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Characteristics and Fatty Acid Composition of Yeast Isolated from the Intestines of Rainbow Trout *Oncorhynchus mykiss*

Taizo Sakata*1, Tsukasa Ohshiro*1, and Shin-ichi Teshima*2

Keywords : Yeast, *Candida*, rainbow trout, fatty acid

Abstract

A species of yeast was isolated from the intestines of rainbow trout *Oncorhynchus mykiss* fed with pelleted diets in two different aquaculture stations. The yeast isolates were found to multiply by budding, not to form spore and any pigment and identified as *Candida* sp. The yeast isolates fermented glucose, galactose, sucrose, fructose, inulin and trehalose but not lactose, mannitol, maltose, inositol, melibiose, cellobiose and starch. The isolates grew poorly on Z-AII agar without glucose, whereas they grew very well on Z-AII agar with glucose (ZAG agar). The representative strain Y91-01 grew in a temperature range of 13 to 34℃, in a pH range of 2 to 9, and in a NaCl concentration range of 0 to 7%. Strain Y91-01 required biotin as incubated in glucose-$(NH_4)_2SO_4$ medium. The total lipids extracted from strain Y91-01 cells contained $C_{16:1}$, $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ as major fatty acids.

Various kinds of yeast strains have been isolated from the alimentary tracts of mammals, birds, insects and crustacea[1]. These yeast strains were known to be obligately or facultatively parasites and in some cases to become opportunistic pathogens for compromised host animals. However, few yeast strains have been reported to be isolated from fish intestines, although Awakura and Kimura[2], and Hatai and Egusa[3] described yeast strains isolated from gastric contents of gastro-tympanites fish. Yoshimizu *et al.*[4,5] reported that in some samples of masu salmon yeast was isolated as one of the predominant organisms in normal microflora of fish intestines.

We have isolated yeast strains from the intestinal contents of rainbow trout

*1 Laboratory of Microbiology, Faculty of Fisheries, Kagoshima University, 50-20 Shimoarata 4, Kagoshima, 890 Japan.

*2 Laboratory of Marine Nutritional Chemistry, Faculty of Fisheries, Kagoshima University, 50-20 Shimoarata 4, Kagoshima, 890 Japan.

Oncorhynchus mykiss fed in aquaculture ponds as a constituent of intestinal microflora[6]. In this paper, yeast strains isolated from rainbow trout intestines were characterized and analyzed for the fatty acid composition of the cellular lipids.

Materials and Methods

Fish samples

Fish samples of rainbow trout *Oncorhynchus mykiss* were obtained from two aquaculture stations. One (Station A) is a private facility located in Kagoshima city. The other (Station B) is Kobayashi branch of Miyazaki Fisheries Experimental Station. Fish were fed with dry pelleted diets in both aquaculture stations. Fish samples were transported within three hours to the laboratory in plastic tanks with aeration pumps.

Isolation of intestinal microflora

Intestinal tracts were removed from fish samples aseptically. Intestinal contents were squeezed out and homogenized in a mortar. Aliquots (0.05-0.1 mℓ) of decimal dilutions of the homogenate of intestinal contents were smeared on Z-AII, ZAG, KS, Z/SPMF, EG, NBS, NBO and NBC agar media as described in the previous papers[7-9]. ZAG agar medium contained glucose (20.0 g / ℓ) in Z-AII agar medium. Z-AII, ZAG, KS and Z/SPMF plates were incubated aerobically at 25℃, while EG, NBS, NBO and NBC plates were incubated anaerobically in an anaerobic jar supplied with N_2 gas and steel wool.

Identification of bacterial groups

Fifty colonies grown aerobically on Z-AII plates for each sample were picked up randomly and purified on the agar plates. Characterization of the aerobic isolates was carried out on the basis of the following properties: cell form, gram stain, motility, pigmentation, oxidase, catalase, glucose fermentation and colonization on KS and Z/SPMF plates as described in the previous papers[8,9]. Identification of aerobic isolates in the genus level was achieved according to the identification scheme proposed by Ezura and Simidu[10]. For anaerobic isolates, 50 strains were isolated from EG, NBS, NBC and NBO and examined with gram stain, cell form, sporulation and aerobic growth.

Characterization of yeast

Yeast strains were isolated on ZAG, EG and NBS plates. Yeast isolates were characterized by using the following tests: gram stain, cell form, sporulation, pigmentation, oxidase, catalase, hydrolysis of starch, casein, tributyrin, tween 80, urea and DNA, fermentation of various carbohydrates, and growth response for temperature, salinity, vitamin and pH. In these tests, ZAG medium was used as the growth

and basal medium for bacteriological tests.

Fatty acid analysis

The frozen and thawed preparations of yeast cells 1.0 g were homogenized in 30 mℓ of chloroform-methanol mixture with a disperser (Ultra Turrax) and cellular lipids were extracted according to the method of Bligh and Dyer[11]. Fatty acids of the total lipids were analyzed by gas-liquid chromatography on a 30 m × 0.25 mm capillary column of SUPELCOWAX™ 10 (bonded wall-coated fused silica column, 0.25 μm film thickness) at 220℃ by using a Shimadzu GC-9APF gas chromatograph with a helium gas flow rate of 60 mℓ/min after methylated with boron trifluoride-methanol complex. Peaks were identified by comparison of retention times with those of authentic standards as reported previously[12].

Results and Discussion

Microflora in the intestines

Fish samples used in the experiment in 1991 are shown in Table 1. The average values of body length and body weight of fish samples from Station A were 20.2 cm and 93.8 g, respectively, while those of fish samples from Station B were 37.9 cm and 581.3 g, respectively. Viable counts of each bacterial genus or group in the intestinal microflora were presented in Table 2. The aerobic microflora was composed of the genera *Plesiomonas*, *Aeromonas*, and *Pseudomonas* and the family *Enterobacteriaceae*. On the other hand, the anaerobic microflora contained *Bacteroides* ATS as well as *Clostridium* spp. as predominant components. Average viable counts of the anaerobic and aerobic microflora were 7.08 and 7.52 (log No./g), respectiveiy. Herbivorous and omnivorous fresh-water fish such as tilapia, carp and goldfish have been reported to possess anaerobic microflora outnumbering aerobic microflora in the intestines[8].

Table 1. Fish samples examined in 1991.

Sample No.	Station	Date	W.T. (℃)	B.L. (cm)	B.W. (g)	I.L. (cm)
R-1	A	4/25	17.5	19.0	70.0	5.5
R-2	A	4/25	17.5	19.0	70.0	6.0
R-3	B	5/16	18.5	40.0	600.0	15.5
R-4	B	5/16	18.5	37.0	540.0	13.5
R-5	A	6/14	21.0	22.0	120.0	12.0
R-6	A	6/14	21.0	21.0	115.0	11.0
R-7	B	7/ 2	18.5	37.0	635.0	17.5
R-8	B	7/ 2	18.5	37.5	550.0	17.5

Abbreviation: W.T., water temperature; B.L., body length;
B.W., body weight; I.L., intestinal lenght.

Table 2. Viable counts of intestinal microflora.

Group	Viable counts (log No./g)							
	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8
(Aerobes)								
Plesiomonas			6.88	6.18	5.51		6.89	6.08
Aeromonas	5.98	6.04	7.72	6.18	6.15		6.30	
Pseudomonas	7.08	7.11	6.84	6.65	5.69		6.30	5.98
Enterobact.	6.46	6.43	6.79				6.30	5.38
Others	7.52	7.00	6.79	7.26	5.69	5.00	7.54	6.98
Total	7.70	7.43	7.88	7.40	6.43	5.00	7.69	7.08
(Anaerobes)								
Bacteroides ATS	6.38		9.08	8.15	6.32	6.45	9.00	7.72
Bacteroides BTS					5.70		5.70	6.68
Bacteroidaceae			7.72					5.32
Clostridium	6.49	6.46	7.72	6.08			7.94	5.32
Spiral bacteria			7.40	6.56			7.57	
Total	6.74	6.46	9.11	8.15	6.41	6.45	9.04	7.76
Yeast	7.08	6.78	7.95	7.36	6.38	5.70	7.56	6.46
Total counts	7.83	7.56	9.18	8.28	6.89	6.53	9.08	7.86

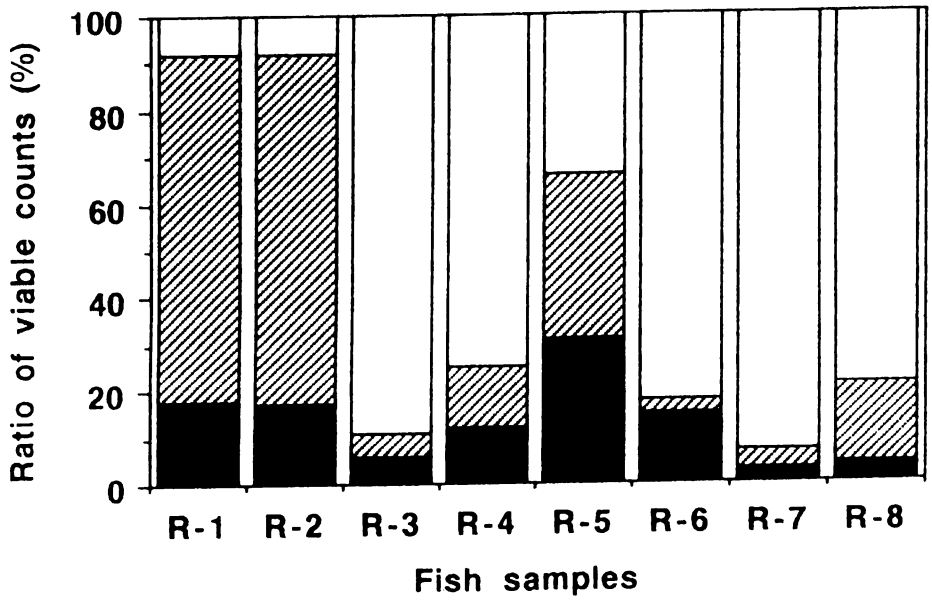

Fig. 1. Variations in viable counts of yeast, aerobic and anaerobic bacteria from the intestinal contents.
■, yeast; ▨, aerobic bacteria; □, anaerobic bacteria.

In this experiment, rainbow trout fed in aquaculture ponds were found to have somewhat more viable counts of anaerobic microflora than those of aerobic microflora in the intestine. Yeast strains were isolated from the intestinal contents of rainbow trout on aerobic ZAG agar and anaerobic EG, NBS, NBO and NBC agar plates. As shown in Fig. 1, the percentage of yeast viable counts to total viable counts in the intestines varied from 3 to 18% and the average percent was 13.3%. In the experiment in 1992 (data not shown), the viable counts of yeast in the intestine of rainbow trout ranged from 0 to 96.3% and the average value was 34.8%.

Characteristics of yeast isolates

All fish samples used in this experiment possessed yeast cells in their intestines. Five representative strains were chosen to be examined for morphological and physiological properties. The main characteristics of five strains were shown in Table 3. All strains tested were found to multiply by asymmetric budding, not to form spore, pseudohyphae and pigment. They did not show hydrolysis of starch, casein, tween 80, DNA, lecithin, chitin and urea with the exception of tributyrin. They could ferment various kinds of carbohydrates including galactose, glucose, sucrose, mannose, fructose, inulin and trehalose but not lactose, mannitol, maltose, inositol, mellibiose, cellobiose and starch. Five strains of yeast isolated in this experiment were considered to be the same species and belong to the genus *Candida*[13] on the basis of main

Table 3. Characteristics of yeast isolates.

Characters	Isolates	Characters	Isolates
Gram stain	+	Fermentation of:	
Catalase	+	Galactose	F
Oxidase	−	Glucose	F
Budding	+	Fructose	F
Sporulation	−	Inulin	F
Pseudohypha	−	Mannose	F
Pigmentation	−	Raffinose	Fw
Hydrolysis of:		Sucrose	F
Starch	−	Trehalose	F
Casein	−	Cellobiose	−
Tributyrin	+	Inositol	−
Tween 80	−	Lactose	−
DNA	−	Maltose	−
Urea	−	Mannitol	−
Growth in Z-AII	+−	Melibiose	−
Growth in ZAG	++	Starch	−

characteristics described in Table 3. The yeast strains grew very poorly in Z-AII broth without glucose but the addition of glucose to Z-AII broth (ZAG medium) resulted in heavy cell densities. Accordingly, the yeast strains were suggested to grow well in the fish intestines when fish were fed with carbohydrate rich diets in aquaculture ponds.

Growth response under various conditions

Growth response of a yeast strain Y91-01 to various temperatures was shown in Fig. 2. Yeast cells were incubated in L-shape test tubes containing 10mℓ of ZAG broth and the growth of yeast cells was expressed as turbidity at 540nm after 2 day incubation. Yeast strain Y91-01 could grow over the temperature range of 13 to 34℃ and the optimal temperature for growth was about 25℃. Strain Y91-01 also could grow over a wide range of pH of 2 to 9 as shown in Fig. 3. As strain Y91-01 grew in ZAG broth, pH of the medium was found to come down resulting from the metabolism of glucose. In the case of *Candida lipolytica* isolated from fish silage, the addition of 2.0% glucose to broth cultures was reported to cause an increase of final cell density and a reduction in final pH of the cultures[14]. The growth of strain Y91-01 incubated in ZAG broth containing various concentrations of NaCl for 4 days at 25℃ was demonstrated in Fig. 4. The yeast strain could grow at high concentrations of NaCl such as 7%. If strain Y91-01 was incubated in the basal medium containing glucose 20.0 g

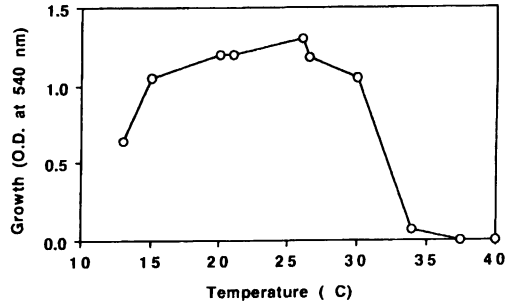

Fig. 2. Effect of temperature on the growth of yeast strain Y91-01.

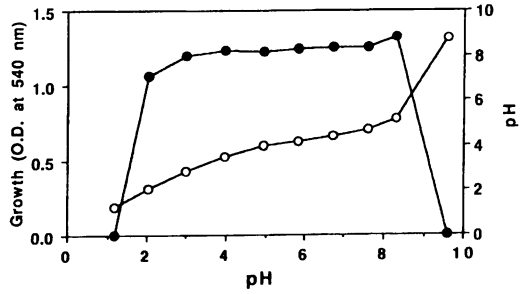

Fig. 3. Effect of pH on the growth of yeast strain Y91-01.
—●—, O.D. at 540nm ; —○—, final pH of the culture.

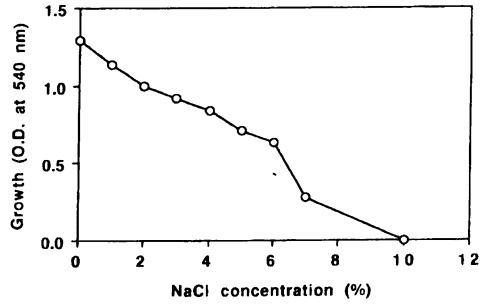

Fig. 4. Effect of NaCl concentration on the growth of yeast strain Y91-01.

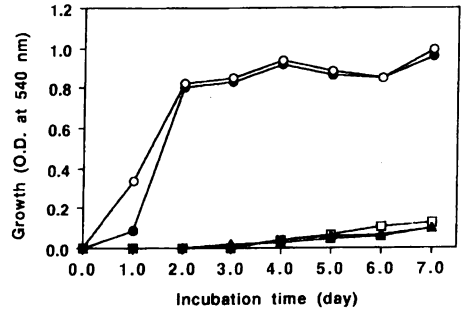

Fig. 5. Effect of vitamins on the growth of yeast strain Y91-01.
□, vitamin B_{12} (100μg/ℓ) ; +, vitamin B_1 (5mg/ℓ) ;
●, biotin (50μg/ℓ) ; ○, vitamin complex ;
▲, basal medium.

and $(NH_4)_2SO_4$ 1.0 g / ℓ , it could grow only slightly. However, the strain could grow notably on addition of biotin to the basal medium as shown in Fig. 5. The growth response of strain Y91-01 indicates that it can grow under extreme conditions such as lower pH and higher concentrations of NaCl, and requires biotin and some carbohydrates specifically for growth as compared with dominant bacterial strains isolated from the intestines of freshwater fish.

Fatty acid composition of cellular lipids

The fatty acid composition of the total lipids extracted from the cells of strain Y91-01 grown on Z-AII and ZAG agar media at 25℃ for 3 days was represented in Table 4. Major fatty acids were $C_{16:1}$, $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ and the ratio of total C_{16} to total C_{18} was 3.26 to 4.07. The percentage of unsaturated fatty acids in total lipids was 75.5 to 84.1%. The fatty acids of a thermotolerant yeast, *Hansenula polymorpha*[15] was reported to contain high proportions of oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids. The main fatty acids of *Candida utils*[16] and *Candida albicans*[17] strains were demonstrated to be $C_{16:0}$, $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$, giving greater proportions of total C_{18} acids to total C_{16} acids in both strains. On the contrary, the

Table 4. Fatty acid composition of the total lipids in yeast strain Y91-01.

Fatty acids	Z-AII agar μg/g (wet)	%	ZAG agar μg/g (wet)	%
14:0	51	2.3	49	1.1
14:1 n-5	15	0.7	26	0.6
15:0	17	0.8	5	0.1
16:0	284	12.7	331	7.7
16:1 n-5	1335	59.5	2704	62.7
17:0	14	0.6	4	0.1
18:0	72	3.2	91	2.1
18:1 n-9	285	12.7	631	14.6
18:1 n-7	38	1.7	206	4.8
Others	45	1.9	120	2.8
Unknown	88	3.9	148	3.4
Total	2244	100	4315	100
UFA (%)	75.5		84.1	
C16/C18	4.07		3.26	

fatty acid composition of yeast strain Y91-01 isolated from the intestine of rainbow trout was found to be characterized by high proportion of $C_{16:1}$.

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