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海洋酵母의 分類(1)

——거머리말과 몇 종의 無脊椎動物로부터——

全 順 培
(全南大學校 文理科大學 生物學科)

Studies on Yeasts Isolated from Marine Substrates (I) :

——From *Zostera marina* and Several Invertebrates——

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ABSTRACT

As a part of taxonomical and ecological studies on the yeasts in marine environments, several kinds of yeasts were isolated from *Zostera marina* several invertebrates (penaeus, Meretrix and Neptunus) and surface sea water, which are collected at the two established sites of estuarine areas; Dolsan island in Youchun district and Baiksu-ri in Youngkwang district. The obtained results can be summarized as follows.

1. Ascosporogenous Yeasts.

Hansenula sp I and Hansenula sp II were isolated from *Zostera marina* and Hanse nula sp I from penaeus.

2. Asporogenous Yeasts.

Trichosporon fermentans, Torulopsis, ernobii and Toruopsis dattila were isolated from Zostera marina, Candida krusei from Meretrix and Neptunus, Torulopsis pinus from surface sea waters, and Rhodotorula aurantiaca from Penaeus.

3. More notable isolations of several species from Zostera marina than the other sources could be assumed as related to the higher sugar concentration of this plant.

INTRODUCTION

Studies on the marine Yeasts for the past decade have been actively developed, and the results obtained from these studies hitherto have indicated that oceans and estuaries are by no means adverse environments for growth and activity for yeasts (Fell et al., 1960; Roth et al., 1962; Meyers et al., 1967; Ahearn et al., 1968). Fell et al (1960) and Roth et al (1962) have suggested that the yeasts present in ocean and estuaries have been introduced by aerial contamination, fresh water inflow, and terrestrial run-off, and that the varied ecological niche of marine environment is

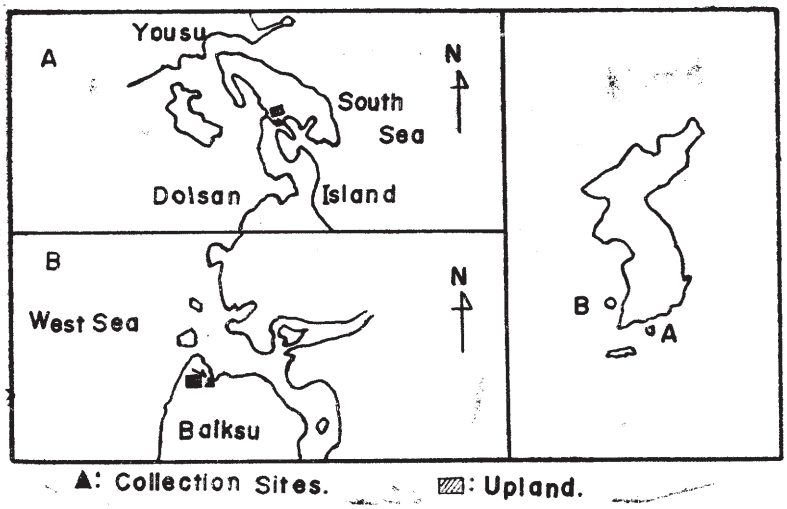


Fig. 1. Sampling locales in Area A and B
→ : Direction of Natural Drainage

suitable for both growth and reproduction of the yeasts. They have also reported that, in many inshore areas,

(1) required nutrients are present,

(2) oxygenation is adequate for the yeasts, and

(3) temperatures are well within the cardinal range of this microorganisms. It was recently reported by Meyers (1970) that Pichia and Kluveromyces were isolated from Spartina alternaria inhabiting in Barataria Bay. Recent investigations have involved the analyses of the overall contribution of these yeasts to marine biology and the taxonomic / physiological relationships of these organisms to their terrestrial counterparts. But the above studies on yeasts throughout our country have not been developed yet and, as a part of overall projects, during the last summer, 1970, this experiment was undertaken to isolate yeasts from Zostera marina which may be a good habitat for yeasts on account of the higher sugar concentration, several invertebrates and surface sea water. And thus, the author presents the results here.

MATERIALS AND METHODS

L. Collection and Isolation

A. The first isolation August 1970

Locality and Sources: Dolsan island in Youchun district 83 Km south of Kwangju city(Fig.1).

One material was Zostera marina dipped in sea water and the other was surface sea water. In this paper references to the ocean sampling include those mentioned in the reports of Ahearn et al., 1968, Meyers et al., 1970 and Roth et al., 1962. The salinity of sample collection sites was 25.9 % and pH of sea water indicated 6.25.

For isolation, living healthy plants was collected and was aseptically introduced into a malt agar of 75 % natural sea water, and the plant placed in a sterile containers was processed in laboratory within 4 days. Approximately 4.0-portion of this wet plant was poured into 60 ml. sterile sea water of 5% glucose. After liquid culture was kept at 25°C for 7 or 8 days, the sediment in the Ehrenmyer flask was streaked on the malt agar plate. On the one hand, 100 ml. of surface sea water was taken and was settled down in bottom of sterile tube by centrifuge. This sediment was also streaked on the malt agar plate.

B. The second isolation: Date; September 1970

Locality and Sources; Baiksu-ri in Youngkwang district 64 km west-north of Kwangju city (Fig. 1).

The materials collected in this region were penaeus, Meretrix and Neptunus, and the salinity was 20.4 % and *P*H was 6.8. These animals were inoculated into a malt agar plate of 75 % natural sea water and also put into Ehrenmyer flask containing 60 ml. natural sea water of 5 % glucose. The incubation was conducted at 25°C for 8 days. The other methods were much the same as previous experiment.

All the primary cultivations of samples were conducted at 25°C and bacteria were controlled by antibiotics (chloramphenicol) and acid *P*H (Meyers et al., 1967).

10 representative yeasts based upon the colonial morphology were selected and purified by a customary palting technique. As for the medium, a malt medium of 15°C Balling and of *p*H adjusted to 4.2-4.5 with 10 % lactic acid, was employed pure colonies were again selected and inoculated in the slant medium

2. Identification

Taxonomic identification procedures followed those recommended by Lodder and Kreger-van Rij (1952) and Wickerham (1951).

A. Characteristics of the vegetative reproduction

The characteristics of the vegetative reproduction are of basic importance. The vegetative cells were observed in malt agar after 3 days at 25°C. The present species were reproduced by budding method.

B. Shape and size of cells

As a matter of fact it is closely connected to the way of vegetative reproduction. The shape and size of the cells was observed in malt agar after 3 days at 25°C. Shape and size of cells is given in Table 2 and Fig. 2 or 3.

C. Ascospore formation

The property to from ascospore is a most important characteristics, because it is decisive for the classification of the yeasts either among the ascosporogenous yeasts or among the asporogenous ones. Ascospores were observed in the modified Gorodokwa agar after 10 days at 25°C. The ascosporogenous yeasts are given in Table 3 and Fig. 2.

D. Pellicle formation

Pellicle formation was observed in malt extract after 3 days at 25°C and finally after 10 days. The results are appeared in Table 2.

E. Fermentation of sugars

For fermentation of sugars, 2 % sugar solution in yeast extract was tested in Einhorn tubes and Durham tubes. These were incubated at 25°C and observed everyday. The final reading was made after 10 days. The results of sugar fermentation are given in Table 3.

F. Potassium nitrate assimilation and Sugar assimilation

The auxonographic method was used in these tests. In potassium nitrate assimilation, the basic agar medium does not contain a sources of nitrogen.

Experimental Result

8 kinds of yeasts from 5 materials were isolated. The results are as follows and the morphological and physiological properties of these species are presented in Table 2 and Table 3.

Ascosporogenous yeasts

Hansenula sp I close to Hansenula anomala (Hansen) H. et p Sydow var. anomala.

This species (Exp. No. 1-B and No. 3-A) was isolated from Zostera marina

Table 1. Occurence of Yeasts in marine substrates

Collection sites	Genera of organisms	Quantity of samples	Identification of yeasts	Exp.N.
Inshore Dolsan island in (Youchun district)	Zostera marina	4 g	Hansenula sp I Hansenula sp II Trichosporon fermentans Torulopsis ernobii Torulopsis dattila	1
ditto	Surface water	100 ml.	Torulopsis pinus	2
Inshore (Baiksu-ri in Youngkwan district)	Meretrix	10 g	Candida krusei	4
ditto	Penaeus	ditto	Hansenula sp I Rhodotorula aurantiace	3
ditto	Neptunus	ditto	Candida krusei	5

and Penaeus. This organism differed from Hansenula anomala var. anomala by its negative assimilation of soluble starch and raffinose, but in all other properties it was typical of this species.

Growth on malt agar: After 3 days at 25°C, cells are round or ellipsoidial, measuring (2.7-4.2)×(3.1-4.9)μ. Growth in malt extract: After 10 days at 25°C, pellicle is thin and usually accompanied by a ring. Streak culture on malt agar: After one month at 17°C, the surface of streak culture is smooth and crenate margin, and the colour is greyish, mat and butyrous. Dalmau plate culture on potato glucose agar: No hypha produce under coverslip at the end of 3 days at 25°C. Sporulation: Ascospores are easily formed on malt agar after one month at 25°C; ascospores are hat-shaped with an oil drop inside; 1-4 per ascus (Fig. 2). Fermentation: Glucose, galactose, sucrose, maltose and raffinose are fermented; lactose is negative. Sugar assimilation: Glucose, sucrose, maltose and ethanol are positive; galactose, lactose, raffinose and soluble starch are negative. Assimilation of potassium nitrate: positive, Ester formation: usually strong.

Hansenula sp II close to Hansenula anomala (Hansen) H. et p Sydow var. schnegii (Weber) Wickerham.

This species (Exp. No. 1-A) was isolated from *Zostera marina*.

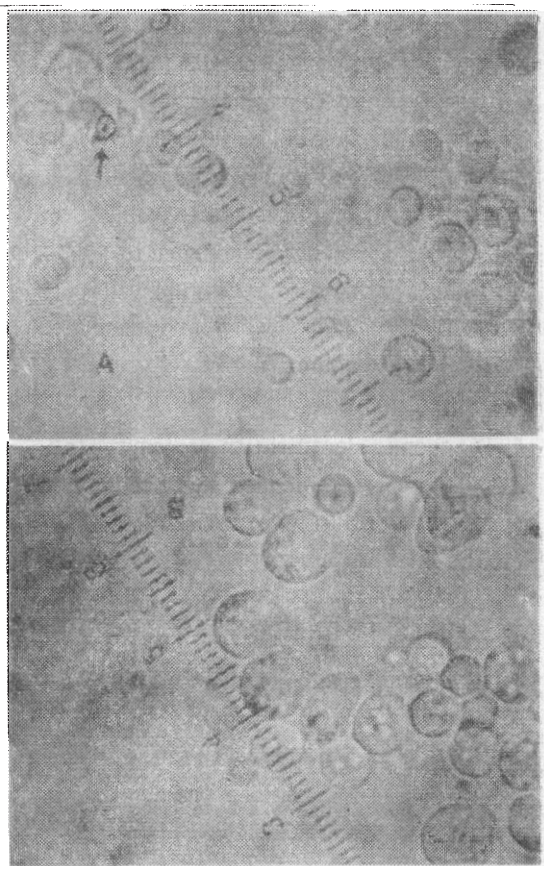


Fig. 2. Ascosporogenous yeasts
A: Hansenula SP I, Spore outline inkedin.
B: Hansenula SP II.

The morphological and physiological characteristics of this orgaisms were almost identical with Hansenula sp I, but pellicle formation in the malt extract was rugose, and weaker fermentation of sucrose was than Hansenula sp I, and the cells are usually elongate. Growth on malt agar : After 3 days at 25°C, cells are round or elongated, measuring (2-5)×(2. 2-8. 0)μ. Growth in malt extract: After 10 days at 25°C, pellicle is thick and usually accompanied by a thick rugose. Streak culture on malt agar : After one month at 17°C, the surface of streak culture is rugose and crenate margin, and the colour is white chalky. Dalmau plate culture on potato glucose agar: Pseudomycelium are branched. The blastos ore were ovoidal and ellipsoidial.

Sporulation: The ascospores are easily formed on malt agar; The spores are hat-shaped with an oil drop inside; 1-4 per ascus (Fig. 2). Fermentation: Glucose, sucrose. galactose, maltose, and raffinose are fermented; lactose is negative. Sugar assimilation: Sucrose, glucose, maltose and ethanol are positive; galactose, lactose, raffinose and soluble starch are negative. Assimilation of potassium nitrate is positive. Esterformation: Positive.

Asporogenous yeasts

Torulopsis dattila (Kluyver) Lodder

This species (Exp. No. 1-D) was isolated from Zosteramarina.

Undoubtly, this species belongs to Torulopsis dattila in morphological characteristics, as well as in behaviors towards sugar fermentation and assimilation. Growth on malt agar: After 3 days at 25°C, cells are round or oval, measuring (3-4)×(3. 8-4. 3)μ. (Fig. 3).

Growth in malt extract: After 10 days at 25°C, sediment is formed and pellicle is a slighting. Streak culture on malt agar: After one month at 17°C, the surface of streak culture is smooth, glossy and entire margin. The colour of streak culture is creamcoloured. Dalmau plate culture on potato glucose agar: No pseudomycelium is formed. Fermentation: Glucose, sucrose and raffinose are fermented.

Sugar assimilation: Glucose, galactose, maltose and raffinose are positive. Assimilation of potassium nitrate: Absent.

Torulopsis ernobii

Lodder et Kreger-van Rij

This species (Exp. No. 1-C) was isolated from Zoster marina.

Growth on malt agar: After 3 days at 25°C, cells are round or oval, measuring (2. 3-3. 0)×(2. 3-5. 0)μ. (Fig. 3). Growth in malt extract: After 10 days at 25°C, sediment is slightly formed and sligh ring formed. Streak culture on malt agar: After one month at 17°C, the surface of streak culture is smooth, entire margin and the colour of streak culture is grey-white. Dalmau plate culture on potato glucose agar: No pseudomycelium is formed.

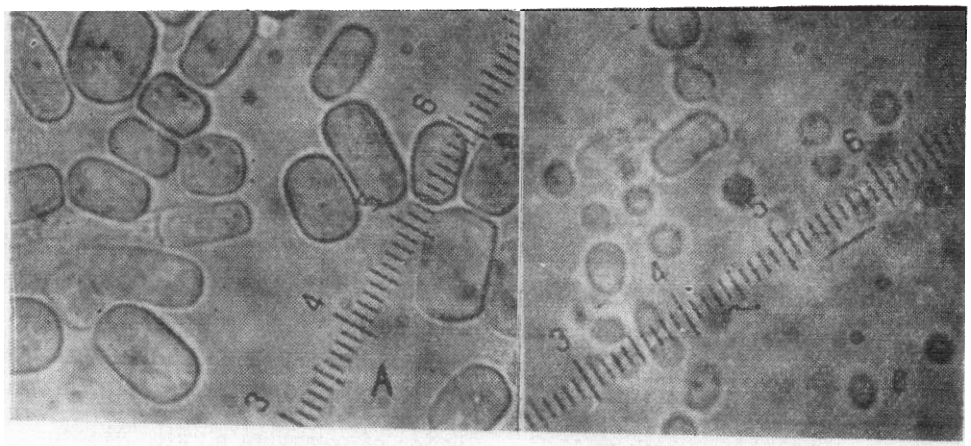
Sugar assimilation: Glucose, sucrose, maltose and ethanol are positive. Assimilation of potassium nitrate: Negative.

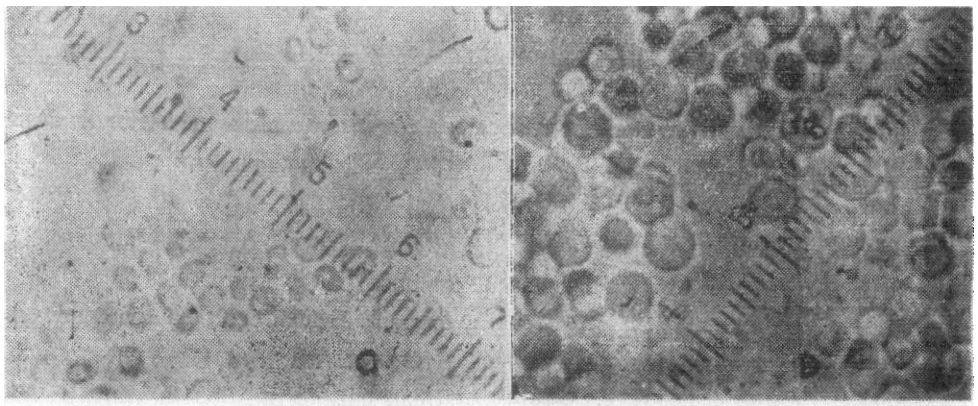
Torulopsis pinus

Lodder et Kreger-van Rij

This species (Exp. No. 2) was isolated from surface sea water.

Growth on malt agar: After 3 days at 25°C, cells are round or oval, measuring (3. 0-3. 2)×(3. 0-3. 5)μ. (Fig. 3). Growth in malt extract: After 10 days at 25°C, thin ring and sediment are formed. Streak culture on malt agar: After one month





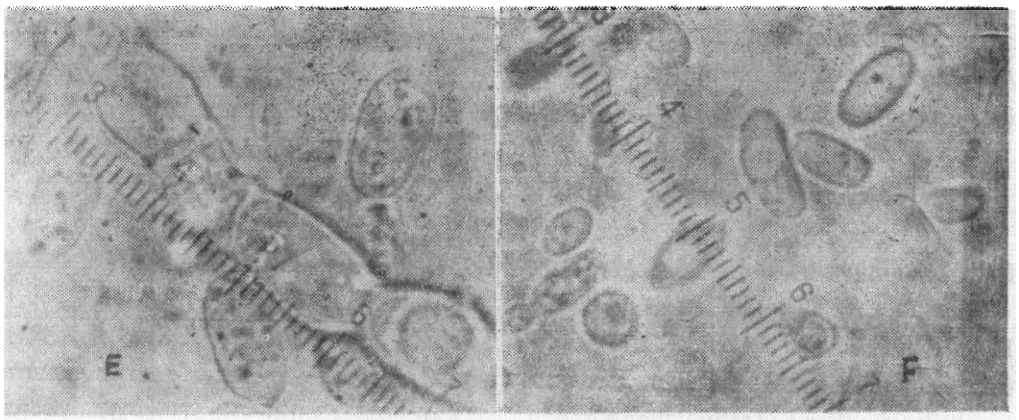


Fig. 3. Asporogenous yeasts
A: Trichosporon fermentans. B: Torulopsis ernobii. C: Torulopsis pinus.
D: Torulopsis dattila. E: Rhodotorula aurantiaca. F: Candida krusei.

at 17°C, the surface of streak culture is smooth, glossy and entire margin. The colour of streak culture is greyish cream-coloured. Dalmau plate culture on potato glucose agar: No pseudomycelium is formed. Fermentation: Absent. Sugar assimilation: Only glucose is positive. Assimilation of potassium nitrate: Absent.

Trichosporon fermentans
Dddens et Lodder

This species (Exp. No. 1-E) was isolated from Zostera marina. Growth on malt agar: After 3 days at 25°C, cells are cylindrical or elongate, measuring (4-5)×(5-11.5)μ (Fig. 3). Growth on malt agar: After 10 days at 25°C, a thick slimy sediment was formed and a thick rugose pellicle formed. Streak culture on malt agar: After one month at 17°C, the surface of streak culture is hairy and entire margin. The colour of streak culture is white-coloured.

Dalmau plate culture on potato glucose agar: In the beginning, abundant formation of blastospore only, later on chains of arthrospores appeared. Fermentation: Glucose and galactose are fermented.

Sugar assimilation: Glucose, galactose and ethanol are positive.

Assimilation of potassium nitrate: Nagative.

Candida Krusei (Cast) Barkhout

This species (Exp. No. 4 and No. 5) was isolated from Meretrix and Neptunus. Growth on malt agar: After 3 days at 25°C, cells are oval or cylindrical, measuring (3.0-4.2)×(5.0-12.5)μ (Fig.3).

Growth in malt extract: After 10 days at 25°C, a rugose pellicle and sidiment were formed. Streak culture on malt agar: After one month at 17°C, the surface of streak culture is flat and dull, and the colour of streak culture is yellowish cream-coloured.

Dalmau plate culture on potato glucose agar: Pseudomycelium and blastospore are

Table 2. Morphology of Yeasts

Exp. No.	Species	Shape	Size	Streak culture	Form & Number of ascospore	PM and TM
1—A	Hansenula Sp II	round or elongated	(2.0—5.0)×(2.2×8.0μ)	rugose white chalky	hat-shaped 1-4 per ascus	PM
1—B, 3—A	Hansenula sp I	round or ellipsoidial	(2.7—4.2)×(3.1—4.9)μ	smooth greyish	hat-shaped 1-4 per ascus	none
1—C	Torulopsis ernobii	round or oval	(2.3—3.0)×(2.3—5.0)μ	smooth grey-white	none	ditto
1—D	Torulopsis dattila	round or oval	(3.0—4.0)×(3.8—4.3)μ	smooth, glossy cream-coloured	ditto	ditto
1—E	Trichospo-ron fermentans	cylindrical or elongate	(4—5)×(5—11.5)μ	hairy white	ditto	TM
2	Torulopsis pinus	round or oval	(3.0—3 2)×(3.0—3.5)μ	smooth, glossy greyish cream-color	ditto	none
3—B	Rhodotorul-aaurantiaca	elongate or cylindrical	(4—6)×(5—13)μ	mucous, glossy orange	ditto	PM
4. 5	Candida krusei	oval or cylindrical	(3.0—4.2)×(5.0—12.5)μ	flat,dull yellowish-cream color	ditto	PM

PM=pseudomycelium TM=true mycelium

Table 3. Physiological characteristics of Yeasts

Exp. No.	Species	Fermentation						Assimilation									Pellicle formation	Ester formation
		Gl.	Ga.	Su.	Ma.	Ra.	La.	Gl.	Ga.	Su.	Ma.	Ra.	La.	Eth.	St.	KNO_3		
1-A	Hansenula sp II	+	V	+	V	+	−	+	−	+	+	−	−	+	−	+	thick rugose	+
1-B. 3-A	Hansenula Sp I	+	+	+	V	+	−	+	−	+	+	−	−	+	−	+	ring	+
1-C	Torulopsis ernobii	+	−	−	−	−	−	+	−	+	+	−	−	+	※	−	slight ring	※
1-D	Torulopsis dattila	+	−	+	−	+	−	+	+	+	+	−	−	+	※	−	ditto	※
1-E	Trichosporon fermentans	+	+	−	−	−	−	+	+	−	−	−	−	+	※	−	thick rugose	※
2	Torulopsis pinus	−	−	−	−	−	−	+	−	−	−	−	−	+	※	−	thin ring	※
3-B	Rhodotorula aurantiaca	−	−	−	−	−	−	+	−	+	+	−	−	−	※	−	ring	※
4・5	Candidakrusei	+	−	−	−	−	−	+	−	−	−	−	−	+	※	−	rugose	※

Gl. =Glucose, Ga. =Galactose, Su. =Sucrose, Ma. =Maltose, Ra=Raffinose, La. =Lactose, Eth. =Ethanol, V. =Variable, St. =Soluble starch, ※=Not experimented.

formed. Fermentation: Only glucose is fermented. Sugar assimilation: Glucose and ethanol are positive. Assimilation of potassium nitrate: Negative.

Rhodotorula aurantiaca (Saito) Lodder

This species (Exp. No. 3-B) was isolated from penaeus. Growth on malt agar: After 3 days at 25°C, cells are elongate or cylindrical, measuring (4-6)×(5-13)μ (Fig. 3). Growth in malt extract: After 10 days at 25°C, sediment and ring are formed. Streak culture on malt agar: The surface of streak culture is glossy and mucous, and the colour of streak culture is vorange-coloured. Dalmau plate culture on potato glucose agar: Pseudomycelium consisting of tree like fermentation is formed. Fermentation: Absent. Sugar assimilation: Glucose, sucrose and maltose are positive. Assimilation of potassium nitrate: Positive.

Discussion

Meyers et al (1970) reported that sexual species of pichia and Kluveromyces, and a sexual yeasts of Cryptococcus lourenti, Rhodotorula rubra and Crytococcus albidus, were isolated from Spartina growth and marshland in Bearataria Bay; and that this grass does provide a varie and rich sources of sugars for yeast habitat. As shown in Table I, the ascosporogenous yeasts of Hansenula spp and a sexual species of Torulopsis ernobii, Trichosporon fermentans and Torulopsis dattila were isolated from Zostera marina, and it could be suggested that this plant containing varied and rich sugars provided a good habitat for yeast population present. Roth et al (1962) isolated Trichosporon cutaneum, Candida spp, Rhodstorula spp, Hansenula spp, Torulopsis spp and Cryptococcus albidus from the intestine of fishes collected in Biscayne Bay. On the one hand,

Ahearn et al (1968) reported that the ascosporogenous yeast, Torulopsis ernobii, were isolated from sea water of South Florida: and that most of these organisms decreased rapidly in numbers within 5-10 miles south of South Florida but Hansenula anomala and Candida krusei was widespread in this region. In this experiment, it can be assumed that Hansenula spp and

Torulopsis ernobii isolated from Zostera marina and Penaeus were originated from adjoining land, and that these species can be widespread in estuarine waters. The original habitat of these species should be investigated in the future. On the other hand, it can be suggested that Torulopsis dattila and Torulopsis pinus were temporally inflowed from adjoining land habitat, because these yeasts have not been reported as a widespread species in estuarine waters. Ahearn et al (1967) indicated that Candida krusei isolated from North sea occur in high numbers in inshore in proximity to urbanized regions and are not found in open ocean waters but Rhodotorula spp and Candida krusei isolated from various sites of South Florida are distributed wide. In this study it can be surmised that Rhodotorula aurantiaca and Candida krusei isolated from Penaeus, Neptunus and Meretrix were widespread in estuarine waters, and so these species can dwell in these animals. Ahearn (1964) demonstrated that Rhodotorula strains isolated from marine environment had an absolute requirement ferp-aminobenzoate, eventhough certain terrestrial strains needed this vitamin only in a stimulatory way. Such an investigation should be studied in the future. Ahearn et al (1968) isolated Trichosporon cutaenum in equal frequency from estuarine waters and fresh waters in South Florida.

But in this study it seems to the author that Trichosporon fermentans isolated from *Zostera marina* was inflowed from adjoining fresh waters. As described above the results investigated from North sea to subtropical sea waters indicated that various marine enviroments can be a normal habitat for yeasts. In such a temperate zone as our country whose seasons are distinctive, the investigation of seasonal distribution of marine yeast population and relationships between marine yeast population and its terrestrial counterparts will be a very interesting subject.

摘 要

海水에 棲息하고 있는 酵母의 分類 및 生態學的 硏究의 一環으로 麗州郡 突山島와 靈光郡 백수 沿岸, 두 곳에서 採集한 거머리말(zostera marina)과 몇 종의 無脊椎動物(새우, 백합, 꽃게), 그리고 表層海水로부터 數種의 酵母를 分離하였다. 그 結果는 다음과 같이 要約할 수 있다.

1. 有胞子 酵母

거머리말로부터 Hansenula SP I과 Hansenula SP II를, 새우로부터 Hansenula SP I를 分離하였다.

2. 無胞子 酵母

거머리말에서 Trichosporon fermentans, Torulopsis ernobii, Torulopsis dattila를 백합과 꽃게에서 Candida krusei, 그리고 表層海水에서 Torulopsis pinus를 分離하였다.

3. 타분리원보다 거머리말에서 여러 種이 分離된 것은 높은 糖含量과 關係 있는 것으로 생각할 수 있었다.

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