A COMPARATIVE TAXONOMIC STUDY ON TWO FORMS OF *CANDIDA PARAPSILOSIS* (ASHFORD) LANGERON ET TALICE

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A comparative study was made on two forms of *Candida parapsilosis*. From the reciprocal absorption experiment of antiserum for heated cells of these yeasts, and gel diffusion precipitation of mannans, the two forms were assumed to possess a small amount of respectively specific antigens in addition to antigens common to both. Factor antiserum 24 prepared from antiserum for *Saccharomyces rosei* agglutinated *C. parapsilosis* form I but not form II, and is considered to be useful for clear-cut discrimination of the two forms. Proton magnetic resonance spectrum of mannans also demonstrated the difference of the two forms. Further, *C. parapsilosis* assimilated L-arabinose, did not excrete excess amount of riboflavin into synthetic media, and did not require thiamine. Since *C. parapsilosis* form II was found to correspond to the imperfect form of *Lodderomyces elongisporus*, these characteristics are useful for the discrimination of the imperfect form of *L. elongisporus* from *C. parapsilosis*.

In 1962, two isolates of asporogenous yeast were obtained from adhesives of a neutralizing tank of L-glutamic acid in monosodium glutamate manufacturing plant, and were identified with *Candida parapsilosis* (1). Later, these two yeasts were found to differ from typical strains of *C. parapsilosis* in the lack of assimilation of L-arabinose, requirement of thiamine, excretion of excess amount of riboflavin, and serological characteristics. One of the authors tentatively dealt with these atypical strains as *C. parapsilosis* form II, and typical strains of *C. parapsilosis* as form I (2).

A precise study was carried out to clarify the taxonomic situation of yeasts belonging to *C. parapsilosis* form II, and these were found to represent the imperfect form of *Lodderomyces elongisporus*.

In the Second Edition of "The Yeasts, a Taxonomic Study" published in 1970,

C. parapsilosis was regarded as the imperfect form of *Lodderomyces elongisporus*(3). However, MEYER and PHAFF (4) reported that these two species were not closely related in DNA-DNA hybridization study.

In the present work, we found that *C. parapsilosis* can be discriminated from *L. elongisporus* and its imperfect form by assimilation of L-arabinose, requirement of thiamine, lack of antigen 24, proton NMR spectrum of mannan, and lack of excretion of excess amount of riboflavin into synthetic media. These characteristics are useful for routine discrimination of the imperfect form of *L. elongisporus* from *C. parapsilosis*.

MATERIALS AND METHODS

Microorganisms. Twenty strains of *C. parapsilosis* form I, two strains of *C. parapsilosis* form II, and a strain of *L. elongisporus* were employed. Source of strains is listed in Table 1. In addition to these strains, *Candida albicans* J-1012 (serotype A) and J-1445 (serotype B), *C. stellatoidea* J-1016, *C. guilliermondii* J-1023, *C. krusei* J-1005, *C. pseudotropicalis* J-1004, *Torulopsis glabrata* J-4002, and *Saccharomyces rosei* J-6013 were used for serological investigation.

Taxonomic characteristics conventionally employed. Taxonomic characteristics were investigated mainly by the method described by WICKERHAM (5) and by those described in the Second Edition of "The Yeasts, a Taxonomic Study" (6). Vitamin requirement and maximum growth temperature were determined by the methods previously described (7).

Extraction and purification of polysaccharides. The yeast cells grown for 48 hr with gentle shaking in yeast extract (0.3%)–malt extract (0.3%)–peptone (0.5%) broth supplemented with 3% of glucose were harvested by centrifugation and washed three times with distilled water. The washed cell debris was suspended in 5 volumes of 2% aqueous potassium hydroxide solution and extracted for 2 hr at 100°. After neutralization with acetic acid, the extract was centrifuged and crude polysaccharide was recovered by the addition of 2 volumes of methanol into the resultant supernatant. The polysaccharide was purified *via* copper complex as described by GORIN and SPENCER (8).

Determination of component sugars of polysaccharide. Polysaccharide was analyzed for its component sugars by thin-layer chromatography using silica gel plate Art. 5721 (Merck, Germany) after being hydrolyzed in 1 N H₂SO₄ (100°, 18 hr) and neutralized with saturated Ba(OH)₂. Ethyl acetate–isopropanol–water (65:22:11) was used as the solvent system for development. The sugars were detected with *p*-anisaldehyde-sulfuric acid.

Determination of proton NMR spectrum. The proton NMR spectrum of polysaccharide was determined in 20% solution of D₂O at 70°, using a JEOL nuclear magnetic resonance spectrometer at 100 MHz. Sodium 3-(trimethylsilyl)propane-sulfonate (DSS) was used as the internal standard.

Table 1. Strains employed.

Strains		Sources
	J-1006	NI 7493
	J-1015	ATCC 10232
	J-1025	ATCC 10322
	J-1104	IFO 0585
	J-1124	IFO 0678
	J-1162	IFO 0640
	J-1172	IFO 0708
<i>C. parapsilosis</i> form I	J-1481	CBS 604, Type
	AJ 4569	IFO 0708
	AJ 4570	IFO 0585
	AJ 4572	Na glutamate manufacturing factory, Y-2-2 (I)
	AJ 4578	" Y-2-10 (I)
	AJ 4583	" Y-3-5 (I)
	AJ 4588	" Y-4-1 (I)
	AJ 4597	Kamaboko, YO-166 (I6)
	AJ 4598	" YO-171 (I6)
	AJ 4602	NI 7542
	AJ 4603	IFO 0640
	AJ 4604	IFO 1068
	AJ 5370	Human origin (Dr. T. Nishikawa)
<i>C. parapsilosis</i> form II	AJ 4571	Na glutamate manufacturing factory, Y-2-1 (I)
	AJ 4575	" Y-2-6 (I)
<i>L. elongisporus</i>	AJ 5470	CBS 2605

Serological characteristics. The methods for the preparation of antisera, the slide agglutination test, and the absorption test of agglutinins followed those described by TSUCHIYA *et al.* (9). Most of factor antisera were obtained from the Iatron Laboratory (Tokyo). Factor antiserum 24 was prepared by the authors. Gel diffusion precipitation was carried out in borate buffer (pH 8.4) (10) using 0.85% of Noble agar (Difco). Undiluted antiserum was placed in the central well and 0.05% solution of purified polysaccharide was filled in the outer wells. The plate was incubated at 10° for 5 days.

RESULTS

Characteristics commonly employed for determination

In the taxonomic characteristics commonly employed, strains tested in the present experiment closely resembled one another. Among 33 carbon compounds used in the assimilation test, difference was found only in one compound, L-arabinose. Twenty strains of *C. parapsilosis* form I, including the strain derived from the type (CBS 604), rapidly assimilated L-arabinose but two strains belonging to *C. parapsilosis* form II and *L. elongisporus* failed to assimilate this sugar (Table 2).

Table 2. Physiological characteristics of *C. parapsilosis* and *L. elongisporus* (I).
Assimilation of carbon compounds.

Carbon compounds	Species		
	<i>C. parapsilosis</i> form I (20 strains)	<i>C. parapsilosis</i> form II (2 strains)	<i>L. elongisporus</i> (1 strain)
Glucose	+	+	+
Galactose	+	+	+
Saccharose	+	+	+
Maltose	+	+	+
Lactose	—	—	—
L-Sorbose	+, w, —	+	L
Cellobiose	—	—	—
Trehalose	+	+	+
Melibiose	—	—	—
Raffinose	—	—	—
Melezitose	+	+	+
Inulin	—	—	—
Soluble starch	—	—	—
D-Xylose	+	+	L
L-Arabinose	+	—	—
D-Arabinose	—	—	—
D-Ribose	+, w, —	w, —	+
L-Rhamnose	—	—	—
Ethanol	+	+	+
Glycerol	+	+	+
Erythritol	—	—	—
Ribitol	+	+	+
Galactitol	—	—	—
D-Mannitol	+	+	+
D-Glucitol	+	+	+
α -Me-D-glucoside	+	+	+
Salicin	—	—	—
K Gluconate	+	+	+
Ca 2-Ketogluconate	+	+	+
DL-Lactic acid	—	—	—
Succinic acid	+, w	+, w	+
Citric acid	+	+	+
Inositol	—	—	—

w, weak; L, latent.

All the strains used required biotin at 25°, and *C. parapsilosis* form II and *L. elongisporus* also required thiamine (Table 3).

Excess amount of riboflavin was excreted into the synthetic media employed for the test of carbon assimilation when *C. parapsilosis* form II and *L. elongisporus* were cultivated, while no prominent pigmentation due to riboflavin excretion was observed in the case of *C. parapsilosis* form I (Table 3).

Table 3. Physiological characteristics of *C. parapsilosis* and *L. elongisporus* (2).

Characteristics	Species		
	<i>C. parapsilosis</i> form I (20 strains)	<i>C. parapsilosis</i> form II (2 strains)	<i>L. elongisporus</i> (1 strain)
Fermentation			
Glucose	+	+	+
Galactose	V	—	—
Saccharose	— or w	—	—
Maltose	— or w	—	—
Lactose	—	—	—
Raffinose	—	—	—
Melibiose	—	—	—
Trehalose	V	L	L
Vitamins required	biotin	biotin, thiamine	biotin, thiamine
Maximum growth temperature (°)	40–44	41–42	41–42
Liquefaction of gelatin	—	—	—
Split of arbutin	—	—	—
Production of "starch"	—	—	—
Urease	—	—	—
Assimilation of nitrate	—	—	—
Production of excess amount of riboflavin	—	+	+

+, positive; —, negative; V, variable; w, weak; L, latent.

Other characteristics including alcoholic fermentation from sugars, maximum growth temperature, splitting of arbutin, *etc.*, were not essentially different among the strains tested (Table 3). These yeasts were also similar to one another in the morphological characteristics though development of pseudomycelium was prominent in some strains and primitive in some other strains.

Serological characteristics

Investigation of serological characteristics revealed that the yeasts employed were divided into two groups. The one is composed of strains belonging to *C. parapsilosis* form I and the other is *C. parapsilosis* form II and *L. elongisporus*.

Reciprocal absorption experiment of antisera with heated cells showed that some difference was present in the surface antigens of the cell envelope between the two groups (Table 4). Unabsorbed antisera for *C. parapsilosis* form I J-1015, *C. parapsilosis* form II AJ 4571, and *L. elongisporus* AJ 5470 intensively agglutinated the cells of all of the strains employed.

Antiserum for *C. parapsilosis* form II AJ 4571 absorbed with heated cells of *C. parapsilosis* form I J-1015 agglutinated cells of *C. parapsilosis* form II and *L. elongisporus* but not any of *C. parapsilosis* form I. When this antiserum was

Table 4. Reciprocal absorption experiments among strains of *C. parapsilosis* and *L. elongisporus*.

Antisera for	Absorbed with	<i>L. elong.</i> AJ 5470	<i>C. parap.</i> form II		<i>C. parap.</i> form I												
			AJ 4571	AJ 4575	J-1015	J-1006	J-1025	J-1104	J-1162	AJ 4570	AJ 4572	AJ 4578	AJ 4583	AJ 4588	AJ 4597	AJ 4598	AJ 4602
<i>C. parap.</i> form I J-1015	<i>C. parap.</i> form II AJ 4571	—	—	—	+	+	±	+	±	+	+	+	+	+	+	±	±
"	<i>L. elong.</i> AJ 5470	—	—	—	±	+	±	+	±	+	+	+	+	+	+	±	±
<i>C. parap.</i> form II AJ 4571	<i>C. parap.</i> form I J-1015	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
"	<i>L. elong.</i> AJ 5470	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>L. elong.</i> AJ 5470	<i>C. parap.</i> form I J-1015	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
"	<i>C. parap.</i> form II AJ 4571	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

+, positive agglutination; —, negative agglutination; ±, ambiguous.

Table 5. Slide agglutination against factor antisera.

Heated cells		Slide agglutination with factor antisera ^a										
		1	4	5	6	8	9	13	13b	24	34	
<i>C. parapsilosis</i> form I	J-1006	††	—	+	—	—	—	††	††	††	—	
	J-1015	††	—	+	—	—	—	††	††	††	—	
	J-1025	††	—	+	—	—	—	††	††	††	—	
	J-1104	††	—	±	—	—	—	††	††	††	—	
	J-1124	††	—	+	—	—	—	††	††	††	—	
	J-1162	††	—	+	—	—	—	††	††	††	—	
	J-1172	††	—	±	—	—	—	††	††	††	—	
	J-1481	††	—	+	—	—	—	††	††	††	—	
	AJ 4569	††	—	±	—	—	—	††	††	††	—	
	AJ 4570	††	—	+	—	—	—	††	††	††	—	
	AJ 4572	††	—	+	—	—	—	††	††	††	—	
	AJ 4578	††	—	+	—	—	—	††	††	††	—	
	AJ 4583	††	—	+	—	—	—	††	††	††	—	
	AJ 4588	††	—	+	—	—	—	††	††	††	—	
	AJ 4597	††	—	+	—	—	—	††	††	††	—	
<i>C. parapsilosis</i> form II	AJ 4598	††	—	+	—	—	—	††	††	††	—	
	AJ 4602	††	—	+	—	—	—	††	††	††	—	
	AJ 4603	††	—	±	—	—	—	††	††	††	—	
	AJ 4604	††	—	±	—	—	—	††	††	††	—	
	AJ 5370	††	—	+	—	—	—	††	††	††	—	
	AJ 4571	††	—	±	—	—	—	††	††	—	—	
	AJ 4575	††	—	±	—	—	—	††	††	—	—	
<i>L. elongisporus</i>	AJ 5470	††	—	±	—	—	—	††	††	—	—	

^a ††, intensive agglutination; +, weak agglutination; ±, ambiguous; —, negative.

absorbed with *L. elongisporus*, antibody factors were completely absorbed.

Antiserum for *L. elongisporus* AJ 5470 absorbed with *C. parapsilosis* form I J-1015 or *C. parapsilosis* form II AJ 4571 gave a result similar to the case mentioned above (Table 4). Antiserum for *C. parapsilosis* form I J-1015 absorbed with *C. parapsilosis* form II AJ 4571 or *L. elongisporus* AJ 5470 agglutinated the cells of *C. parapsilosis* form I though agglutination was not so clear in some cases, but it did not agglutinate those of *C. parapsilosis* form II and *L. elongisporus*. These results indicated that these two groups possessed a small amount of respectively specific antigens in addition to the large amount of antigens common to both groups.

Agglutination by factor antisera demonstrated a more clear difference between the two groups (Table 5). All the strains employed were agglutinated by factor antisera 1, 5, 13, and 13b. Twenty strains belonging to *C. parapsilosis* form I were agglutinated also by factor antiserum 24 but *C. parapsilosis* form II and *L. elongisporus* were not.

When factor antiserum 24 was absorbed with heated cells of *C. parapsilosis* J-1015, agglutinins intensively reacting with *S. rosei* remained (Table 6). Therefore, antibody factors of factor antiserum 24 which agglutinate *C. parapsilosis* form I are antibodies induced by partial antigens of antigen 24.

Gel diffusion precipitation reaction of antisera for representative strains of *C. parapsilosis* form I and form II, and *L. elongisporus* against alkali-extracted polysaccharides of these yeasts is shown in Fig. 1. These polysaccharides were composed of only D-mannose.

Table 6. Specificity of factor antiserum 24 prepared from antiserum for *Saccharomyces rosei*.

Antiserum for	Absorbed with	Antigens											
		<i>C. albicans</i> J-1012 ^a	<i>C. albicans</i> J-1445 ^b	<i>C. tropicalis</i> J-1017	<i>C. stellatoidea</i> J-1016	<i>C. guilliermondii</i> J-1023	<i>C. parapsilosis</i> J-1015	<i>C. krusei</i> J-1005	<i>C. pseudotropicalis</i> J-1004	<i>T. glabrata</i> J-4002	<i>S. rosei</i> J-6013	<i>C. parapsilosis</i> form II AJ 4571	<i>L. elongisporus</i> AJ 5470
<i>S. rosei</i> J-6013 (IFO 0428)	<i>C. albicans</i> J-1012 ^a	—	—	—	—	—	≠	—	—	—	≡	—	—
"	<i>C. albicans</i> J-1012 ^a + <i>C. parapsilosis</i> J-1015	—	—	—	—	—	—	—	—	—	≡	—	—

^a Serotype A.

^b Serotype B.

Fig. 1. Schematic illustrations of agar gel diffusion precipitation with mannans from *C. parapsilosis* and *L. elongisporus*.

A, antiserum for *C. parapsilosis* form II AJ 4571 was placed in the central well; B, antiserum for *C. parapsilosis* form I J-1015 was placed in the central well; C, antiserum for *L. elongisporus* AJ 5470 was placed in the central well. Antigens were placed in the peripheral well in each case.

When antiserum for *C. parapsilosis* form II AJ 4571 was placed in the central well, a clear precipitation line common to mannans of *C. parapsilosis* form I, form II, and *L. elongisporus* was formed against each of mannans placed in the peripheral wells (Fig. 1A). In addition to the precipitation line mentioned above, a weak line was formed against each of mannans from *C. parapsilosis* form II and *L. elongisporus*. The latter was not observed in the case of mannans from *C. parapsilosis* form I.

Fig. 2. Proton NMR spectra (H-1 region) of mannans from *C. parapsilosis* and *L. elongisporus*.

Arabic numerals indicate τ values.

When antiserum for *L. elongisporus* AJ 5470 was employed, similar results were obtained (Fig. 1C).

When antiserum for *C. parapsilosis* form I J-1015 was used, two kinds of precipitation line formed (Fig. 1B). The one was common to mannans from *C. parapsilosis* form I, form II, and *L. elongisporus*. The other was formed against mannans from *C. parapsilosis* form I but not against *C. parapsilosis* form II or *L. elongisporus*.

Proton NMR spectra of alkali-extracted mannans from six representative strains are shown in Fig. 2. All of the mannans tested demonstrated four signals with similar τ values in the range of H-1 region (Fig. 2). Differences were found, however, in the intensity of signals with τ values of 4.71–4.77 and 5.03–5.10. Namely, the signal of τ 4.71–4.77 was more intensive than that of τ 5.03–5.10 in mannans from *C. parapsilosis* form I (AJ 4570, AJ 5370, and J-1015), while the latter was more intensive than the former in the case of *C. parapsilosis* form II

(AJ 4571 and AJ 4575) and *L. elongisporus* (AJ 5470). An additional minor signal with τ value of 4.82–4.86 was observed in mannans from *C. parapsilosis* form II and *L. elongisporus* though it was not clearly detected except for *L. elongisporus* (Fig. 2).

DISCUSSION

In 1952, RECCA and MRAK (11) isolated a yeast forming long oval ascospores from orange concentrate, and described it as a new species of the genus *Saccharomyces*, *S. elongasporus* RECCA et MRAK. VAN DER WALT (12) eliminated this yeast from *Saccharomyces* and established a new genus *Lodderomyces* by the reason of the formation of long oval ascospores. He considered that *L. elongasporus* was the perfect form of *C. parapsilosis* because *L. elongasporus* agreed sufficiently closely with *C. parapsilosis* morphologically and physiologically with the exception of its ability to form ascospores.

In the Second Edition of "The Yeasts, a Taxonomic Study" published in 1970, the genus *Lodderomyces* was adopted but specific epithet of *L. elongasporus*, the only species of the genus, was corrected as "*elongisporus*." This yeast was regarded as the perfect form of *C. parapsilosis* yet. In 1971, however, MEYER and PHAFF (4) reported that DNAs of this proposed pair of yeasts were not closely related in DNA-DNA hybridization experiment though guanine+cytosine ratios were similar. This result clearly indicated that *L. elongisporus* and *C. parapsilosis* were not closely related and that the former was not the perfect form of the latter. These two species, however, closely resemble each other in taxonomic criteria commonly employed. Therefore, imperfect form of *L. elongisporus* freshly isolated from natural sources is difficult to be distinguished from *C. parapsilosis* unless DNA homology study is carried out. In the present experiment, we found that *L. elongisporus* including its imperfect form could be discriminated from *C. parapsilosis* in serological characteristics, proton NMR spectrum of alkali-extracted mannans, lack of assimilation of L-arabinose, requirement of thiamine, and in the excretion of excess amount of riboflavin into synthetic media. Reciprocal absorption experiments of antisera and subsequent agglutination experiment showed that cell surface antigens of these two species differed from each other though the difference is not so distinct in some cases (Table 4). A clear difference was found in agglutination against factor antiserum 24 prepared from antiserum for *Saccharomyces rosei* and gel diffusion precipitation of mannans (Table 5, Fig. 1). Factor antiserum 24 agglutinated the cells of *C. parapsilosis* form I but neither those of *C. parapsilosis* form II nor *L. elongisporus* (Table 5). Serological investigations clearly demonstrated that these two species have respectively specific antigens in addition to antigens common to both species.

Gel diffusion precipitation reaction (Fig. 1) and proton NMR spectrum (Fig. 2) of alkali-extracted mannans indicated that the chemical structure of

the cell wall mannan, a cell wall component usually responsible for specificity of antigens of yeast species, is different in these two species.

In the present experiment, all of the strains belonging to *C. parapsilosis* form I rapidly assimilated L-arabinose, while those of *C. parapsilosis* form II and *L. elongisporus* did not (Table 2). According to the standard description of *C. parapsilosis* in the Second Edition of "The Yeasts, a Taxonomic Study" (13), assimilation of L-arabinose is positive or negative. Strains having the latter property are assumed to belong to the imperfect form of *L. elongisporus*. *C. parapsilosis* var. *quercu* (14) also does not assimilate L-arabinose. This variety was not included in the present investigation.

C. parapsilosis form II and *L. elongisporus* required thiamine in addition to biotin but *C. parapsilosis* form I does not require thiamine (Table 3). Excess amount of riboflavin was excreted and turned the color of the media yellow after 1 to 3 weeks of cultivation when *C. parapsilosis* form II and *L. elongisporus* were grown in the synthetic media employed for the carbon assimilation test (Table 3). This phenomenon was not observed in the case of *C. parapsilosis* form I.

As mentioned above, *C. parapsilosis* and *L. elongisporus* are distinct species without any close relationship, as clearly demonstrated by MEYER and PHAFF (4) in DNA-DNA hybridization study, in spite of a close resemblance in taxonomic criteria commonly employed (3, 12) and similar guanine+cytosine content in DNA (4, 15). Strains tentatively dealt with as *C. parapsilosis* form II by the present author (2) represent the imperfect form of *L. elongisporus*. Negative assimilation of L-arabinose, requirement of thiamine, and excretion of excess amount of riboflavin are useful for practical discrimination of the imperfect form of *L. elongisporus* from *C. parapsilosis*, and agglutination test with factor antiserum 24 and use of proton NMR spectrum of mannan are considered to be more practical than DNA-DNA hybridization study for routine identification.

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