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Ecological Studies on Yeasts in the Waters of the Yeong San River Estuary

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榮山江 汽水域의 酵母에 關한 生態學的 硏究

全 順 培

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ABSTRACTS

With 156 water samples collected from 39 locations in the Yeong San River estuary during the 12-month period from March 1976 to February 1977, the seasonal distribution of yeast and the distributional pattern of yeast on salinity gradient have been investigated. An overall average number of yeast ranged from 52 to 487 viable cells (c.f.u.) per 100ml water sample. The highest count of yeast was obtained in spring while the lowest value came in summer. 933 yeast and one yeast-like fungus pertaining to 14 genera and 83 species were recovered, of which *Candida* were 29%, *Debaryomyces* 17.3%, *Rhodotorula* 16%, and *Saccharomyces* 14%, respectively. *Debaryomyces hansenii* and *Rhodotorula glutinis* were dominant forms in all locations as well as throughout the year. The population size of total aerobic bacteria, the amount of terrestrial inputs, and some of geographical and/or climatic factor appear to reflect the seasonal distribution of yeast as well as the composition of yeast species in an estuarine environ. Average number of yeast, species diversity, and particularly the number of fermentative and pseudomycelium-producing yeasts increased with decreasing salinity whereas nitrate-utilizing yeasts showed opposite trend, suggesting that salinity gradient can be used as a feasible detector for the distributional pattern of yeast in estuarine habitat.

INTRODUCTION

Since the study on marine yeasts by Kriss *et al.*(1952) an ever-increasing numbers of papers on the yeasts in marine environs have been published during the last decades. These results have demonstrated that yeasts are commonly encountered in the sea(Moss & Morris, 1968; Roth & Fell, 1968) as well as in the open sea (Bahnweg & Sparrow, 1971; Fell & Phaff, 1968).

In estuarine and inshore water, a considerable amount of yeast populations is usually dominated by the species of *Candida*, *Rhodotorula*, *Debaryomyces* and *Cryptococcus*(Uden & Fell, 1968) Enumeration and identification in estuarine waters have also shown a wide range in number and species but a similarity in genera. The influence of sewage discharge and terrestrial runoff on the yeast population of estuarine areas was established, wherein some species are more prevalent in estuary than in the open sea(Uden & Fell, 1968; Uden *et al.*, 1958; Uden & Sousa, 1961; Uden & Sousa, 1957; Uden & Branco, 1963). Uden and Fell(1968) concluded that those of species found in sewage and polluted waters were also the dominant taxa in the estuarine waters. Ahearn *et al.*,(1968) have indicated that strictly oxidative species of *Candida*, *Cryptococcus*, *Rhodotorula* and the sexual yeast, *Debaryomyces hansenii*, were the most widespread in all the aquatic habitats of south Florida, and the predominant forms in waters

of relatively low organic matter. Lazarus and Koburger(1971) also showed that genera of yeasts in Biscayne, Florida, a highly populated region(Fell et al., 1960), were similar to those in the Suwanee River estuary, a less inhabitated areas, but their predominant species differed from those in the Suwanee River estuary. The predominant genera in this river were *Candida* and *Rhodotorula*, and the frequently isolated species was *Cryptococcus laurentii*.

Woollett and Hedrick(1970) quantitatively determined and compared the yeast population according to the different types and degree of population in southern part of Lake Michigan. They showed that very few yeasts in the fresh water were able to use nitrate as nitrogen sources while most of yeast in water with a high inputs of domestic wastes was pseudoproducing as well as fermentative. However, the seasonal change in yeast populations and their distributional pattern on the continuous spectrum of salinity in a river-dominated estuary have not been reported yet. An attempt was made to investigate the seasonal distribution and densities of yeast populations, together with their distributional pattern on salinity gradient in the Yeong San River estuary and the adjacent littoral zone.

MATERIALS AND METHODS

Collection Station

During the 12-month period from March 1976 to February 1977, 156 water samples were colle-

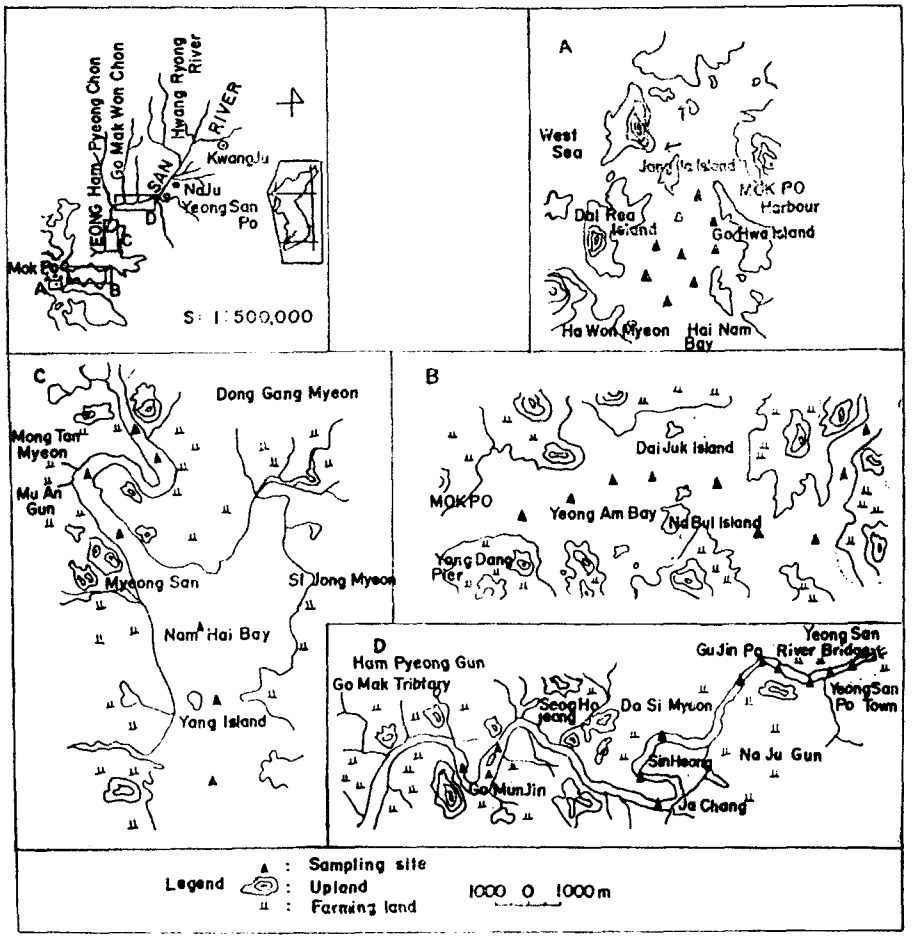

Fig. 1. Study region in the Yeong San River estuary.
Symbols: A, Station A; B, Station B
C, Station C; D, Station D

cted from the four locations in the Yeong San River estuary (Fig. 1). Those were chosen to represent salinity gradient, potential inputs of terrestrial runoff, and geographical distribution. The Yeong San River estuary is river-dominated as well as meandered, involving the great difference between the rise and fall of tide, and drains 3.142km^2 with approximately 36,000 population, including two towns of over 6,000. About 50% of the river basin of agricultural areas, mainly consisting of rice paddy, and the remainder is mostly of rural forest areas.

Four sampling stations had been set up and desingnated as A, B, C, and D, respectively. Station A is bordered by Go Wha Island to the north and open to the Yellow Sea through the narrow channel between Dal Rhee Island and Hwa Won Myun, Hae Nam Gun, and located west of Hae Nam Bay. The influence of terrestrial runoff and waste discharge on this water was the least, compared with the turbidity of water and the number of bacteria. Station B was a zone of admixture of estuarine and fresh water. The sampling area extend from about 8km upstream from Mok Po harbour to the station, from where Man Wol tributary emptied into the Yeong San River mouth, 2km from the harbour. Its drainage was sparsely inhabitated. Station C was Nam Hae Bay, located north of the head waters of Yeong Am Bay, including Ham Pyeong tributary north. Being located between the narrow channel at north and south side, Nam Hae Bay was much like a large lake. This was also joining site of natural water, flowing from Sam Po, where vineyards peach ones were studied about 24km ahead. This drainage basin was also a sparsely inhabitated rural region. Station D was an extremely meandering channel. Included in this station was a sizable amount of farmland, mainly consisting of rice paddy between both sides of this region, and brewery factory located nearby, and Yeong San town. Judging from salinity, this sector was characteristic of fresh water. Just as in the case of Station C, vineyards and peach orchards were located nearby.

Sampling and Isolation of Yeasts

All samplings were made on main stream as well as during incoming tide. Surface water samples were collected three times at each sites with 1 m extension apparatus specially constructed to remotely open and close 1,000ml sterile bottles, which was a modification of Ahearn's model(Ahearn *et al.*, 1968). Most collections were made at depth of 30cm below the surface. Sampling was done aboard a small boat(3.5 ton). Samples bottles were transported in an ice-chest maintaining the temperature from 4 C to 5 C, to the laboratory with the period collection and final processing generally ten hours and not twelve ones. After completely mixing three 1,000ml water samples collected at the same interval of distance in approximately 500m apart, the quantity of water filtered varied with turbidity of the samples and prior information. 10, 20, 30, 50, and 100ml aliquots at Station A and B; 5, 10, 20, 30, and 40ml at Station C; and 3, 5, 20, 30, and 40ml at Station D were filtered through 0.45μm cellulose ester membrane(Millipore filter) respectively, which were then implanted on three kinds of isolation media(Ahearn *et al.*, 1968), The composition of each medium were as follows: medium A consisted of 2.3% nutrient agar, 0.1% yeast extract, 0.4% peptone, 1% glucose, and 0.05g chloramphenicol, whereas medium B contained 0.3% malt extract, 0.5% yeast extract, 0.5% peptone, 1% glucose and 0.05g chloramphenicol. These two media prepared with the filtered natural water which were collected at each sampling stations. Following sterilization, the melted and cooled media were acidified to pH 6.5 with sterile 10% lactic acid to retard bacterial growth as well as rapid spreading of terrestrial fungi. On the other hand, medium C consisted of 2.3% nutrient agar, 0.1% yeast extract, 0.4% peptone and 1% glucose in filtered natural water. This medium was employed for the total counts of bacteria.

The membrane filters through which water had been drawn were placed-one dish per each aliquots-upon solidified agar plates and incubated for 72 hr or longer if the molds had not overgrown the yeast colonies. Plates containing im-

planted filters were incubated at 20 C, which allowed development of growing yeast but deferred excessive proliferation of mold. Numbers of individual colonies were counted microscopically after 72 or 120 hr. The numbers of yeast colonies appearing on the five membranes were combined together and harmonic mean was estimated. Representative colonies were selected and inoculated into yeast extract-malt extract agar (YM agar), incubated for 6 days, and streaked into the same media to chek purity. Final transfer was then made onto YM slants. After incubation, the isolates were stored at 4 C and transferred to stock culture medium approximately every 8 weeks for subsequent stduy and identification. Water samples were diluted 1:10 sterile saline water for the enumeration of total baceria. Colonies appearing on the spread plates were counted after incubation at 20 C for 3 days.

Identification of Yeasts

Identification of yeast was performed by using the methods by Lodder(1970) and Barnett *et al.*, (1979). Chaarcteristics of vegetative cells and their reproduction on solid media were examined on YM agar(Wickerham, 1951), whereas their features in liquid media were observed in 2% glucose-yeast extract-peptone water. Formation of pseudomycelium, true mycelium and ballistospore were performed on the slide culture procedures, together with the Dalmau plate (Wickerham, 1951), and mirror image(Lodder, 1970). Spore formation was made on Gorodokwa agar, potato glusose and YM agar. The presence of spore was observed microscopically, and in case of doubt, confirmed by staining method of Kufferath carbol-fuchsine. A multiple inoculation device(Beech *et al.*, 1955) was employed for carbon assimilation test. For confirmation, the method of Wickerham(1951). using a liquid synthetic medium in test tube, was applied to all new strains and in case of doubt. In all cases, the basal medium for assimilative utilization of carbon sources was yeast nitrogen base(Difco) while that for the utilization of nitrogen compounds was yeast carbon base (Difco).

Survival Test

The ability of yeast species to survive in natural water was assessed. Cells were grown in liquid media consisting of 1.0% glucose, 2.0% peptone and 0.1% yeast extract in distilled water for 48 hr at 25 C with 60 strokes/min. The yeasts were washed twice with phosphate buffer(pH 6.5), after which washed cells were inoculated into the 100ml of natural water which were filter-sterilized. Cell suspensions were incubated at 25 C with constant agitation and regularly examined for viability for peroids up to 4 weeks by standard membrane filter procedure.

Miscellaneous Procedures

The indices for both species diversity and dominance were calculated by the equation of Shannon-Wiener (1963) and Simpson (1949).

RESULTS

Survey of Habitat

The Yeong San River estuary lie topographically as well as physicochemically between fresh water environment and that of the sea. Since the upstream in this estuary is not only shallow but also narrow, much of sediments with significant inputs of fresh water from adjoining sluices, can be observed to be resuspended. Moreover, it has the heavy rainfall during summer months, together with the strong north wind against incoming sea water during winter. Therefore, the rate of dilution of estuarine water by terrestrial runoff which may be much affected by the rainfall, may cause to have a different range of salinity. The distinctive four seasons in Korea reflect the diverse temperature, which is characteristic of the subtropical during summer, of the subfrigid during winter, and of

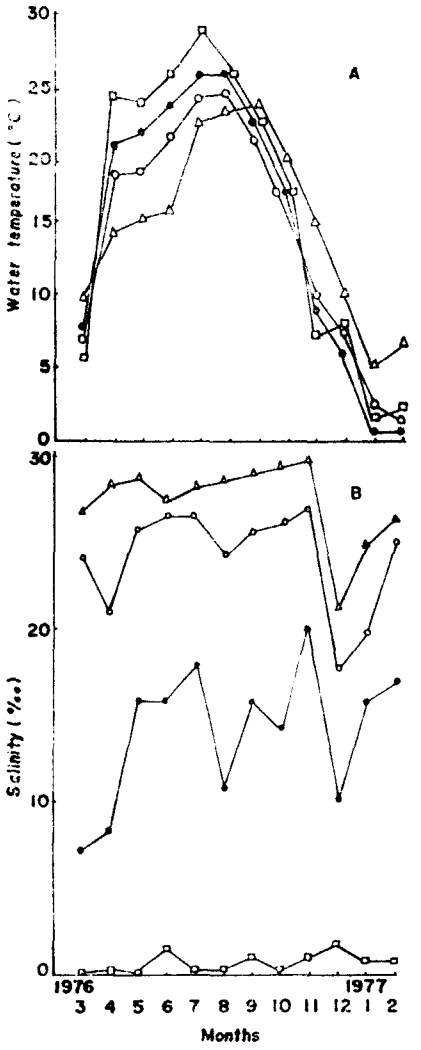

Fig. 2. The seasonal changes of temperature(A) and salinity(B) at four different sampling stations. Symbols:
△—△, Station A; ○—○, Station B
●—●, Station C; □—□, Station D

the temperate zone during spring and autumn, respectively. Therefore, the aforementioned conditions have been given without pretentions to dissolve the problems of the very complex distribution of yeast in estuarine environ.

Water temperature, salinity and pH were determined on boat by salinothermo conductivity meter(YSY model, Fisher) and pH meter (Beckman, chemat). Water temperature and salinity observed at the four stations during the 12-months were given in Fig. 2. The water temperature at four stations showed a similar trend to gradually increase during the period from March to July, while a rapid decline was observed during the period from August to November. However, during winter, Station B, C and D showed more rapid decrease in water temperature compared with that of Station A. On the other hand, salinity varied between 0‰. and 31‰. The regional ranges were in the 21～31‰ at Station A, 17～27‰ at Station B, 4～17‰ at Station C and 0～2‰ at Station D, respectively. Station B, C and D showed some

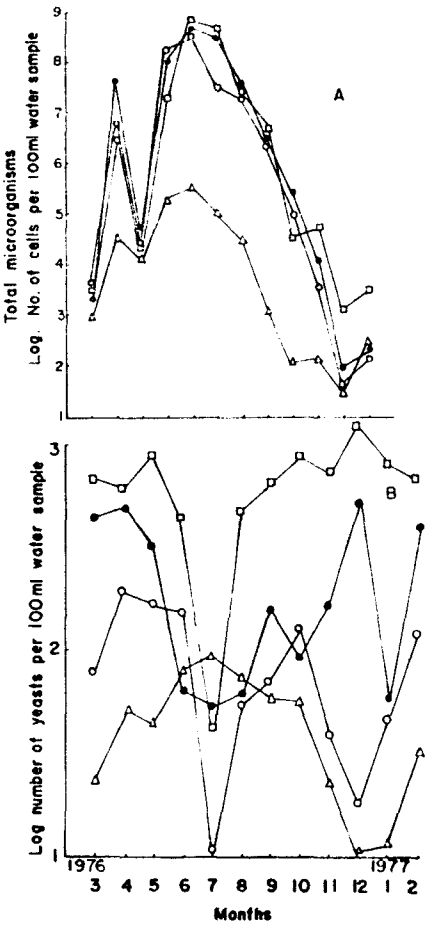

Fig. 3. The seasonal variation of yeasts(A) and tota aerobic bacteria(B) at four sampling stations. Symbols:
△—△, Station A; ○—○, Station B
●—●, Station C; □—□, Station D

decline in it during December. pH varied between 6.4 and 8.1. The regional range was 7.2 and 7.9 at the Station C, and between 6.6 and 8.0 at the Station D, respectively.

Seasonal Variation of Total Bacteria and Yeasts

Fig. 3 indicates the general or overall population level of total bacteria and yeasts at four stations. The highest counts of total bacteria were obtained from Station D while the lowest came from Station A, where salinity was high. The seasonal change followed similar pattern: the total bacteria showed a rapid decline in their number during the period of September January and May, whereas they rapidly increased in August, showing a little rise in April and exhibiting the highest level in summer. On the other hand, the yeast population level was the highest at Station D while the lowest was observed at Station A, where yeast number increased or decreased parelleling the water temperature profile(Fig. 2). At Station B, C and D, however, no readily discernable pattern was noted for such changes but follow similar trend: the overall number of yeast usually lower in summer than in spring and winter, showing a rapid decline in July. Thus, it appears that yeast population had inverse relationship to total bacteria.

To examine the yeast constituents of total bacteria was estimated during the 12month period. The result is shown in Table 1. A very high ratio of yeast was observed during the period from October through next March during which water temperature varied between 20 C and 2 C while a low value of it was between June and August. On the other hand, its value was much less at Station A compared with that of the other stations, suggesting that Station A could be much less affected by terrestrial runoff.

Quantitative and Qualitative Aspects of Yeast Population

The combined total of yeast from four stations are summerized in Table 2 and Fig. 4. Overall average of yeast in the Yeong San River estuary and the adjacent littoral zone ranged from 52 to 489 viable cells (c.f.u.)/100ml. 933 yeasts of 31722 isolates were identified as 82 yeast species in 13 genera and as one species of yeastlike fungus, of which *Candida* were 29%, *Debaryomyces* 17.3%, *Rhodotorula* 16%, *Saccharomyces* 14%, and other genera were within 10%.

Seasonal Variation of Sexual and Asexual Yeasts

Table 1. Seasonal change of of population ratio between yeast and total aerobic bacteria in the river water of the four sampling sites

Months / Stations	Yeast counts vs total bacterial counts					
	1976 3	4	5	6	7	8
A	2.8	1.7×10^{-1}	4.5×10^{-1}	3.5×10^{-2}	7.5×10^{-2}	7.6×10^{-2}
B	2.3	3.2×10^{-3}	5.1×10^{-1}	1.2×10^{-5}	1.9×10^{-6}	1.7×10^{-4}
C	2.7×10^{1}	1.1×10^{-3}	7.7×10^{-1}	7.4×10^{-5}	1.3×10^{-5}	2.5×10^{-5}
D	1.9×10^{1}	1.8×10^{-2}	2.7×10^{-1}	2.4×10^{-3}	9.6×10^{-6}	1.3×10^{-4}

Months / Stations	Yeast counts vs total bacterial counts					
	1976 9	10	11	12	1977 1	2
A	2.3×10^{-1}	5.2	2.3×10^{1}	3.9	4.0×10^{-1}	1.3
B	2.3×10^{-4}	8.1×10^{-3}	5.4×10^{-2}	4.2×10^{-1}	1.9×10^{2}	7.9×10^{1}
C	6.6×10^{-4}	2.6×10^{-3}	1.8×10^{-5}	4.5	7.0×10^{1}	2.0×10^{1}
D	2.3×10^{-3}	2.1×10^{-2}	1.6	2.8	5.8×10^{1}	2.4×10^{1}

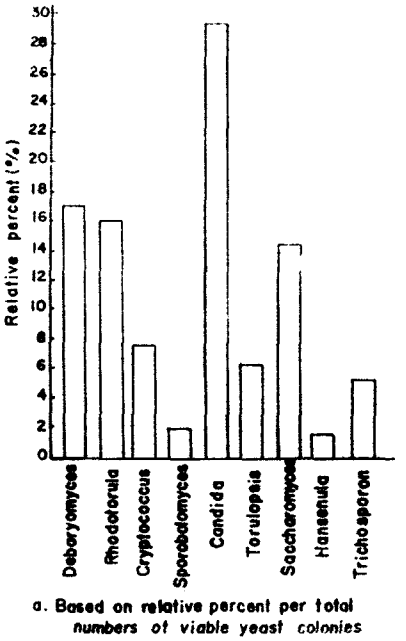

Fig. 4. The relative abundance of major yeast genera in the water of the Yeong San River estuary.

The seasonal distribution of asexual and sexual yeast is shown in Fig. 5. The dominant genera of asexual yeasts were *Candida* and *Rhodotorula*. They showed a generally similar trend to rapidly decrease in summer. Most of *Candida* showed an increase in autumn and winter while *Rhodotorula* exhibited a relatively evenly distribution except for July and October. *Torulopsis* and *Trichospron* occurred mainly in winter and early spring. However, *Kloeckera* was obtained only in autumn. On the other hand, the dominant form of sexual yeast was *Debaryomyces*, which occurred mainly during the period of both February-January and August-December. *Saccharomyces*, *Pichia* and *Hansenula* were obtianed mainly between August and December.

Regional Distribution of Yeasts

The number of relative incidence are presented in Table 2 and Fig. 6. 5~100 cells per 100ml water samples were obtained from Station A. The dominant species were *Debaryomyces hansenii* (DI=0.44) and *Rhodotorula* (DI=0.23). The average number of yeasts from

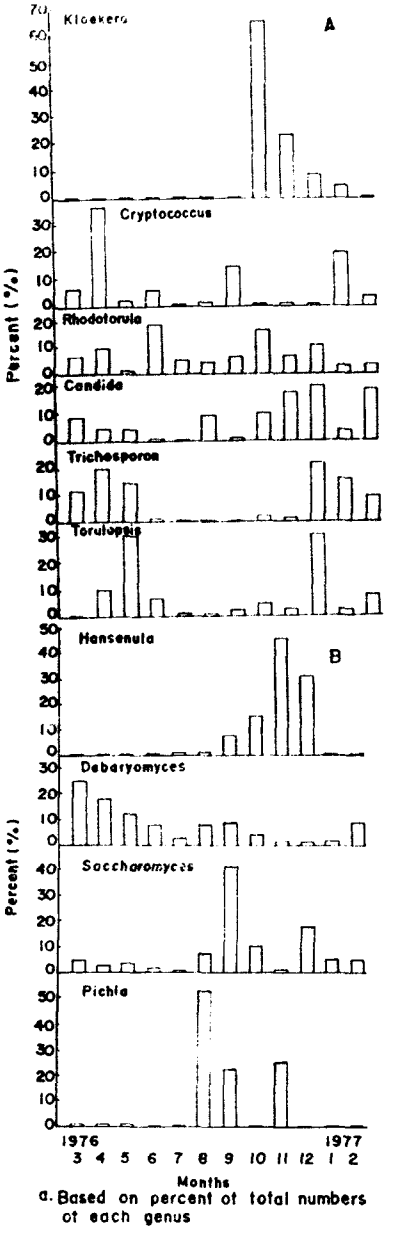

Fig. 5. The seasonal variation of asexual yeasts(A) and sexual yeasts(B) in the water of the Yeong San River estuary.

Station B ranged from 9~197 cells per 100ml water sample. This value, accordingly, showed some increase as compared with that of Station A. Species diversity (H=1.74) exhibited 1.3 fold increase as compared with $\bar{H}$=1.43 of Station A. However, genera composition showed a similarity. The dominant species in this region

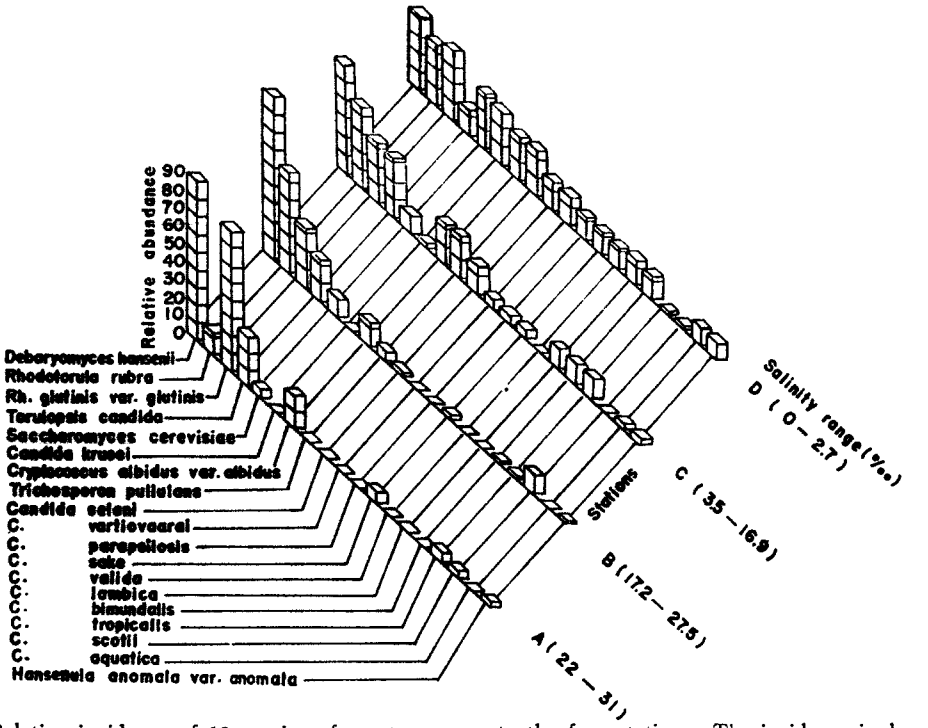

Fig. 6. Relative incidence of 19 species of yeast common to the four stations. The incidence is based on 36 water samples per each station.

were *Debaryomyces hensenii* (DI=0.42) and *Rhodotorula glutinis* (DI=0.16) although their dominance showed somewhat low value compared with that of Station A. On the other hand, 37~511 cells per 100ml water sample were obtained from Station C,showing three-fold increase compared with that of Station B. The dominant species were *Debaryomyces hansenii* (DI=0.15) and *Trichosporon pullulans* (DI=0.13), and species diversity was $\bar{H}$=2.8, showing 1.6-fold increase compared to Station B. At this station, additional species occurred that were absent in Station A and B: among these, the highest counts were of *Candida lambica*, *C. gelida*, *C. parapsilosis* and *C. vartiovaarai*. Most of these species were obtained during early spring and winter. The average count of yeast from Station D was 43~1164 per 100ml water sample, showing an approximately threefold increase with that of Station C. Species diversity, however, was a little less value ($\bar{H}$=2.6) than that of Station C. The predominant species were *Saccharomyces cerevisiae* (DI=0.13) and *Debaryomyces hansenii*(DI=0.1). From this station, animal-associated yeasts, i.e., *Candida guilliermondii*, *C. krusei* and *C. tropicalis*, occurred at high incidence.

The Effect of Salinity on the Distribution of Yeasts

The salinity gradient by distance from downstream to upstream and the effect of salinity on the distribution of major yeast genera are reflected in Fig. 7 and 8. These indicate that *Debaryomyces* and *Rhodotorulla* exhibited the least variation in coefficient variance (21.4~22.5%) throughout all the distance (Table 3), suggesting the ability of these genera to survive in all the environ. However, *Saccharomyces* showed the highest value, indicating the limited distribution to water of low salinity. Another comparison of several yeasts common to four stations was made with the coefficient variance.

Table 2. The regional distribution of yeast population in the river water sampled at four stations. Counts were made for each sampling site and combined together to show in the table

Species	Accumulative counts per thirty-six 100ml water				
	Stations				Total No.
	A	B	C	D	
Debaryomyces hansenii	823	1,416	1,393	2,154	5,786
Rhodotorula glutinis	542	509	358	1,317	2,726
Rh. graminis	50	264	29	36	379
Rh. rubra	183	186	337	1,157	1,863
Rh. pilimanae	—	1	300	—	301
Rh. pallida	—	—	—	7	7
Torulopsis candida	96	32	503	306	937
T. lactis-condonsii	—	—	14	83	97
T. holmii	—	—	6	423	429
T. apis	—	—	—	4	4
T. stellata	—	—	2	4	6
T. globosa	—	—	98	40	138
T. etchellsii	—	—	—	56	56
T. mogii	—	—	—	129	129
T. domercqii	—	—	—	35	35
T. fusisanensis	—	—	—	24	24
T. magnoliae	—	—	—	77	77
T. inconspicuus	—	—	3	—	3
Cryptococcus albidus	67	145	597	682	1,491
Cryp. infirmo-miniatus	6	33	224	72	335
Cryp. laurentii	—	3	—	39	42
Cryp. luteous	—	—	50	1	51
Cryp. macerans	—	—	—	95	95
Cryp. uniguttulatus	—	—	—	35	35
Trichosporon pullulans	—	9	1,142	440	1,591
Trich. variable	62	100	—	—	162
Trich. capitatum	—	—	26	5	31
Trich. cutaneum	—	—	—	5	5
Candida sake	2	33	518	296	849
C. guilliermondii	6	6	12	259	283
C. solani	—	5	615	897	1,517
C. bimundalis	—	26	541	518	1,085
C. lambica	—	—	321	226	547
C. salmonicola	—	30	230	41	301
C. tropicalis	4	—	40	67	111
C. parapsilosis	—	—	357	524	881
C. krusei	—	—	4	815	819
C. aquatica	—	—	16	56	72
C. valida	—	—	—	330	330
C. vartiovaarai	—	—	423	308	731

Species		Accumulative counts per thirty-six 100ml water				Total No.
		Stations				
		A	B	C	D	
C.	***scotii***	6	74	—	14	94
C.	***pelliculosa***	—	4	3	10	17
C.	***beechii***	—	1	—	86	87
C.	***diddensii***	—	1	70	—	71
C.	***gelida***	—	—	615	—	615
C.	***claussenii***	—	—	4	86	90
C.	***zeylanoides***	—	—	50	46	96
C.	***fabiani***	—	—	39	124	163
C.	***boidini***	—	—	27	96	123
C.	***intermedia***	—	—	—	74	74
C.	***membranaefaciences***	—	1	—	—	1
C.	***melinii***	—	—	33	—	33
C.	***diversa***	—	—	—	22	22
C.	***shehatae***	—	—	—	42	42
C.	***utilis***	—	—	4	14	18
C.	***curiosa***	—	—	—	24	24
C.	***diffluens***	—	—	—	29	29
C.	***ingens***	—	—	—	287	287
C.	***brumptii***	—	—	—	14	14
C.	***viswanathi***	—	—	7	86	93
C.	***slamanticensis***	—	—	12	6	18
Saccharomyces	***cerevisiae***	3	131	59	2,591	2,784
Sacch.	***telluris***	—	—	—	120	120
Sacch.	***saitoanus***	—	67	—	1,127	1,194
Sacch.	***globous***	—	—	—	1	1
Sacch.	***exoguus***	—	—	2	—	2
Sacch.	***chevalierii***	—	—	—	14	14
Sacch.	***uvarum***	—	—	—	38	38
Hansenula	***anomala***	5	7	13	226	251
H.	***beijerinckii***	—	—	—	111	111
H.	***silvicola***	—	—	6	—	6
H.	***ciferrii***	—	2	—	—	2
Pichia	***ohmeri***	—	—	—	10	10
P.	***ethchellsii***	—	—	—	11	11
Kloeckera	***apiculata***	—	18	9	71	98
K.	***corticis***	—	—	—	32	32
K.	***javanica***	—	—	—	5	5
Sporobolomyces	***roseus***	—	—	4	427	431
Sp.	***hispanicus***	—	—	12	—	12
Sp.	***gracis***	—	—	—	47	47
Endomycopsis	***burtonii***	—	—	—	29	29
Bullera	***alba***	—	—	100	—	100
Aureobasidium	***pullulans***	—	—	—	52	52
Total No.		1,855	3,104	9,228	17,535	31,722

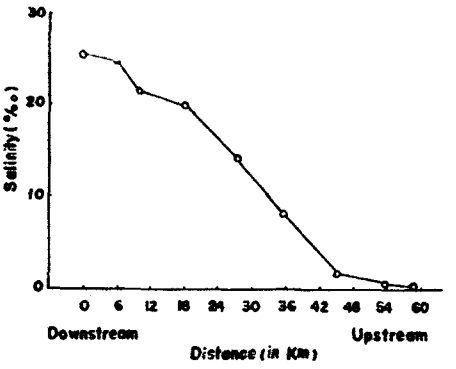

Fig. 7. The relationship between distance from the sea water and salinity of the river water.

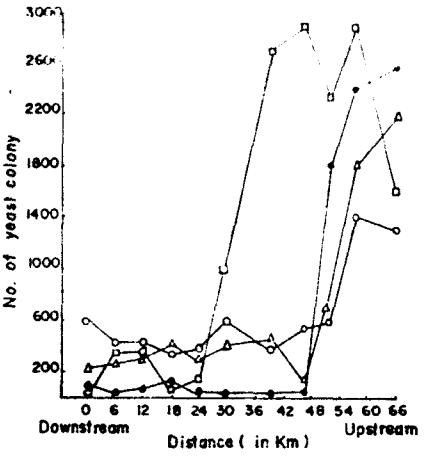

Fig. 8. The effect of salinity on the distribution of major yeast gener.
Symbols: ○—○, *Debaryomyces*;
△—△, *Rhodotorula*
□—□, *Candida*;
●—●, *Saccharomyces*

This is shown in Table 4. This indicates that ***Debaryomyces hansenii*** was most unifromly distributed throughout the year, while ***Saccharomyces cerevisiae*** and most of ***Candida*** gradually decr-

Table 3. Effect of distance from sea water region(river mouth) on the distribution of yeast genera in the water of the Yeong San River estuary
a: The numbers are based on the incidences of yeasts from 36 water samples per each site throughout the year

Genera	River mouth				Distance in Km			Upstream			Mean	Coefficient of Variance (%)
	0	12	18	24	30	38	46	52	56	64		
Candida	4	7	5	7	16	22	19	25	25	25	15.5	54.45
Cryptococcus	1	2	6	2	5	4	5	9	6	5	4.5	49.77
Rhodotorula	12	8	14	12	10	13	7	9	13	14	11.2	21.42
Debaryomyces	11	11	9	8	7	7	7	7	6	6	7.9	22.25
Torulopsis	4	1	4	6	7	6	6	3	7	7	5.1	37.66
Trichosporon	1	1	1	3	5	4	6	5	3	1	3.0	61.46
Saccharomyces	2	2	2	2	0	3	2	6	5	8	3.2	64.35

Table 4. Stability of yeast distribution throughout the year in the water of the four different sampling sites

Species	Stations							
	A		B		C		D	
	M	C.V	M	C.V	M	C.V	M	C.V
Candida krusei	—	—	—	—	0.083	337.0	1.25	108.93
C. solani	—	—	0.083	337	0.5	129.1	0.666	187.27
C. vartiovaarai	—	—	—	—	0.17	223.8	0.666	127.60
C. parapsilosis	—	—	—	—	0.17	223.8	0.500	173.20
C. guilliermondii	—	—	—	—	0.166	333.0	0.333	187.20
C. lambica	—	—	—	—	0.333	141.5	0.583	130.20
C. tropicalis	0.170	223.8	0.083	337	0.333	187.3	0.166	333.00

Cryptococcus albidus	0.750	688.0	0.416	153.8	0.666	111.9	1.080	103.24
Rhodotorula glutinis	2.333	47.5	0.916	113.3	1.000	81.0	1.580	60.26
R. rubra	0.420	229.3	1.583	87.3	1.427	78.7	1.417	95.42
Debaryomyces hansenii	2.670	17.7	2.583	24.78	1.833	48.9	2.080	63.47
Torulopsis candida	0.920	78.42	0.666	127.62	1.000	100.0	0.583	109.80
Trichosporon pullulans	—	—	0.167	331.80	0.750	129.1	1.083	344.30
Saccharomyces cerevisiae	0.170	223.8	0.250	173.2	0.330	141.6	1.333	55.90
Hansenula anomala	0.083	337.0	0.083	337.0	0.083	337	0.416	118.50

Note: Mean is based upon the frequency of occurrence of yeasts species from 36 water samples per each station throughout the year. C.V: Coefficient of variance —: Not available

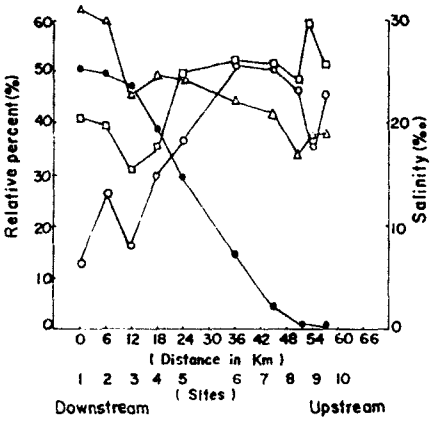

Fig. 9. The effect of salinity of the river water on the distribution of nitrate utilizable, fermentative, and pseudomycelium-producing yeasts. Relative percent is based on occurrence of yeasts at each site.
Symbols: △—△, nitrate utilizable yeasts
□—□, fermentative yeasts
○—○, pseudomycelium-producing yeasts
●—●, Salinity(‰)

eased in this value with the remoteness of distance from sea water. On the other hand, the distribution of yeasts capable of utilizing potassium nitrate, of producing pseudomycelium and of fermenting sugars was examined in relation to salinity. The result is presented in Fig. 9. This result showed that the increase in numbers of KNO_3-assimilative yeasts parelleled that of salinity while pseudomycelium-producing and fermentative yeasts showed an opposite trend.

Survival of Yeasts in the Three Different Stations

A differential ability of ceratin yeasts to survive in three aquatic environs is given in Table 5. This result showed that *Debaryomyces hansenii*, *Rhodotorula glutinis* and *Rh. rubra* survived for prolonged periods in sea water while

Table 5. Survival of yeasts in fresh, estuarine and marine waters

Time in days	Fresh (0.07‰)				Estuarine (17‰)				Marine (26‰)			
	0	5	15	30	0	5	15	30	0	5	15	30
	Numbers of viable cells/ml											
Debaryomyces hansenii	1,000	520	880	380	890	200	500	280	1,020	580	980	470
Rhodotorula rubra	268	383	258	493	182	205	178	138	201	212	208	104
Rh. glutinis	1,030	980	870	760	940	980	820	483	1,021	1,039	582	380
Trichosporon pullulans	1,830	1,760	1,580	1,980	2,000	1,800	1,200	2,100	1,210	1,280	808	98
Candida solani	1,228	1,028	840	—	1,129	1,230	980	320	1,219	840	380	—
C. lam[illegible]ca	2,803	2,080	2,380	1,898	2,903	830	908	303	2,763	830	400	20
C. tropicalis	1,828	1,530	683	38	2,020	1,560	780	28	2,080	3,290	1,528	813
C. parapsilosis	1,121	1,580	1,230	601	1,211	1,380	980	302	1,080	1,621	2,000	48
C. vartiovaarai	2,230	2,580	1,920	406	2,218	1,986	760	48	2,210	2,310	960	38
Saccharomyces cerevisiae	600	2,000	200	1,000	580	1,280	850	1,121	570	680	560	118
Cryptococcus albidus	21,380	6	4,028	—	—	—	—	—	2,228	2,320	215	125

Candida lambica, *C. vartiovaarai*, *C. parapsilosis*, *C. solani* and *Trichosporon pullulans* maintained their ability to some extent to survive in fresh or saline water but showed a trend to decrease in sea water. On the other hand, *Saccharomyces cerevisiae* and *Cryptococcus albidius* showed an intermediate survival compared with the above two groups.

Water Temperature and Animal-associated Yeasts

Potential distribution of yeasts in association with animals or man was examined (Table 6 and Fig. 10). The majority of species which can be able to grow at 37 C were *Candida tropicalis*, *C. guilliermondii*, *C. krusei*, *Saccharomyces cerevisiae*, *C. parapsilosis* and *Rhodotorula rubra*. Their distribution was limited to Station D, and most of them was frequently isolated in summer during which water temperature was between 19~29 C.

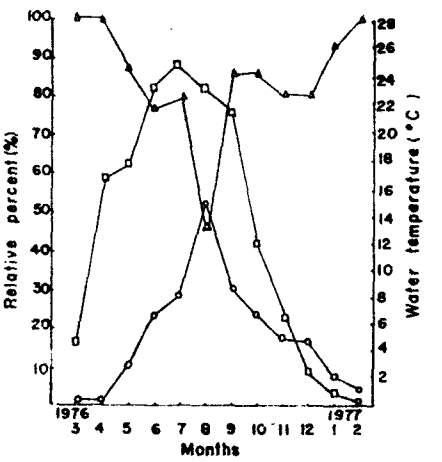

Fig. 10. The relationship between river water temperature and the distribution of animal-associated yeasts. The relative percent is based on isolates per each month.
Symbols: ○—○, yeasts capable of growing at 37°C
△—△, yeasts isolated at 2) C
□—□, water teamperature profile

Table 6. Effect of salinity of the river water on the distribution of yeast species common to sewage water

	Accumulative counts per three 100ml water			
	Stations			
	A	B	C	D
	Salinity range(‰)			
	(22~31)	(17~28)	(4~17)	(0~1)
Candida guilliermondii	6	8	135	780
C. tropicalis	4	5	52	150
C. parapsilosis	0	0	508	1,960
C. krusei	0	11	28	6,309

DISCUSSION

An overll average of 52~487 cells(c.f.u.) per 100ml water sample showed the intermediate value between some other determination of yeast concentration in the estuarine water and that of the polluted fresh water. From studies of two temperate estuaries of Portugal(Taysi & Uden, 1964) and of the estuary of swedish westcoast Norkrans, 1966a), 2~51 cells per 100ml water sample have been reported. On the other hand, Ahearn *et al.*,(1968) reported that a range 29~4700 cells per 100ml water occurred in the polluted fresh water of the River Miami, Florida and of the River Grand Calumet,Illinois-Indiana border.

The dominant species were *Debaryomyces hansenii* and *Rhodotorula glutinis*. These were followed by *Rh. rubra*, *Saccharomyces cerevisiae*, *Trichosporon pullulans*, and *Cryptococcus albidius*. The following yeasts, i.e., *D. hansenii*, *Rh. glutinis*, *Rh. rubra* and *Cryptococcus albidius*, corresponded fairly well with the results of other investigators(Bhat & Kachwalla, 1955; Fell & Uden, 1963; Fell, 1967; Meyers et al., 1967a; Norkrans, 1966a), who showed that similar

species were the predominant forms in the estuarine environs. On the other hand, most of *Candida*, *Saccharomyces cerevisiae*, and the other *Rhodotorula* species have been frequently isolated from the sediments of the Suwanee River estuary (Lazarus & Koburger, 1974) and the polluted fresh water of the River Calumet and Miami (Ahearn et al., 1968; Woollett & Hedrick, 1970). However, an exception appears to be *Trichosporon pullulans*. Its isolation from other estuaries has not been reported yet, except for its recovery with low incidence from the fresh water of the River Calumet (Wollett & Hedrick, 1970), suggesting that the Yeong San River estuary harboured a variety of species, reflecting the geographical feature and the distinctively seasonal changes in Korea.

The number of total bacteria at Station B, C and D (Fig. 3) increased in summer but the corresponding increase in the number of yeasts did not occur (Fig. 3). Such relatively low portion of yeasts to total bacteria might depend on anti-yeast action by bacterial and other sources (Ahearn, 1973; Buch & Meyers, 1965; Norkrans, 1966a), a long generation time of yeast compared with that of bacteria, and a relative reduction in number of yeast to bacteria by dilution factor, caused by heavy rainfall during this period. In contrast to this, the increase in number of yeast according to the thermo-gradient of water temperature (Fig. 2) was observed at Station A, suggesting that the yeasts in sea water could be chracterized by water temperature. This has been reported by Seshadri and Sieburth (1975), who pointed out that the yeasts in sea water followed a trend with maxima in summer and with minima in winter. On the other hand, total bacteria showed a general tendency to decrease with the increasing salinity from Station D to B, with a rapid decline in Station A. The observed decrease may be due to bactericidal action of sea water (Choe & Kim, 1970; Gerba & Mcleod, 1976; Pramer *et al.*, 1963) and partly due to the dilution of terrestrial bacteria by inflowing fresh water.

When comparison with the seasonal variation of yeasts from this estuary, it is noteworthy that *Kloeckera*, mainly *K. apiculata*, which are known to be usually associated with ripe grapes (Hansen, 1881), did not occur during the period of March September but were recovered mainly during autumn, suggesting that this species could be originated from the soil in similar areas and reflect the seasonal distribution. Similarly, a significant number of *Torulopsis* was isolated during the period of spring and autumn. Most of *Torulopsis* have been reported to occur at high incidence on such diverse substrates as flowers, slim-fluxes and fruits (Norkrans, 1966b; Seshadri & Sieburth, 1975; Hansen, 1881), indicating that the seasonal pattern of this yeast in this water might be related to that of the terrestrial counterparts. On the other hand, *Cryptococcus infirmominiatus* and *Cryp. albidius* were isolated mainly during the period of early spring-winter. These yeasts are known to be psychrophilic (Norkrans, 1966b), which have been reported to occur in Arctic and Antarctic region (Fell & Phaff, 1968; Goto *et al.*, 1969; Kobayasi *et al.*, 1967). Their distribution restricted to this cold season might be due to good growth at low temperature. A high density and frequency of *Candida*, mainly consisting of *C. solani*, *C. vartiovaarai* and *C. lambica* was observed in spring and winter during which a heavy turbulance of water was caused by a strong north wind against incoming sea water. The increase in number of these species appears to be caused by yeast flora on sediments. Similar results have been reported by Woollett and Koburger (1970), who showed that these yeasts were the most common species on sediment of Suwanee River. The distribution of *Debaryomyces hansenii* seems to be associated with algal blooms since this yeast was abundant in spring as well as in early autumn. Similar results were reported by Meyers *el al.*, (Meyers et al., 1970; Meyers et al., 1967b), who showed that *D. hansenii* occurred in association with algal bloom in North Sea. However, further investigation for this field will be required since we have not any data available to specifically correlate the number and/or yeast species with algal flora.

The comparison of yeasts isolated from four

stations showed that the density of yeast, its flora and species composition were characteristic of each stations: *Debaryomyces hansenii* and *Rhodotorula glutinis*, which was salt tolerant and abundant in marine and estuarine water (Ahearn *et al.*, 1968; Chun & Park, 1976; Genelius & Norkrans, 1970; Meyers *et al.*, 1967a, b; Moss & Morris, 1968; Norkrans, 1966a; Phaff *et al.* 1966; Roth *et al.*, 1964; Taysi & Uden, 1964), was most frequently isolated from Station A (Fig. 6), whereas *Rh. rubra*, more frequently isolated from rural region rather than from urban areas (Spencer *et al.*, 1970; Ahearn, 1973), occurred at high incidence in Station B than in other stations, reflecting the rural region. On the one hand, *Candida lambica*, *C. gelida* and *C. vartiovaarai* were abundant yeasts at Station C. Particularly, *Trichosporon pullulans* was significantly isolated from this station, indicating the association of this yeast with plant materials (Cooke *et al.*, 1970; Ahearn, 1973) since in vitro study showed that some strains of this yeast had a strong cellobiose assimilation as well as a cellulolytic ability (Chun & Park, 1979; Dennis, 1972). Furthermore, the apparent occurrence of *Torulopsis* and *Sporobolomyces roseus*, associated with plant materials or cereals, indicates that this station was affected by a significant inputs of water containing plant remnants. On the other hand, the yeast population at Station D was much larger than any other stations. The presence and persistence of strongly fermentative yeast and animal-associated yeasts was suggestive of a highly eutrophi cated water body.

Animal-associated yeasts were obtained mainly in summer as well as in Station D, indicating the importance of water temperature (Kawqakita & Uden, 1965) and salinity for their distribution. The number of these yeasts decreased as salinity increased (Table 6) while *Debaryomyces hansenii* and *Rhodotorula glutinis* increased (Fig.6 and 8). This might be explained at their differential survival in the water of different range of salinity (Table 5). The decrease of *D. hansenii* and *Rh. glutinis* with the remoteness of distance from sea water suggests that tidal current might bring these species into upstream (Table 4). Similar results were reported by Taysi and Uden (1964) and Norkrans (1966a), who suggested that the predominant marine yeasts in an estuarine waters might be originated from sea water.

The distributional pattern of fermentative and pseudomycelium yeasts in fresh water have been observed in relation to the concentration of domestic wastes (Woollet & Hedrick, 1970), but salinity gradient factor has not been applied to the yeast distribution in river-dominated estuary. In this study, the number of fermentative and pseudomycelium-producing yeasts increased with decreasing salinity whereas nitrate-utilizing yeasts showed an opposite trend, suggesting that salinity gradient can be used as a feasible detector for the distributional pattern of yeast in estuarine habitat.

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摘 要

1976年 3月부터 1977年 2月까지 滿 12個月동안 榮山江 汽水域 39個 地所로부터 採水한 156個 water sample로서 酵母의 季節的 分布와 鹽度包配에 따른 이들 酵母의 分布型을 調査하였다. 酵母의 全體 平均値는 100ml當 52에서 487個의 生菌數를 보여주었고 가장많은 個體數는 春季에 있었고 가장 적은 個體數는 夏季에 있었다. 14屬 83種에 해당하는 933個의 酵母와 한種의 酵母樣 곰팡이가 分離되었는데 其中 *Candida*가 29%, *Debaryomyces*가 17.3%, *Rhodotorula*가 16%, *Saccharomyces*가 14%이었고 나머지 다른 屬들은 10% 以內이었다. *Debaryomyces hansenii* 와 *Rhodotorula glutinis*가 全水域에서는 물론 年中 優點種이었고 총 好氣性細菌集團의 크기, 陸水流入量, 그리고 몇가지 지형적 기후적 요소들이 汽水환경에서 酵母의 季節的 分布는 물론 種의 組成을 反映하는것 같다. 酵母의

平均數, 種의 多樣性 特히 醱酵性 및 爲菌系形成 母酵들의 數는 鹽度가 감소함에 따라 增加한 反面 질산염 利用酵母들은 위와 相反된 경향을 보여주었는데 이는 汽水域에서의 酵母의 分布型을 認識하는데 鹽度勾配가 바람직한 看破 요소로서 利用 될 수 있음을 시사하고 있다.

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