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Ubiquinone compounds of the strains in *Leucosporidium scottii* and its related taxa

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담자균 酵母 *Leucosporidium scottii*와 관련 分類群菌株의 ubiquinone 물질

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ABSTRCT: Ubiquinone compounds of strains in *Leucosporidium scottii, Leucosporidium fellii, Leucosporidium lari-marini,* and *Rhodosporidium fluviale* were analyzed by the high performance liquid chromatography. The Q-9 or Q-10 compounds, independent on mating or self-sporulating type, were determined in the analyses of *Leucosporidium scottii* strains. Particularly, there is heterogenous in the quinone compounds in the same strain of *L. scottii.* These results showed reassessment of the quinone compounds as a taxonomic criterion; *L. fellii* had the Q-9 compound, *L. lari-marini* the Q-8 compound, and *R. fluviale* the Q-10 compound, Taxonomic position of *L. lari-marini* should be elucidated on the basis of analysis of Q-compound with other aspects.

KEYWORD: Ubiquinone compounds, *Leucosporidium, Rhodosporidium.*

Similarity in the matabasidial morphology (Fell and Tallman, 1984ab), the life cycle (Fell and Tallman, 1984ab), and some chemotaxonomic characteristics (Gorin and Spencer, 1970; Nakase and Komagata, 1971 a, b, c; Yamada and Kondo, 1972 a, b; Yamada and Kondo, 1973; von Arx and Weijman, 1979; Sugiyama *et al.*, 1985; Hamamoto *et al.*, 1986) has been seen between the genus of *Rhodosporidium* and *Leucosporidium.* Particulaly, the occurrence of colorless variant from *Rhodosporidium toruloides* strain YK 212 (Joo *et al.*, 1988) indicates a relationship between these two genera would be very closely related. The discrimination of these two genera, based on the production of carotenoid pigments, is considered to taxonomically be inadequate.

Chemotaxonomic characterization for the genus *Rhodosporidium* has been well-documented, whereas that for genus *Leucosporidium* have not yet been sufficient. Isoprenoid quinones widespread in the microorganism are reported to be important in the functioning of the electron transport system in respiration. Also, this is considered to be adopted as a useful taxonomic criterion for yeasts and yeast-like fungi at the generic level (Yamada and Kondo, 1973; Yamada *et al.*, 1976; Yamada *et al.*, 1983; Sugiyama *et al.*, 1985, 1987; Suzuki and Nakase, 1986).

In this report, ubiquinone compounds of strains in *Leucosporidium scottii, Leucosporidium fellii, Leucosporidium lari-marini,* and *Rhodosporidium fluviale* were determined by high performance liquid chromatography and the taxonomic significance of these compounds was discussed, here.

Materials and Methods

Strains used: Twenty-two strains of *Leucosporidium scottii,* and the respective type strain of *Leucosporidium fellii, Leucosporidium lari-marini,* and *Rhodosporidium fluviale* were employed in these studies. Their Latin names, strain designation, and

Table I. Strains used for ubiquinone analysis

Species*	Strain		Other strain designations					Source	Sexuality***
			YK	AJ	ATCC	CBS	IFO		
L. scottii	CBS	5931^{AT}	1009	14164	22182		1924	ex water	A1B1
	CBS	2300						ex air	A1B2
	CBS	6562							A1B2
	CBS	6561							A2B1
	CBS	5930^{T}			22181	5930	1923	ex water	A2B2
	IFO	1287	721			2281		ex chilled beef	A2B2
	CBS	4026							A2B2
	ATCC	26897						Alaska glacier	A2B2
	CBS	8039							A3B1
	CBS	8038							A3B2
	CBS	8040							A4B2
	CBS	8036							A5B1
	IFO	1212**	722	14170	10572	614		ex soil	A5B1
	CBS	8037							A5B3
	CBS	5932			22183		1925	ex water	SS
	IFO	0736	723	5023					SS
	CBS	6868						ex water	?
	IFO	9475					9475		?
	IFO	1528							?
	IFO	1529							?
	CBS	8162						ex rotten truck of *Eucryphia cordifolia*	?
	CBS	8188						ex thallus of *Fucus distichus*	?
L. fellii	CBS	7287^{T}							SS
L. lari-marini	CBS	7420^{T}							SS
R. fluviale	CBS	6568^{T}							SS

**L. Leucosporidium; R. Rhodosporidium.*
***Strain derived from the holotype of *Candida scottii*.*
****SS: self-sporulating, : unknown, T: Strain derived from the holotype, AT: Strain derived from the allotype.*

data for the sexuality are listed in Table I. A superscript "T" in this Table indicates the strain derived from the holotype.

Cultivation and harvest of cells: The culture broth consisted of 3 g, yeast extract (Difco Labs., Detroit, Michigan, U.S.A.), 3 g, malt extract (Difco Labs.), 5 g, peptone, 10 g, glucose, and 1,000 m*l*, distilled water. The pH of this broth was adjusted to 8.0, to prevent the formation of extracellular polysaccharides (Nakase and Komagata, 1971 a). The cells were cultivated in 5 litre flasks containing 1 litre of broth. Incubation was made for 4 days at 20℃, with shaking. Strains in which the optimal temperature was below 17℃ were cultivated on solid medium composed of the same ingredients supplemented with 1.5 % agar for 7 to 10 days. The cells were harvested by centrifugation at 4,400×g for 5 min at 5℃ and washed twice

Table II. Ubiquinone profiles of strains in *Leucosporidium scottii, L. fellii, L. lari-marini,* and *Rhodosporidium fluviale.**

Species	Strain		Ubiquinone homologs					Sexuality
			Q-6	Q-7	Q-8	Q-9	Q-10	
L. scottii	CBS	5931^{AT}				+**		A1B1
	CBS	6562			13	**85**	1	A1B2
	CBS	6561		1	29	**68**	1	A2B1
	CBS	5930^{T}				+**		A2B2
	IFO	1287				+**		A2B2
	CBS	8039		1	31	**66**	1	A3B1
	CBS	8038		1	25	**72**	1	A3B2
	CBS	8037		1	22	**60**	16	A5B3
	CBS	6868			1	**97**	2	?
	CBS	5932			19	**81**		SS
	IFO	0736					+**	SS
	CBS	2300			1	19	**80**	A1B2
	CBS	4026			1	8	**91**	A2B2
	ATCC	26897			3	26	**71**	A2B2
	CBS	8040				20	**80**	A4B2
	CBS	8036			2	19	**77**	A5B1
	IFO	1212^{T}***			7	**45**	**48**	A5B1
	IFO	9475			1	28	**71**	?
	IFO	1528			1	6	**93**	?
	IFO	1529				4	**95**	?
	CBS	8162			2	15	**83**	?
	CBS	8188			6	**41**	**53**	?
L. fellii	CBS	7287^{T}			8	**90**	2	SS
L. lari-marini	CBS	7420^{T}		3	**96**	1		SS
R. fluviale	CBS	6568^{T}			2	22	**76**	SS

*The boldfaced numbers indicate the major ubiquinone system.
**Determined by Yamada *et al.* (1972a, b, 1973).
***Strain derived from the holotype of *Candida scottii*.
T: Strain derived from the holotype, AT: Strain derived from allotype.

with saline-EDTA buffer (0.15 M NaCl. 0.1 M ethylenediaminetetraacetic acid, disodium salt, pH 8. 0). The havested cells were stored in a freezer at -20℃ until used.

Extraction of quinone compounds: First, to remove lipids, the cells (wet weight ca. 10 g) suspended in 20 m*l* of distilled water, were saponified in the presence of 2.5 g of pyrogallol, 10 g of potassium hydroxide, and 80 m*l* of methanol for 1 h at 90℃. Their suspensions were transferred to 4 polycarbonate tubes. The quinones were extracted twice from intact cells using 10 m*l* of hexane for 30 min with shaking for each tube. After centrifugation. the upper hexane layer was transferred to a new tube and washed twice with same volume of distilled water to remove potassium hydroxide. The only hexane upper layer was transferred to a flask of 50 m*l* volume and evaporated

Table III. Profiles and confirmation of ubiquinone homologs of strains in *Leucosporidium scottii* determined.*

Strain	Ubiquinone homologs					Ubiquinone**
	Q-6	Q-7	Q-8	Q-9	Q-10	
CBS 5931^{AT}		2	37	**60**	1	Q-9
IFO 1923-S**			13	**85**	2	Q-9
CBS 5930^{T}			11	**87**	2	Q-9
IFO 1287			1	13	**86**	Q-9
CBS 2281			1	16	**83**	Q-9
IFO 0736		1	8	**40**	**51**	Q-9

*The boldfaced numbers indicate the major ubiquinone system.

**Strain kept and the ubiquinone systems determined by Y. Yamada's laboratory of Shizuoka University (Yamada and Matsumoto, 1988).

Table IV. Ubiquinone systems of a single cell isolate from *Leucosporidium scottii* IFO 1212^{T}* (=CBS 614^{T}).

Strain	Ubiquinone homologs		
	Q-8	Q-9	Q-10
IFO 1212-1	6	39	55
IFO 1212-2	1	9	90
IFO 1212-3		6	94
IFO 1212-4	6	13	86
IFO 1212-5		6	94
CBS 614-1	2	19	78
CBS 614-2	7	34	58
CBS 614-4	3	27	69
CBS 614-5	2	21	76

*Strain derived from the holotype of *Candida scottii*.

to dryness under reduced pressure by rotary evaporator, resuspended twice in 10 m*l* of acetone and evaporated to dryness, and then resuspended in 2 m*l* of acetone and dried in dassicator.

Purification of quinone compounds by thin layer chromatography: Samples resolved in 0.02 m*l* of acetone were applied as a narrow band (1-2 cm long) to preparative thin-layer chromatography (Kieselgel GF 254 nach Stahl Type 60 plates, Merck). The plate was developed with a mixture of petroleum benzine-diethyl ether (9:1 V/V) as a solvent, and purified quinone compounds were revealed by the brief irradiation with ultraviolet light (Irie super light, 2, 573 A). The quinone compounds were scraped from the TLC-plate and transferred to Eppendorf tubes and then resolved in 1 m*l* of acetone. After shaking and centrifugation, the acetone layer was transferred to sample vials and finally dried in dessicator.

Determination of quinones: The ubiquinone compound was determined by the retention time in High Performance Liquid Chromatography (HPLC) using a mixture of methanol-isopropyl ether (3/1, v/v) as an eluent compound. The column of HPLC for analysis of quinone compound was Zorbax ODS (4.6 X 250 mm i.d.). Here, the ubiquinone (Q) homologues with the 7, 8, 9 and 10 isoprene units were designated as the Q-7, Q-8, Q-9 and Q-10 compounds, respectively.

Single cells isolation: Single cells were isolated using micromanupulator (Narishi Co., Japan) under light microscope.

Examination of morphological, physiological and biochemical characteristics: The methods as same as described by Kreger-van Rij (1984) were used.

Electrophoretic comparison of enzymes: Electrophoretic comparison of enzymes was made as the same methods as those described by Yamazaki and Komagata (1981).

Results and Discussion

The profiles of quinone compounds are obtained in the twenty-five strains assigned to *L. scottii, L. fellii, L. lari-marini* and *Rhodosporidium fluviale,* as shown in Table II.

All strains tested have the ubiquinone compounds. *Leucosporidium scottii* mating type strains CBS 6562, CBS 6561, CBS 8039, CBS 8038 and CBS 8037 had the Q-9 compound, but *L. scottii* mating type strains CBS 2300, CBS 4026, ATCC 26897, CBS 8040, and CBS 8036 had the Q-10 compound. *Leucosporidium scottii* self-sporulating type strain CBS 5932 had the Q-9 compound. *Leucosporidium scottii* CBS 6868 of which mating type has not yet determined had the Q-9 compound, *L. scottii* IFO 9475, IFO 1528, IFO 1529, and CBS 8162 of which mating type have also not yet dete-

Table V. Physiological and biochemical characteristics of a single cell isolate from *Leucosporidium scottii* strain IFO 1212*.

	1212-1	1212-3	1212-4	Fell *et al.* (1969)	Barnett *et al.* (1983)	Kreger-van Rij (1984)
Fermentation						
Glucose	−	−	−	−	−	−
Maltose	−	−	−	−	−	−
Lactose	−	−	−	−	−	−
Galactose	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
Assimilation						
Galactose	+	+	+	+,−	−,D	V
Sucrose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Cellobiose	+	+	+	+	+,−	+
Trehalose	+	+	+	+	+	+
Lactose	+S	+S	+S	+,−	+,−	V
Melibiose	−	−	−	−	−	−
Raffinose	+	+	+	+	+	+
Melezitose	+	+	+	+	+	+
Soluble starch	−	−	−	−	−	−
D-Xylose	+	+	+	+	+	+
L-Arabinose	+	+	+	+,−	+,−	V
D-Ribose	+	+	+	+,−	+,−	V
L-Rhamnose	+	+	+	+	+,−	+
Erythritol	−	−	−	−	−	−
Ribitol	+	+	+	+,−	+,−	V
D-Mannitol	+	+	+	+	+	+
Succinic acid	+	+	+	−	+	−
Citric acid	+	+	+	−	+	−
α-Methyl−	+	+	+	+	+,D	+
D-gluside						
Nitrate	+	+	+	+	+	+
Splitting of arbutin	+	+	+	+	+	+
Growth on vitamin free medium	+	+	+	+	+,D	+
Growth on 50% (W/W)						
glucose-yeast extract agar	−	−	−	−	−	−
Growth at 5℃	−	−	−	−	ND	−
10℃	W	W	W	−	ND	−
17℃	+	+	+	+	ND	+
20℃	+	+	+	+	ND	+
26℃	+	+	+	+	−	+
30℃	W	W	W	+	−	+
37℃	−	−	−	−	−	−
Starch formation	−	−	−	−	−	−
Urease	+	+	+	ND	+	+
DNase	+	+	+	ND	ND	ND
Diazonium Blue B reaction	+	+	+	ND	+	ND

*Abbreviation: D, delayed; V, variable; S, slow; ND, not described; W, weak.

Table VI. Comparison of the electrophoretic *Rm* values of enzymes in a single cell isolate from *Leucosporidium scottii* IFO 1212

Strain	*Rm* values							
	FA[a]	6PGDH[b]	MDH[c]	HK[d]	PGm[e]	G6PDH[f]	GDH[g]	Fmase[h]
IFO 1212-1	ND[i]	0.43	0.28	0.12	0.51	0.27	0.14	0.30
			0.33	0.35				
IFO 1212-3	ND	0.43	0.28	0.12	0.51	0.27	0.14	0.30
			0.33	0.35				
IFO 1212-4	ND	0.43	0.28	0.12	0.51	0.27	0.14	0.30
			0.33	0.35				
CBS 614	ND	0.43	0.28	0.35	0.51	0.27	0.14	0.30
			0.33	0.49				
IFO 0736	ND	0.41	0.33	0.31	0.50	0.30	0.14	0.28

[a]FA indicates Fructose-1,6-bisphosphate aldolase, [b]6PGDH indicates 6-Phosphogluconate dehydrogenase, [c]MDH indicates NAD dependent Malate dehydrogenase, [d]HK indicates Hoxokinase, [e]PGm indicates Phosphoglucomutase, [f]G6PDH indicates Glucose-6-phosphate dehydrogenase, [g]GDH indicates Glutamate dehyrogenase, [h]Fmase indicates Fumarase, [i]ND, not detected.

rmined had the Q-10 compound. *Leucosporidium scottii* IFO 1212 (mating type; A5B1) and CBS 8188 (mating type; unknown) had almost the same volume of Q-9 and Q-10. *Leucosporidium fellii* type strain CBS 7287 had Q-9 compound. *Rhodosporidium fluviale* type strain CBS 6568 had the Q-10 compound. *Leucosporidium lari-marini* CBS 7420 had the Q-8 compound.

Leucosporidium scottii IFO 1212 possessed a ubiquinone compound composed of both Q-9 and Q-10 as stated the above. Its ubiquinone compound had been already analyzed as the Q-10 by Yamada and Kondo (1972 b). Therefore, quinone compounds in strains of which quinone compound determined by Yamada and Kondo (1972 a, b, 1973) were re-examined. The results are shown in Table III. *Leucosporidium scottii* CBS 5931 and IFO 1923, and CBS 5930 (=IFO 1923) had the Q-9 compound. These results were the same as those determined by Yamada and Kondo (1972 a, b). But *Leucosporidium scottii* IFO 1287 and CBS 2281, both strains derived from the same origin, were equipped with Q-10 on the contary to the data of Yamada and Kondo (1973). *Leucosporidium scottii* IFO 0736 had a ubiquinone compound comprised of both Q-9 and Q-10.

Single cells were isolated by micromanupulator from *Leucosporidium scottii* IFO 1212 equipped with the Q-9 and Q-10, to check its purity, and its quinone compounds were analyzed, again. The results of its ubiquinone analysis are presented in Table IV. Of single cell isolates from *Leucosporidium scottii* IFO 1212, one cell isolates had Q-9 as the same amount as the Q-10 compound, another cell isolates had only Q-10 compound, and the intermediate type in ubiquinone homologue also existed. The quinone compound of single cell isolates from *Leucosporidium scottii* CBS 614 derived from the same origin with *L. scottii* IFO 1212 was also determined. As the result, the single cell isolates from *Leucosporidium scottii* CBS 614 had Q-10 as the major component, and Q-9 as the minor component.

Physiological and biochemical characteristics of single cell isolates from *Leucosporidium scottii* IFO 1212 were the same as those in the descriptions provided by Fell *et al.* (1969), Barnett *et al.* (1983). and Kerger-van Rij (1984) (Table V), and all single cell isolates from *Leucosporidium scottii* IFO 1212 showed identical enzymetic patterns for 6PDGH, MDH, HK, PGm, G6PDH, GDH, and Fmase (Table VI). All single cell isolates from *Leucosporidium scottii* IFO 1212 in this experiment were considered to be identical as this results.

In the strains of *Leucosporidium scottii,* the ubiquinone compounds were heterogeneous (Yamada and Kondo, 1972a, b, 1973); viz, *L. scottii* CBS 5931, IFO 1287, mating type strains, had the Q-9 compound and *L. scottii* IFO 1212 (mating type A5B1, indicated by Fell and Tallman, 1982) and IFO 0736, self-sporulating strain, had the Q-10 compound. On the other hand, *Leucosporidium scottii* CBS 5931 and IFO 1287, mating type strain, did not have xylose in their cells, but *L. scottii* IFO 1212 and IFO 0736, self-sporulating type strains, contained xylose (Sugiyama *et al.*, 1985). These facts were correlated with the results obtained from the electrophoretic comparison of enzymes (Yamazaki and Komagata, 1982). Sugiyama *et al.* (1985) concluded that *Leucosporidium scottii* was included, at least, in two different species. In this experiment, the quinone compounds of *Leucosporidium scottii* were more diverse. Because both mating type strains and self-sporulating type strains have the Q-9 or Q-10 compound, and then, there is heterogenous in the the quinone compound of same strains. More detailed studies about the biosynthesis of quinone compounds and re-assessment of the quinone compounds as a taxonomic criterion are needed. Taxonomic position of *L. lari-marini* CBS 7420 having the Q-8 compound should be elucidated on the basis of polyphasic investigations from micromorphology to chemotaxonomy.

摘 要

담자균효모 *Leucosporidium scottii, Leucosporidium fellii, Leucosporidium lari-marini, Rhodosporidium fluviale*의 ubiuqinone계를 고속액체크로마토그래피에 의해 결정하였다. *Leucosporidium scottii* 균주는 교배형 또는 self-sporulating 형에 관계없이 Q-9 또는 Q-10계를 가지고 있었다. 특히 동일 균주에서도 ubiquinone계의 변이가 관찰되었다. 이는 분류지표로서 중요시 되는 ubiquinone계의 재평가를 시사하는 새로운 사실로 간주된다. *Leucosporidium fellii*는 Q-9, *Leucosporidium lari-marini*는 Q-8, *Rhodosporidium fluviale*는 Q-10을 함유하고 있었다. *L. lari-marini*의 분류학적 위치는 다각적 연구에 기초하여 해명되어야 한다.

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