海洋酵母のステロール組成

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Sterol Composition of Marine Occuring Yeast

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The present study describes the sterol compositions of 5 species of marine occuring yeast. The crude sterols were isolated from the unsaponifiable materials by a digitonine-precipitation method or a column chromatography (alumina). The sterols were identified by gas-chromatography, ultraviolet absorption, infrared spectroscopy, and mass spectrometry.

Rhodotorula mucilaginosa, *Cryptococcus albidus*, and *Sporobormyces salmonicolor* were found to contain ergosterol and campesterol. In the case of *Toruropsis dattila* and *Toruropsis famata*, the unknown digitonine-precipitable substance (RRT on SE-30, 1.34) was detected in addition to the above 2 sterols.

On the presence of sterols in marine animals and plants, many reports have been found.[1] On the other hand, it was demonstrated that some marine crustacean and mollusk are incapable of synthesizing their sterols from acetate or mevalonate although they contain a variety of sterols in their body.[2-11] SHIMAYA *et al.* reported that the zooplanktons, *Artemia* and *Daphnia*, utilized the marine yeast as a diet, and that the advantage as a diet was similar to that of the phytoplanktons, *Chlorella sp.* and *Scenedesmus sp.*[12] These results may suggest that a part of sterols in marine invertebrates, especially in herbivorous ones, probably originates from a diet such as phytoplanktons, marine microorganisms, and sediments etc. However, little attention have been paid to the sterols in microorganisms, especially on marine yeast no report has been shown.

The present paper describes the sterol compositions of serveral species of marine yeast.

Materials and Methods

Marine yeast. *Toruropsis dattila, Toruropsis famata, Rhodotorula mucilaginosa, Cryptococcus albidus*, and *Sporobormyces salmonicolor* were incubated on a reciprocal shaker at 28°C for 48 hours. The incubation medium contains the following solutes: 8 g of molasses, 1 g of polypeptone, 0.5 g of yeast extract, and 1000 ml of the natural sea water filterated with a glass filter (No. 5).[12] The cells were harvested and washed with distilled water four times by centrifugation (4000 rpm, 15 min.). The average yield of the cells was 0.7–1.0 g of fresh weight per 1000 ml of incubation medium.

Isolation of sterols. The wet cells were homogenized in acetone with a Teflon homogenizer for 5 minutes, and then acetone was removed under reduced pressure. The homogenized cells were saponified with alcoholic potassium hydroxide solution (95%

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ethanol- 50% aqueous potassium hydroxide (10:4)) at 80°C for 3 hours, and extracted with ether by a usual manner. The residues were re-saponified by the same method. The crude sterols were obtained by a digitonine precipitation method[13] or a column chromatography[2] (alumina, grade II).

Gas-chromatography (GLC). The composition of sterols was investigated by GLC. During the present study, Shimadzu model GC-3AF gas-chromatographic unit with a hydrogen flame ionization detector and glass column was used. The column packings used were 1.5% SE-30, 1.0% XE-60, and 1.0% NGS-1.0% XE-60 (1:1). The identification of sterols was carried out by comparing the relative retention times (relative to cholestane or cholesterol) to that of authentic sterols. In GLC on 1.0% NGS-1.0% XE-60 (1:1), the sterols were trimethylsillylated and subjected to GLC. The details of the operating conditions in GLC were described previously.[14]

Specta analysis. Ultraviolet-absorption spectrum was measured by use of a Shimadzu multipurpose spectrophotometer MPS-50L. Infrared absorption spectrum was obtained with a Nippon Bunko DS-301 spectrometer in chlorofrom. Mass spectrum was measured on the Hitachi RMU-60 instrument (chamber voltage, 70 eV).

Results and Discussion

The contents of sterols isolated from the marine yeast are shown in Table 1. The composition of sterols was determined by GLC and the results are shown in Table 2.

Table 1. Sterol contents of marine yeast.

Marine yeast	Fresh weight	Unsaponifiable materials		Sterols	
	g	g	%	mg	%
Toruropsis dattila	78.0	1.290	1.66	81.7	0.110
Toruropsis famata	45.0	0.596	1.32	39.8	0.088
Rhodotorula mucilaginosa	29.4	0.047	0.16	5.0	0.018
Cryptococcus albidus	56.0	0.152	0.27	9.5	0.017
Sporobormyces salmonicolor	51.9	0.370	0.71	5.9	0.011

Table 2. Sterol compositions of marine yeast.

Marine yeast	Composition (%)		
	Ergosterol	Campesterol	Unknown component*
Toruropsis dattila	70	2	18
Toruropsis famata	95	1	4
Rhodotorula mucilaginosa	79	21	—
Cryptococcus albidus	69	31	—
Sporobormyces salmonicolor	77	23	—

* The relative retention time (relative to cholestane) on SE-30 was 1.34.

GLC on SE–30 indicated that the sterols from 5 species of yeast contain 2 or 3 components. Two of these sterols were identical with authentic ergosterol and campesterol in relative retention times in GLC on SE–30, XE–60, and NGS– XE–60 (1:1). All yeast examined in this study contained the above 2 sterols although a little variation was perceived in the amounts among the species. In the case of *Toruropsis*, the unknown digitonine-precipitable substance (RRT on SE–30, 1.34) was detected in addition to the above sterols.

The UV-absorption spectra of the sterols from *R. mucilaginosa, C. albidus,* and *S. salmonicolor*, revealed the typical absorbancy (at 272, 282, and 294 mμ)[16] of a $\Delta^{5,7}$-sterols. The peaks at 282 mμ are indicative of a conjugated dien in ring B.[16] The molecular coefficient of the crystal (E_{215} 900) after correction for $\Delta^{5,7}$ was more characteristic of Δ^5 (E_{215} 700) than Δ^7 (E_{215} 3000).[17] In the case of *C. albidus,* the infrared and mass spectra were measured on the mixture of 2 sterols. The results are shown in Figs. 2 and 3. The mass spectrum of the crystal showed the 2 molecular ions at m/e 396 (M_1^+) and 400 (M_2^+) corresponding to ergosterol and campesterol, respectively. The peasks at m/e 229 and 231 (M^+—side chain +42)[18,19] confirmed the absence of 4,4′-substitution such as lanosterol and rejected the possibility of a 14-methyl group. The other prominent peaks were interpreted as follows: m/e 385 (M_2^+—CH_3), 381 (M_1^+—CH_3), 382 (M_2^+—HOH), 378 (M_1^+—HOH), 367 (M_2^+—CH_3+HOH), 363 (M_1^+—CH_3+HOH), 273 (M_2^+—R_2, R_2= alkyl side chain of campesterol), 271 (M_1^+—R_1, R_1=alkyl side chain of ergosterol),

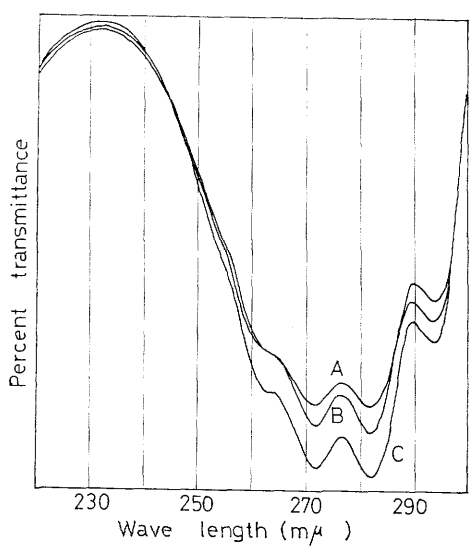

Fig. 1. Ultraviolet absorption spectra of the sterols isolated from the three species of marine yeast.
A: *Sporobormyces salmonicolor*
B: *Cryptococcus albidus*
C: *Rhodotorula mucilaginosa*

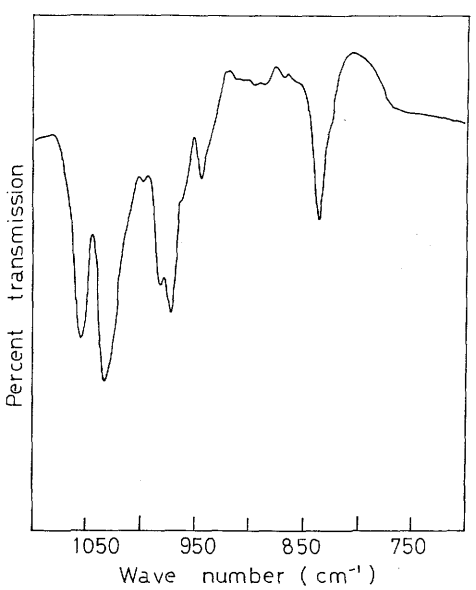

Fig. 2. Infrared spectrum of the sterols isolated from *Cryptococcus albidus*

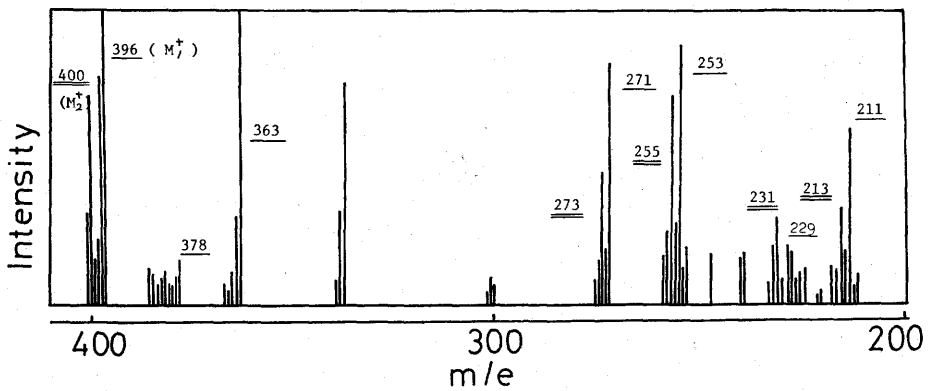

Fig. 3. Mass spectrum of the sterol isolated from *Gryptococcus albidus*.
Peaks m/e; corresponding to campesterol
Peaks m/e; corresponding to ergosterol

255 ($M_2^+-R_2+HOH$), 253 ($M_1^+-R_1+HOH$), 231 ($M_2^+-R_2+42$), 229 ($M_1^+-R_1+42$), 213 ($M_2^+-R_2+42+HOH$), and 211 ($M_1^+-R_1+42+HOH$).

The result in IR-absorption spectrum supported also the presence of ergosterol and campesterol. The abosorption at 970 cm^{-1} revealed the presence of a trans double bond at C–22 in the sterol side chain.[20] A frequency of 1050 cm^{-1} for Δ^5 rather than ν_{max} 1033 cm^{-1} for Δ^7 suggested the presence of Δ^5-sterols.[21] Moreover, the absence of significant absorption at 890 cm^{-1} eliminated the possiblity of methylene ($CH_2=CRR'$) in side chain.[22] In addition, the lackness of doublet absorption at 958 and 950 cm^{-1} rejected the presence of $\Delta^{5,24}$-sterols.[23]

On the basis of the above data, it was concluded that *C. albidus* contains ergosterol and campesterol. However, the configuration of the methyl group at C–24 left undetermined. It is a well-known fact that yeast, some of protozoa and unicellular algae contain ergosterol mainly. In the present study, it was found that the marine occuring yeast also contain ergosterol in a relatively high amount as well as terrestrial type yeast. Campesterol, which was first isolated from the plant, *Brassica campestris*,[24] was also found in the marine green algae (Hosojuzumo), *Chaetomorpha crassa*.[25] However, it has not yet been isolated from yeast. The authors believe that the present report is the first case showing the presence of campesterol in yeast.

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