomycetous species based on the cell wall and septa structures, DBB reaction, urease activity, composition of the carbohydrate of the cells, and the GC content of DNA.

The results of pyro-PA, DBB, and urease tests in ascomycetous and ba-

Table 7. The occurrence of pyrophosphatidic acid, DBB reaction, and urease activity in yeast genera.

Yeasts	Genera	Species	Strains	Pyro-PA	DBB	Urease
Ascomycetous						
Teleomorph	23	28	34	—	—	—
Anamorph						
<i>Brettanomyces</i>		8	8	—	—	—
<i>Kloeckera</i>		5	14	—	—	—
<i>Myxozyma</i>		2	2	—	—	—
<i>Oosporidium</i>		1	1	—	—	—
<i>Saitoella</i>		1	2	—	—	—
<i>Trigonopsis</i>		1	1	—	—	—
<i>Candida</i> (A)		38	43	—	—	—
<i>Trichosporon</i> (A)		5	9	—	—	—
Basidiomycetous						
Teleomorph						
<i>Cystofilobasidium</i>		1	2	+	v	+ /w
<i>Filobasidiella</i>		1	2	+	+	+
<i>Filobasidium</i>		3	3	+	+	+
<i>Leucosporidium</i>		3	3	+	+	+
<i>Rhodospiridium</i>		6	19	+	+ /v	+ /w /—
<i>Sporidiobolus</i>		4	4	+	+ /v	+ /w
Anamorph						
<i>Bullera</i>		5	8	+	+ /v	+ /w
<i>Cryptococcus</i>		13	14	+	+	+
<i>Phaffia</i>		1	1	+	+	+
<i>Rhodotorula</i>		8	19	+	+ /v	+ /w
<i>Sporobolomyces</i>		7	12	+	+ /v	+ /w
<i>Sterigmatomyces</i>		1	1	+	+	+
<i>Candida</i> (B)		21	24	+	+ /v, w	+ /v
<i>Trichosporon</i> (B)		4	15	+	+	+

+, positive; w, weak; v, less definite; —, negative.

sidiomycetous yeast genera are shown in Table 7. Generally, for the yeast species (or genera) in which pyro-PA was detected, the DBB reaction and urease activity were positive. The species (or genera) having those characteristics belong to the basidiomycetous yeasts. In contrast, for the species (or genera) in which pyro-PA was not found, DBB coloration and urease activity were usually negative. The species (or genera) having those characteristics belong to the ascomycetous yeasts. When the pyro-PA test is compared with both the DBB and urease tests, it is a comparatively simple procedure and the presence of pyro-PA is detected without difficulty. We consider that discriminating ascomycetous from basidiomycetous yeast can be done more distinctly by using the pyro-PA tests. Therefore, this test is recommended as a routine and practical procedure for discriminating between ascomycetous and basidiomycetous yeast species or genera.

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