

水産?酵食品の酵母に関する研究（第2報）：各種塩 辛中の真正酵母菌の菌学的性質に就いて（その1）

銭谷，武平
九州大学農学部水産学教室

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水産醱酵食品の酵母に関する研究(第2報)

各種塩辛中の真正酵母菌の菌学的性質に就いて(その1)

銭 谷 武 平

Yeasts occurring in fishery-fermentation products

Part 2. On the general features of true yeasts
in various kinds of "Shiokara" (1)

Buhei Zenitani

緒 言

塩辛類の酵母に就いては喜多¹⁾及び木村・小谷²⁾両氏の研究があり、いづれも *Torula* 属とした。著者は第1報に於て各種塩辛類から多数の真正酵母菌を分離しその主要なものは *Zygosaccharomyces* 属及び *Debaryomyces* 属に入ることを確めた。

本邦に於ける醱酵食品のうち味噌・醤油の酵母に就ては諸氏の報告あり高橋・湯川両氏は醤油酵母の、茂木氏は味噌酵母の菌学的性質一般を密かにし其の特性をも明らかにされた。尙又此等食品の風味の醸成に役立つもの並に有害な菌種をも確めた。塩辛及び其類似品は動物蛋白乃至其分解物を主とし糖質に比較的乏しく前記農産醱酵食品とは其組成に著しい差異があり又味噌・醤油類は絲状菌酵素の作用が顕著なるに比し塩辛類は概ね自己消化酵素に依存する。従つて塩辛酵母(塩辛中の酵母類を総括して塩辛酵母と呼ぶことにする)は味噌・醤油酵母とは其趣を異にすると想像され *Debaryomyces* 属酵母の非常に多いことも一つの特色と推定し得る。

著者は塩辛酵母の特性を明かにし且分類学的位置を定めるために 15 試料から分離した真正酵母菌の 23 菌株に就て形態学的並に生理学的性質一般に関する研究を行つたので報告する。本報では形態的並に培養的性質に就て記載する。

実 験 の 部

供 試 酵 母

前報告の各試料から分離した真正酵母 23 菌株に就て試験した。その所属並に菌株は次の如くである。

The true yeasts used in the experiments.

Debaryomyces.....A₁, A₂, B₁, C₁, C₂, C₃, O₅, P₂, Q₃, Q₄, R₄, S₂, S₃, S₄.

Zygosaccharomyces.....E₁, F₁, F₂, N₁, R₁ and R₃.

Hansenula.....C₄.

Torulasporea.....M₃.

菌 学 的 性 質

I. 形 態 学 的 性 質

1. 細胞の形態：一増殖細胞は麦芽汁(Bllg. 15')の試験管培養を用いて 25°C, 6日間培養した沈澱酵母に就いて細胞の形態、大き及び細胞の内容等を観察した(Table 1).

Table 1. Vegetative cells of "Shiokara" yeasts.

Strain	Form	Round	Oval or ellipsoid	Note
A ₁	Round to oval	5.5—6.5	4.0—6.4×6.4—9.6	Lemon shape rare
A ₂	Round to oval	4.0—5.6	4.0—4.8×4.8—5.6	
B ₁	Round predominantly, some oval	3.2—4.8	3.2—4.0×6.0—6.4	
C ₁	Ditto	3.2—6.4	3.2—4.8×4.8—7.2	
C ₂	Round	4.8—6.4		
C ₃	Ellipsoid predomi., some round	4.0—5.6	4.0—4.8×5.6—8.0	
C ₄	Round	3.2—4.8		
E ₁	Round predominantly, some round	4.8—6.4	4.8×8.0	
F ₁₋₂	Round	4.8—8.0		
M ₁	Ellipsoid predominantly, round rarely	3.0	2.4—4.0×3.2—4.8	Irregular form rare
N ₁	Round	5.0—8.0		
O ₀	Round to short oval	4.0—5.6	4.0—4.8×4.8—5.6	
P ₂	Round to short oval	5.5—7.2	4.0—5.5×4.8—6.4	
Q ₂	Round	5.6—6.4		Max. 7.2, min. 4.8
Q ₃	Round to short oval	4.8—5.5	3.2—4.0×4.8—5.5	
Q ₄	Round to oval	3.2—4.8	3.2—4.0×5.5—6.4	Some sausage shape
R ₁	Round	4.8—8.0		
R ₂	Round	4.8—8.0		
R ₄	Round to short oval	4.2—6.4	4.0—4.8×4.8—6.4	
S ₂	Oval to short ellipsoid, round rarely	4.8—5.4	3.2—4.8×4.8—6.4	
S ₃	Round to short ellipsoid	4.8—6.4	4.8—5.4×5.4—6.4	Some lemon shape
S ₄	Round predominantly, some short oval	4.0—6.4	3.2—4.8×4.8—6.4	

2. 液体培養：一新鮮培養から麦芽汁 (Bilg. 15') の試験管培養に接種し、25°C に放置して1週間に亘つて培養液の濁濁、皮輪、皮膜、沈渣及び酸酵状況に就て観察した (Table 2).

Table 2. Fluid culture at 25°C (Wort, Bilg. 15').

Strain	Note
A ₁	After 6 days, clear, slight ring and a little sediment resembling sand, slow growth.
A ₂	After 6 days, clear, slight yeast ring and sediment.
B ₁ P ₂	Thin film after 4 days, clear and greyish-white film after 6 days.
C ₁₋₄ S ₃	Thin film and turbid after 4 days, abundant sediment after 6 days.
C ₂	Very folded film after 2 days, clear, ring and sediment. Slight gas.
C ₃	Turbid and film after 2 days, abundant sediment and ring, slight gas by shaking.
E ₁	Pellicle after 4 days, clear, film and a little sediment after 6 days.
E ₁₋₂	Clear, slow growth and a little sediment.
M ₃	Turbid under surface, slight ring after 3 days, white ring and sediment after 6 days.
N ₁	Clear, bottom fermentation after 3 days, fermentation continued for 6 days, slight ring after 10 days.
O ₀	Folded, climbing greyish white film and flocculent, turbid and abundant sediment.
Q ₂	Gaseous fermentation continued for 6 days and turbid, sediment.
Q ₃	Grey thin film after 1 day, abundant sediment after 3 days and climbing greyish white film after 6 days.
Q ₄	Pellicle after 24 hours and dull grey film after 6 days.
R ₁	Clear, slight gas after 24 hours, fermentation continued for 6 days, moderate sediment and clear.
R ₂	Vigorous fermentation and turbid after 3 days, gas, ring and pellicle after 6 days.
R ₄	Thin film after 2 days, abundant sediment after 6 days.
S ₂	Clear, greyish white ring and sediment.
S ₄	Clear, floating pellicle and a little sediment after 6 days.

3. 割線培養：一新鮮培養から麦芽汁斜面寒天（Bllg. 12°）に割線接種し 25°C に放置して聚落の状態並に構造を観察した（Table 3）.

Table 3. Streak culture at 25°C (Wort agar, Bllg. 12°).

Strain	Note
A ₁	Yellowish grey, rough, raised and wavy edges.
A ₂	Porcelain white, smooth, flat and wavy edges.
B ₁	Sebaceous, smooth, raised and entire. Old culture with granular surface.
C ₁₋₄	Sebaceous, smooth, slight verrucose, raised and wavy edges.
C ₂	Grey~white, slight glistening, rugose and lobated edges.
C ₃	Sebaceous, smooth, raised and wavy to lobated edges.
E ₁	Sebaceous, raised and entire. Old culture with granular surface.
F ₁₋₂	Butyrous, smooth, raised and entire.
M ₃	Butyrous, smooth, raised and wavy edges.
N ₁	Butyrous, smooth, slight brownish in the center, raised and entire.
O ₉	Dull, greyish white, rugose, flat and wavy edges.
P ₂	Dull, yellowish grey, rough; flat and wavy edges.
Q ₂	Butyrous, smooth, raised and large wavy edges.
Q ₃	Grey~white, glistening, flat, smooth and wavy edges.
Q ₄	White glistening, flat, smooth and wavy edges.
R ₁	Butyrous, smooth, raised and entire.
R ₃	Butyrous, slightly folded at the border.
R ₄	Dull, yellowish, slowly raised and wavy edges.
S ₂	Yellow, waxy, smooth, slowly raised, slightly folded at the border and eroee.
S ₃	Dull, greyish white, moist, flat, slightly glistening at the border and wavy edges.
S ₄	Greyish white, glistening, smooth, white in the center, slowly raised and entire.

4. 穿刺培養：一新鮮培養から麦芽汁膠（Bllg. 10°, Gelatine 20%）の試験管に穿刺し、室温に 1~2 ヶ月間放置して穿刺口上並に溝内の発育状況及び溶膠性を観察した（Table 4）.

Table 4. Stab culture with wort gelatine.

Strain	Liquefaction (days)	Note
A ₁	-(50)	After 5 days irregular mass at the surface, slow growth.
A ₂	-(50)	After 30 days circular, folded in the center and radial sectors.
B ₁	-(50)	After 30 days circular, growth at the surface only.
C ₁₋₄	-(50)	After 30 days grey circular, white powdery at the border and wavy.
C ₂	+(7)	{White irregular, distinctive concentric rings and radial sectors slightly, the surface growth penetrating into the substrate.
C ₃	+(50)	{After 30 days, gelatine liquify bottle-like shape, and gas along the course of the stab.
E ₁	+(50)	A little merged in the center.
F ₁	+(50)	Slight gas in the stab.
F ₂	+(50)	Merged in the center.
M ₃	-(30)	Yellowish white, raised and some gas in the stab.
N ₁	-(70)	Yellow colony at the surface and large gas in the stab.
O ₉	+(70)	{Dirty brownish, irregular circular, grey at the border, merged in the center and many gas bubbles along the course of the stab.
P ₂	+(70)	Yellowish white, but greyish white at the border, slightly merged.
Q ₂	+(70)	{Dirty white, growth along the course of the stab and gas. Merged in the center.
Q ₃	+(70)	Lemon yellow, slightly merged.
Q ₄	-(70)	Dull, grey white, flat and no growth in the stab.
R ₁	-(70)	Greyish yellow, raised and growth in the course of the stab and gas.
R ₃	-(70)	Dirty white, gas bubble in the course of the stab.
R ₄	-(30)	Grey white flat, but yellow in the center, no growth in the stab.
S ₂	-(30)	Yellowish white, raised and no growth in the stab.
S ₃	-(30)	Dry greyish white and folded.
S ₄	-(30)	Yellowish white circular, raised and no growth in the stab.

以上試験結果から見て *Zygosaccharomyces* 属菌種は他属菌種に較べて細胞は大きく総て球形であり *Debaryomyces* 属菌種は細胞の形態等に於て菌株毎に可成り差異があつた。液体培養に於て *Zygosaccharomyces* 属菌種は概ね酸酵を営むが酸酵開始に2~3日を要し且緩慢な酸酵を1週間以上に互り継続した。又 *Debaryomyces* 属酵母の過半数は産膜性酵母であつた。穿刺培養に於いて溶膠性を示す菌種は少く其作用も微弱であつたが *C.* 菌の發育は著しく特徴的で膠中瘤状に没入して發育した。

Table 5. Form and texture of giant colonies of "Shiokara"-yeasts.

Strain	Form (diam. mm)	Color	Elevation and Crater	Concentric rings	Radial sector	Edges
A ₁	Circular 18-20.	Dull, tan in the center, dull, grey-white at border and shiny in the middle.	Raised.	3, indis.	Dis. in edges.	Wavy
A ₂	Circular 11.	Dull, light yellow in the center, grey at the border.	Slow raised.	None.	Some wave-like line.	Entire
B ₁	Circular 12.	Resembles A ₁ .	Little merged crater.	1.	None.	Entire
C ₁	Circular 10.	Dull grey-white.	Broad crater	None.	Numerous at border.	Wavy
C ₂	Irreg. circle. 22.	Dry, powdery surface.	Merged in the center.			Wavy
C ₃	Circular 9.	Yellow, waxy in the center, white powdery at border.	Smooth.	None.	Dis. in edge.	Erosee
C ₄	Circular 13.	Whitishyellow in center, grey-white at border.	Resemble C ₁ .	1 in flat part		Entire
E ₁	Circ. 5.	Grey-brown sebaceous.	Crater.	None.	None.	Entire
F ₁	Circ. 6.	Dull, grey.	Raised in the center.	1.	None.	Entire
F ₂	Circular 3.	Brownish grey-white.	Slow raised.	1.	None.	Entire
M ₂	Circular 6.	Yellowishgrey in center, grey at border.	Slow raised.	Some dis.	None.	Entire
N ₁	Circular 12.	Yellowishgrey, grey at border.	Double crater.			Wavy
O ₂	Circ. 13.	Dry, white powdery.	Flat.	2 dis.	Streamy.	Erosee
P ₂	Circular 11.	Greyishwhite, moist, glossy.	Slight elevat. in the center.	Some dis.	Numerous dis.	Wavy
Q ₁	Circ. 11.	Brownish in the center, grey-white at the border, yellow waxy in middle.	Triple raised crater	Divided in 3 zones by color.	None.	Entire
Q ₂	Circular 16.	Dry, yellow waxy in center, grey-white at border.	Flat.	Some dis.	Folded in center.	Wavy
Q ₄	Irreg. circ. 15.	Moist, yellow waxy in the center.	Flat, slight crater.	None.	Dis. in margine.	Irreg. to lobate
R ₁	Irregular.	Moist, grey-white.				Lobate
R ₂	Circular 12.	Yellowish grey, dull, moist, coarsely granular.	Raised double crater.		Numerous indis.	Wavy
R ₄	Circular 15.	Moist, glistening, grey-white.	Flat.	Dis. at the margine.	Irregular folded.	Entire
S ₂	Circular 8.	Brownish yellow in center, grey at border.	Pulvinate.	3.	Some indis.	Erosee
S ₃	Circular 16.	Yellowish grey in center, white ciliate.	Crater, slowly elevated	1.	Folded numerous.	Erosee
S ₄	Circular 18.	Brownish in the center, waxy at the border.	Raised generally.	None.	Numerous at border.	Wavy

Note; Dis. or dis. distinctly. indis. indistinctly.

5. 巨大聚落：— Stelling-Dekker の分類は本試験を標識に採用していない。然し乍ら巨大聚落は種の特徴をよく示すものとして Hansen 以来一般に広く採用されている。従つて既知菌種との分類査定上の必要から本試験を行つた。

培養基—麦芽汁膠 (Bllg. 12°, Gelatine 20%) を 150 cc容の三角コルベンに 2 cm の深さに入れ常法の如く殺菌し之に新鮮斜面培養又は液体培養から白金線又は毛細管を用いて接種した後コロニーの充分發育するのを待つて観察記載した。其結果は Table 5 の如くである (写真参照)。

Debaryomyces 属の巨大聚落は分類上に大した意義を有しないと長西氏⁴⁾が述べられているが今回分離した *Debaryomyces* sp. (C₂) の麦芽汁膠に於ける發育状況は極めて特徴のある年輪状の皺を作り膠中に瘤状に侵入して發育した。

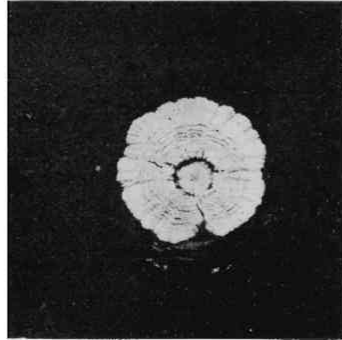


Fig. 1. Giant colony of *Debaryomyces* sp. C₂ on wort gelatine for 80 days.

要 旨

此の研究は 1) 分類学的研究に必要な酵母類の標識を得る為及び 2) 塩辛酵母の特性を確める為の二つの目的の為に遂行した。本報に於ては各種試料から分離した真正酵母菌 (23 菌株) の形態的並に培養的性質の記載をした。

特徴を総括すると次の如くである。即ち *Zygosaccharomyces* 属菌は殆んどすべて球形細胞であるが *Debaryomyces* 属は 1~2 個の油粒を含む球形乃至卵又は楕円形細胞である。麦芽汁培養に於て皮膜又は酵母輪を形成する酵母菌は殆ど総て *Debaryomyces* に属する。塩辛酵母のうち醱酵性菌株は殆ど総て *Zygosaccharomyces* 属であつて其醱酵力は旺盛ではないが持続的である。晒膠穿刺培養では稀に例外はあつたが溶膠性は認めなかつた。*Debaryomyces* 属の C₂ 菌の巨大聚落は特色ある多数の同心円を有し既知の *Debaryomyces* 属のものとは相違している。

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(九州大学農学部水産化学教室)
(長崎大学水産学部製造学教室)

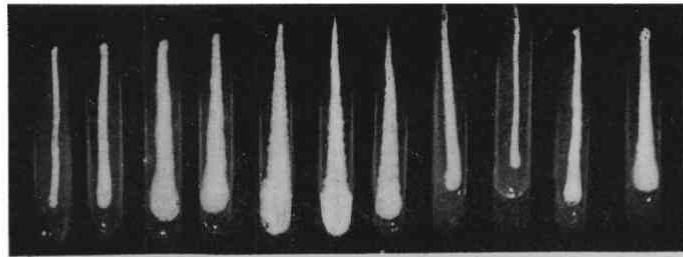
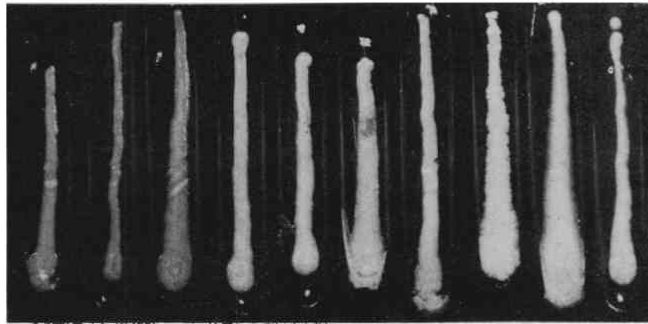
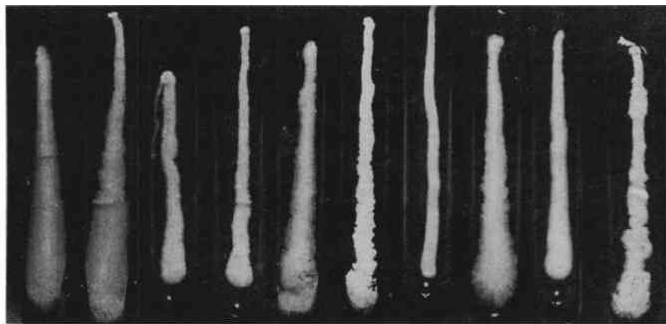
A₁. A₂. B₁. C₁. C₂. C₃. C₄. D₁. E₁. F₁. F₂.K₁. M₁. M₂. M₃. N₁. N₂. O₂. O₃. P₂. Q₂.Q₃. Q₄. R₁. R₂. R₄. S₁. S₂. S₃. S₄. S₅.

Fig. 2. Streak culture of "Shiokara" yeasts on wort agar.
(6 days at 25°C.)

R é s u m é

The present investigation was undertaken with a two-fold purpose: 1) to obtain the synopsis of yeasts for the taxonomic studies, and 2) to determine the specific features of "Shiokara"-yeasts. In this report the description of morphological and cultural characteristics of true yeasts (23 strains isolated from 15 samples) was made.

The summarized characteristics are as follows: almost all of the species of *Zygosaccharomyces* have a round cell while those of *Debaryomyces* have a round, egg-shaped or ellipsoid cell containing one or two oil drops (Table 1). Most film- or ring-forming yeasts belong to *Debaryomyces* (Table 2). The fermentable strains of "Shiokara"-yeasts belong almost all to *Zygosaccharomyces* and their fermentability is continuous, though not vigorous. The streak culture is shown in Table 3 and photograph. In gelatin stab cultures the liquefaction could not be observed except in some rare cases (Table 4). The giant colony of *Debaryomyces* sp. *C*₂ is different from any of *Debaryomyces* already described, the former showing many characteristic concentric rings (Table 5 and photograph).