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Analyses of cellular carbohydrates in *Leucosporidium scottii* and its related texa of basidiomycetous yeasts by the high performance liquid chromatography

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담자균 酵母 *Leucosporidium scottii*와 관련 分類群 菌株의 HPLC 에 의한 細胞糖質 分析

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ABSTRACT: Cellular carbohydrates were hydrolysed and analyzed in the strains of *Leucosporidium scottii* and its related species of basidiomycetous yeasts by HPLC methods without any derivatization. Xylose was detected from the hydrolyses of the cellular carbohydrates of *L. lari-marini*, but not from those of three strains of *L. antarcticum*, *L. fellii*, and *Rhodosporidium fluviales*. not also from those of six strains of *L. scottii* contrary to other data reported. *L. antarcticum* and *L. lari-marini* were considered to be placed on the different genus of *Cystofilobasidium* or *Mrakia*, as based on the numerical analyses.

KEYWORDS: Cellular carbohydrates, *Leucosporidium*, *Rhodosporidium*.

Analyses of cellular carbohydrates were reported to be useful in the taxonomy of yeast and yeast-like fungi (von Arx and Weijman, 1979; Weijman, 1979ab; Weijman and de Hoog, 1985; Weijman and Rodrigues de Miranda, 1983; Weijman *et al.*, 1982; Sugiyama *et al.*, 1985; Suzuki and Nakase, 1988). The presence of xylose in the cellular componants, even in little amounts, was reported to be important in the taxonomic aspects. Sugiyama (1985) indicated the heterogeneity of presence of xylose within the species of *Leucosporidium*. He analyzed the cellular carbohydrates of species of genus *Leucosporidium* by gas chromatography (GLC) with the derivatization. Recently, Suzuki and Nakase (1988) developed new method for analysis of xylose in hydrolysates of whole cell, using High Performance Liquid Chromatography (HPLC) without derivatization.

The cellular carbohydrates were hydrolyzed and analyzed in the strains of *L. scottii* and its related species, by HPLC methods here. The taxonomic evaluation was made, as based on this result.

Materials and Methods

Strains examined: Six strains of *Leucosporidium scottii*, four type strains of *L. antarcticum*, *L. fellii*, *L. lari-marini*, and *Rhodosporidium fluviale* were used. Their Latin names and strain designations are listed in Table I.

Cultivation: Yeasts were cultivated at a 500 m*l* Erlenmeyer flask containing 200 m*l* of YM broth supplemented by 2 % glucose on the rotary shaker (250 rpm/min). Cultivation was carried out at optimal temperature (17~25℃). Cells grown on a YM agar slant for 3-5 days were used as the inoculum. After 4-5 days, the cells were harvested by centrifugation (6,000 rpm), and washed twice with the deionized water.

Acid hydrolysis of whole cells: Cells (dry weight 0.1 g) dried twice with acetone were suspended in 1 m*l* of 2 M trifluoroacetic acid in a test tube

Table I. Yeast strains used to analyze cellular carbohydrate composition in the whole cells

Species	Strains	Other strains designations						
		YK	AJ	ATCC	CBS	IFO	NRRL	UBC
Leucosporidium antarcticum	YK 1005^{T}			22177	5942	1917		
Leucosporidium scottii	CBS 5931^{AT}	1009	14164	22182		1924		
	IFO 1923^{T}			22181	5930			
	IFO 1287	721			2281		Y-1786	
	IFO 1212^{T}*	722	14170	10572	614		Y-1497	
	IFO 0736	722	5023					
	IFO 9474							670
Leucosporidium fellii	CBS 7287^{T}							
Leucosporidium lari-marini	CBS 7420^{T}							
Rhodosporidium fluviale	CBS 6568^{T}							

T: Strain derived from the holotype, AT: Strain derived from the allotype.
*Strain derived from holotype of *Candida scottii*.

with a teflon-sealed screw cap (13×100 mm), and heated at 100℃ for 3 h in a metal block bath. After cooling, the hydrolysates were filtered with filter paper (Toyo No. 2 filter paper) and evaporated to dryness at 50-60℃. The residue was dissolved in 0.5 m*l* of distilled water, neutralized immediately with small amounts of Amberlite IRA 410 (OH form) which was washed twice by 10 % NaOH and many times by distilled water, filtered with a disposable filter unit (Shodex DT ED-13, Showa Denko, K.K., Tokyo, Japan), and used as sample injected to High Performance Liquid Chromatography.

HPLC analysis of whole cell hydrolysates: The HPLC analysis was done on an SSC 3000 Flow system connected with a column oven (Senshu Scientific Co., Tokyo, Japan) using two different column; one was a Shodex SP 1010 column (Showa Denko, K.K., Tokyo, Japan) with water (HPLC grade)as the eluate system at a flow rate of 0.8 ml/min and temperature at 80℃. The other was a Shodex RS pak DC-613 (Na form) column (Showa Denko, K.K., Tokyo, Japan) with acetonitrile-water (80 : 20, v/v, HPLC grade) as the mobile phase at a flow of 0.8 ml/min at 75℃. A refractive index detector (ERC-7520, Erma Optical Works, Ltd., Tokyo, Japan) was used to detect the cellular carbohydrates. A chromatopac C-R3A (Shimadzu Corp., Kyoto, Japan) was used as a recorder.

Cluster analyses: Relative Euclidean distances were calculated by the method of Luwig and Reynolds (1988) based on the cellular carbohydrate compositions. Cluster analyses were done using these relative Euclidean distances.

Principal component analyses: Principal Component analyses were done by the method of Austin and Orloci (1966) and Orloci (1967, 1973, 1978).

Results and Discussion

The cellular carbohydrate composition of the whole cell hydrolysates is summarized in Table II. Three type strains of *L. antarcticum,* and *L. fellii,* and *Rh. fluviale* did not contain xylose in the cells. Six strains of *L. scottii* also did not contain xylose in the cells. Sugiyama *et al.* (1985) reported that *L. antarcticum* YK 1005, *L. scottii* IFO 1212 and IFO 0736 contained xylose in the cells. Those results didn't agree with the data of this experiment. The presence of xylitol in the whole cell hydrolysates may be miss-identified as xylose by the GLC mehtod, Because it is impossible to distinguish the xylitol present in the hydrolysates before derivatization from the xylitol converted from xylose by the reduction of the hydrolysates with the GLC method. In this experiment, the small amounts of xylitol were detected in *L. anta-*

Table II. Cellular carbohydrate composition of strains in *Leucosporidium* spp. and *Rhodosporidium fluviale*.

Species	Strain	Cellular carbohydrate composition (mol%)							
		glu	xyl	gal	man	gly	mann	rib	xylit
Leucosporidium antarcticum	YK 1005^{T}	84.8	0	2.8	10.4	1.3	0.2	0.2	0.3
Leucosporidium scottii	CBS 5931^{AT}	46.0	0	5.4	44.1	1.5	0.7	1.9	0.4
	IFO 1923^{T}	40.5	0	9.2	47.8	1.2	0.2	0.8	0.2
	IFO 1287	46.5	0	7.6	41.8	1.4	0.4	2.0	0.3
	IFO 1212^{T}*	51.9	0	5.2	40.0	1.5	0.1	0.9	0.4
	IFO 0736	27.0	0	6.7	61.9	2.1	0	1.9	0.4
	IFO 9474	51.7	0	0	46.1	1.0	0.3	0.6	0.3
Leucosporidium fellii	CBS 7287^{T}	46.8	0	12.2	36.2	2.8	0.6	1.1	0.4
Leucosporidium lari-marini	CBS 7420^{T}	85.1	0.3	2.2	8.6	2.3	0	1.2	0.3
Rhodosporidium fluviale	CBS 6568^{T}	33.9	0	5.9	58.4	1.0	0.2	0.2	0.3

Abbreviations: T, strain derived from the holotype; AT, strain derived from the allotype; T*, the type strain of *Candida scottii*; glu, glucose; xyl, xylose; gal, galactose; man, mannose; gly, glycerol; mann, mannitol; rib, ribose; xylit, xylitol.

Table III. Matrix of paired distance between strains of *Leucosporidium* spp. and *Rhodosporidium fluviale*.

	1	2	3	4	5	6	7	8	9	10
1		51.49	58.33	49.79	44.33	77.54	48.77	46.92	2.41	70.03
2			7.74	3.24	7.28	26.08	8.23	10.57	52.92	18.83
3				8.72	14.39	19.73	14.60	13.64	59.8	12.93
4					6.28	28.03	10.27	7.45	51.22	20.99
5						38.22	8.04	9.56	45.80	25.76
6							30.13	32.93	78.98	8.04
7								16.57	50.29	22.43
8									48.26	26.52
9										71.54
10										

Note: The numbers above the diagonal are paired distances calculated by the method of Luwig and Reynolds. 1, *L. antarcticum* YK 1005^{T}; 2, *L. scottii* CBS 5931^{AT}; 3, *L. scottii* IFO 1923^{T}; 4, *L. scottii* IFO 1287; 5, *L. scottii* IFO 1212^{T}*; 6, *L. scottii* IFO 0736; 7, *L. scottii* IFO 9474; 8, *L. fellii* CBS 7287^{T}; 9, *L. lari-marini* CBS 7420^{T}; 10, *R. fluviale* CBS 6568^{T}.

rcticum YK 1005, and L. scottii IFO 1212 and IFO 0736. Therefore, the evidences obtained here demonstrate that the taxa assigned to the genera *Rhodosporidium* and *Leucosporidium* do not contain xylose in the cells.

Suzuki and Nakase (1988) found that the type strains of *Sporidiobolus salmonicolor* did not contain xylose in the cells on the contrary with the data of Sugiyama *et al.* (1985), and also pointed out that the miss-identification of xylitol as xylose was a cause. From the results of Suzuki and Nakase (1988) and this study, the strains with relatively small amounts of xylose like *Rhodotorula minuta* YK 131 and *Rhodotorula muscorum* YK 1020 should be re-determined by the HPLC method. On the other hand, *L. lari-marini* CBS 7420 (type strain) contained xylose in the cells.

The paired distances of all the ten strains considered in this analysis are presented in Table III. Dendrogram was contructed by cluster analy-

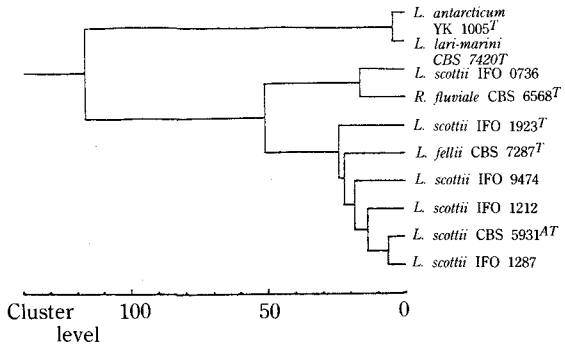

Fig. 1. A distance matrix dendrogram produced from the cellular carbohydrate compositions of *Leucosporidium* spp and *Rhodosporidium fluviale.*

ses based on this matrix of pair distances, as shown in Fig 1. Glucose, and xylose are known to be principal components which influence on the affinities of these compared strains. Based on only glucose and xylose compositions, these strains are correlated with each other at level of 65 %. There are two major groups: one group includes *L. antarcticum* and *L. lari-marini,* the other includes *L. scottii, R. fluviale* and *L. fellii.* The latter group splits into two subgroups. *L. antarcticum* is similar with *Rhodosporidium* and *Leucosporidium* species in the same characters as xylose content, ubiquinone systems, inositol assimilation, starch formation and basidial form but shares the same characters as high cellular glucose content, low cellular mannose content with *Cystofilobasidium* or *Mrakia.* Therefore, the taxonomic position of this species should be well elucidated. Especially, the phylogenetic placement of this species inferred from sequence comparisons of small-subunit Ribosomal RNAs should be required. *L. lari-marini* described by Saez and Nguyen (1989) is similar with *Cystofilobasidium* or *Mrakia* species and differs from *Leucosporidium* species in a number of characters, including assimilation of inositol (Saez and Nguyen, 1989), production of starch (Saez and Nguyen, 1989), Q-8 as major ubiquinone homolog (Joo, unpublished data), and presence of xylose in the cells (the present study). From these characteristics, transfer of this species to the genus *Cystofilobasidium* or *Mrakia* can be proposed, because the two genera *Mrakia* and *Cystofilobasidium* are similar in morphological and chemotaxonomic characteristics. On the basis of further studies on these two genera, the taxonomic position of *L. lari-marini* will be determined.

In conclusion, the results obtained in this study reinforce Weijman and Golubev's statement (1987): Occurrence of xylose is an accepted criterion to discriminate between ascomycetous and basidiomycetous yeasts.

摘 要

담자균효모와 관련분류를 균주의 菌體糖組成을 수식없이 원래 상태로 분석할 수 있는 HPLC법을 사용하여 분석하였다. *Leucosporidium lari-marini*는 균체당 xylose를 함유하고 있으나 *Leucosporidium antarcticum, Leucosporidium scottii, Leucosporidium fellii, Rhodosporidium fluviale*는 균체당 xylose를 함유하지 않았다. *Leucosporidium scottii*에서 종래 xylose 함유가 보고된 균주에서 xylose를 확인할 수 없었다. 균체당 조성에 기초한 수리분석결과와 다른 성질을 함께 고려하면 *L. antarcticum*과 *L. lari-marini*는 *Cystofilobasidium*속이나 *Mrakia*속에 이적되어야 함이 시사되었다.

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