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韓國 木浦 沿海岸海水內의 酵母 分布 및 集團密度와 海藻類上의 酵母相

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The Distribution and Population Densities of Yeasts and their Epiflora on Seaweeds in Inshore Waters of Mok-po, Korea

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ABSTRACT

The distribution and population densities of yeasts including their epiflora on seaweeds were estimated over two months in inshore waters of Mok-po, Korea. Nine species of 48 isolates were obtained from this area. *Rhodotorula*, *Torulopsis* and *Debaryomyces* that are widespread in estuaries were of common occurrence in this water body. The highest counts belonged to *Torulopsis candida* which was predominated in temperate estuarine zone. The distribution of seaweeds seems to be correlated with the population densities of yeasts. Two species of marine algae harbored yeasts during May and July, 1975. Among seaweeds isolates, the highest numbers were of the species *Rhodotorula glutinis* var. *glutinis*. A dominant colonization of this strain on Chlorophyceae and Phaeophyta and a role of water temperature for the growth of yeasts are discussed.

INTRODUCTION

Since the report of Kriss and Novozhilova (1954), a wealthy information had been compiled on yeasts and yeast-like organisms in the natural body of water, sediments, and various diverse substrates. Fell and van Uden (1963) revealed that population densities increased with the degree of admixture with polluted river water and that animal-associated yeasts (*Candida tropicalis*, *C. krusei*, *C. albicans* and *Torulopsis grabrata*) were numerous in polluted area of Biscayne Bay. In temperate estuaries of river Tagos and Sados, and adjacent Atlantic Ocean, Portugal, the exclusively or predominated isolates were *Candida intermedia*, *C. lambica*, *C. silvicola* and *Torulopsis candida*, and water temperature was a selective factor for yeast growth (Taysi and van Uden, 1964). Yeast species in inshore waters largely consisted of *Candida*, *Rhodotorula* and *Debaryomyces* (van Uden and Fell, 1968 ; Hoppe, 1972).

The intertidal algae have been reported to exude considerable amounts of their photosynthetates(Moebus *et al.*, 1974). The seaweeds could, therefore, serve as an important reservoir for yeasts which are commonly encountered in inshore waters. There are reports on yeast colonization on living seaweeds(Seshardri and Sieburth, 1971, 1975; Seshardri, 1972; Chun, 1974). The purpose of our study has been to investigate the population densities of the yeasts encountered in inshore waters and their epiflora of seaweeds in Mok-po, Korea.

MATERIALS AND METHODS

Sea-water; Surface sea-water samples were collected in sterile 1-liter bottles at eight stations. One station was in the estuary of the River Yeoung Sang and the others in the littoral zone of the Mok-po (Fig. 1). Sample collection was made two times between May and July, 1975. The methods of isolation referred to by Ahearn *et al.* (1968) and Fell and van Uden (1963) were used.

Seaweeds; Algae were collected in intertidal zone at Station 2. The methods of Seshardri and Sieburth (1971) were referenced except for isolation medium. In this study, Medium II of Ahearn *et al.* (1968) was employed routinely for isolation of yeasts from living seaweeds. Counting and identification of yeasts were made references to Seshardri and Sieburth (1971) and Lodder's The Yeasts (1970).

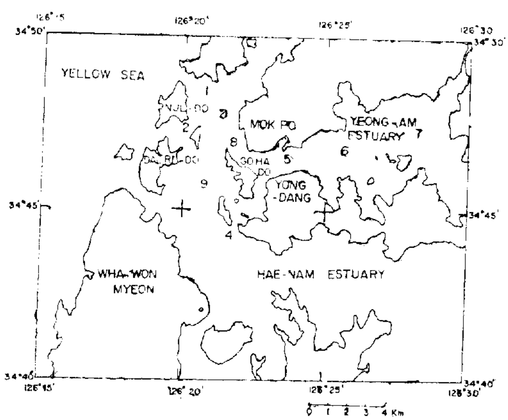

Fig. 1. Sampling locales

Survival test: The ability of *Rhodotorula* and *Debaryomyces* to survive in 5% algal extract was assessed. Cells were grown under constant agitation for 24 hr at 24—28°C in YM broth. The cells were harvested, washed in distilled water and suspended in 300m*l* of filter-sterilized 5% algal extract. Cell suspension were mix-cultured at 25--28°C with constant agitation and regularly examined for viability for periods up to 7 weeks by standard membrane filter procedure.

RESULTS

Nine species of 48 isolates were obtained from inshore waters of Mok-po. Thirty-two water samples contained from 14—554 viable yeasts per 100m*l*; the highest counts came from Station 1, were the seaweeds were more abundant and the inflow of sewages was greater than any other station, while the lowest was obtained from Station 4, where the pollution was relatively lightier and fewer algae inhibited this area than other station(Table 1). About 8°C rise of water temperature made viable numbers some 3-fold increase (Table 1).

Table 1. Number of viable yeast species per 100m*l* water samples

Date of sampling	Mean temperature (°C)	Stations								Average
		1	2	3	4	5	6	7	8	
12 May '75	14.8	82	124	46	10	22	14	14	16	32.8
20 July '75	23.0	554	110	50	20	58	28	14	328	116.2

Table 2. Colony counts of yeast species in 100m*l* water samples at each station

Species	Stations							
	1	2	3	4	5	6	7	8
Rhodotorula glutinis var. *glutinis*	64	8	28	4	18	6	8	2
Debaryomyces hansenii	134	60	36	6	10	10	10	2
Torulopsis candida	430	166	6	20	48	30	10	330
T. apis						6		
T. spharica					2			
T. grabrata								1
T. sp.								8
Candida guilliermondii var. *guilliemondii*	8	22						1
C. scottii					2			
C. lipolytica var. *lipolytica*			4					
Numbers of species isolated	4	3	4	3	4	4	3	6

Table 3. Percentage frequency of yeast species in inshore waters of Mok-po

Species	Percentage frequency* (32 water samples) %
Rhodotorula glutinis var. *glutinis*	75
Debaryomyces hansenii	75
Torulopsis candida	69
Candida guilliermondii var. *guilliermondii*	12
Torulopsis apis	6
T. spharica	ditto
Candida lipolytica var. *lipolytica*	ditto
Torulopsis grabrata	ditto
Candida scottii	ditto

* Based on incidence of occurrence in total numbers of samples

From all stations, 9 species were obtained (Table 2). The yeasts most uniformly distributed among eight stations were of the species *Debaryomyces hansenii*, *Rhodotorula glutinis* var. *glutinis* and *Torulopsis candida* (Table 2). Among these isolates, the highest counts were of *Torulopsis candida*, while the fewest species was *Torulopsis grabrata* (Table 2). Other next fewer were of the genera *Candida* and *Torulopsis*. Of these, *Candida scottii* and *Torulopsis spharica* were rare species in temperate sea waters.

Yeasts from inshore waters of Mok-po are listed in Table 3, in order of their decreasing percentage frequency. The species isolated at<10% have included *Torulopsis apis*, *T. spharica*, *T. grabrata*, *Candida lipolytica* var. *lipolytica* and *C. scottii*. All of these strains were isolated from the mainstream of estuary river mouth.

The data from a survey of the yeast populations on seaweeds and their surrounding waters at Station 2, Mok-po inshore, are summarized in Table 4. All two species of marine algae habored yeasts during May and July, 1975. Among yeast

Table 4. A survey of yeasts on seaweeds and in their surrounding waters

Source	Species	No. of yeasts			
		per gram algae (wet weight)		100ml surrounding water	
		May	July	May	July
Ulva pertusa	*Rhodotorula glutinis* var. *glutinis*	7040	74160	—	80
	Debaryomyces hansenii	20	640	200	400
	Torulopsis candida	—	—	1040	720
Undaria pinnatifida	*Rhodotorula glutinis* var. *glutinis*	23280	120	—	—
	Torulopsis candida	—	200	—	40
	T. sp.	—	—	80	—

isolates, the highest counts were of the species *Rhodotorula glutinis* var. *glutinis*. The next order was *Debaryomyces hansenii*, while the lowest was *Torulopsis candida*. During this period, the population changes of *Rhodotorula* and *Debaryomyces* on *Ulva pertusa* paralleled those in the surrounding waters. When compared with that of *Ulva*, the former strain had a sharp decrease in its numbers on *Undaria pinnatifida* during July. The yeast numbers on the green alga *Ulva pertusa* increased to some ten folds per gram, while their counts on brown alga *Undaria pinnatifida* decreased to about two hundreds per gram.

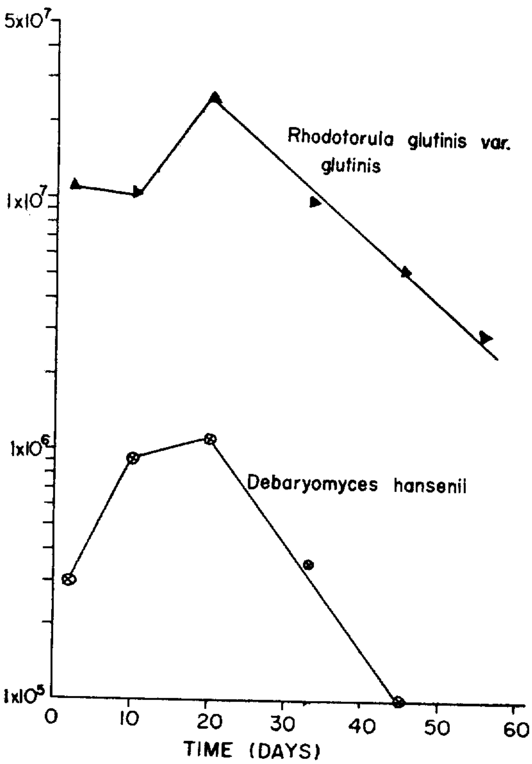

Fig. 2. Semilogarithmic survival curves of the yeast *Rhodotorula* and *Debaryomyces* in 5% algal extract.

A comparison of the yeasts *Rhodotorula glutinis* var. *glutinis* and *Debaryomyces hansenii*, which were predominantly isolated from seaweeds, was made with their survival in 5% algal extract, *Ulva pertusa* (Table 4). These strains showed a similar lag period, but *Rhodotorula* strain has higher numbers than *Debaryomyces* one, with longer survival of former than the latter (Fig. 2).

DISCUSSION

The species common in both river mouth and adjacent zone belonged to the genera *Debaryomyces* and *Rhodotorula*, known to be widespread in seas. This result was in agreement with that of Kriss and Novozhilova (1954), Fell and van Uden (1963) and Fell (1967). Benthic marine algae have been reported to exude as much as 30% of their photosynthesites during immersion (Khailov and Burlakova, 1969). The release during reimmersion of desiccated plants is, however, considerable (Moebus *et al.*, 1974). The highest counts of yeasts at Station 1 at Mok-po in Korea (Table 1) may be, therefore, due to greater amount of exudation of this materials from seaweeds abundant in this area than any other station. In contrast, the lowest came from Station 4 and their decrease is attributed to the increasing distance from coast and a low concentration of organic materials.

The apparent role of water temperature as a factor for the population growth of yeast was observed at this area. This observation confirms the findings of van Uden and Castelo Branco (1963) and Taysi and van Uden (1964). *Candida tropicalis*, *C. krusei* and *C. albicans* occurred in the digestive tract of man and other warm-blooded animal (van Uden and Farinha, 1958). *Torulopsis grabrata*, another intestinal yeast, has been isolated from the Indian Ocean (Bhat and Kachwalla, 1955), Lake Okeechobee in southern California (Ahearn *et al.*, 1968). Kowakita and van Uden (1965) have showed that the optimal temperature for yeast growth may be a limiting factor in the survival of animal associated-yeasts in temperate regions. The apparent absence of *C. krusei*, *C. albicans* and *C. tropicalis* at the inshore waters of Mok-po is particularly significant, since salinity and water temperature, *per se*, do not appear to be deleterious or restrictive to prevent their existence and also the inflow of sewages from adjacent urban or factories was considerably great. Taysi and van Uden (1964) have reported that the maximum temperature for the growth of *Candida tropicalis*, *C. krusei* and *Torulopsis grabrata* was 10°C higher than that of *T. candida*. In this study, the hundred folds increase in numbers of *T. candida* was obtained at the inshore of Mok-po, a temperature of about 8°C rising from 15°C in May to 23°C in July. If Taysi and van Uden (1964) suggested that the growth rate for this strain would be lowered at elevated temperature, one would expect that its numbers would be lower at this area than that of Tagos river mouth, but its growth rate was higher. In contrast, *Torulopsis grabrata*, though in few if at all, was found at lower value than its maximum growth temperature. The absence of animal associated-yeasts at Mok-po, as compared with their presences at Nam-hae (Chun, 1974), suggested to the authors that the combined action of other biological factors such as competitors, predation, anti-yeast materials and phages might well effect their suvival in this waters, although the reason for the discrepancy between this observation and those reports earlier is not presently known. Therefore, other factors mentioned above need closer analysis.

Seaweeds are the most potential habitat due to their rich content and variety of carbohydrates (Percival and McDowell, 1967). There are many reports on the abundant occurrence of yeasts on decomposing seaweeds (Madson *et al.*, 1923; Tomiyasu and Zenitain, 1956 a,b; Bunt, 1955; Suehiro, 1960, 1962; Hagen and Rose, 1961; Fell and van Uden, 1963; van Uden and Castelo Branco, 1963).

Seshardri and Sieburth (1971 and 1975) and Chun (1974) have reported that yeasts may be actually colonized on living seaweeds. However, phenolic materials, known to inhibit the growth for them are readily exuded from seaweeds under living conditions (Sieburth, 1969). By using Cholondny filtrations as a double-filter culture procedure, which reduced the inhibitory polyphenols (Seshardri and Sieburth, 1971), population densities of yeasts were increased for 2 or 3-folds. According to their report (1975), 95% of the 362 isolates were strains of *Candida*, while *Rhodotorula* from Chlorophyceae accounted for 3 to 35 percent of them. Therefore, there are some differences in yeast species between their observation and the authors', although the same method as Millipore filtration was employed. On the other hand, *Candida tropicalis*, *Rhodotorula glutinis* var. *glutinis* and *Trichosporon cutaneum* which were isolated from sewage and activated sludges, could metabolize inhibitory phenols (Nisaburo Nei, 1971). If this strain, *Rhodotorula*, would have more easily access to seaweeds surfaces through prior induction to these inhibitory polyphenols exuded from them, one would expect that population densities of this species would be more increased and that its survival period would be more extended than any other seaweed isolates. The exclusive domination of this strain on the surface of *Ulva* and *Undaria* and its greater survival value in this study suggest that such may be the case (Table 4 and Fig. 2).

Seshardri and Sieburth (1975) revealed that the population changes of yeasts in the water surrounding the seaweeds paralleled those on the plants and that their numbers had a general trend to follow the increasing and decreasing water temperature. To what exent *Rhodotorula* and *Debaryomyces* on *Ulva* coincide with the result of Seshardri and Sieburth (1975), but an exception appears to be a sharp decrease in population of former strain on *Undaria* during July, 1975. During this period, this plant was decomposing. Therefore, it might be stated that there might exist other factors except phenol substances. In this respect, much closer investigation will be needed in the future.

The authors are planning to conduct a study on this problem.

摘 要

韓國 木浦 沿岸 海水中의 酵母의 分布 및 集團密度와 海藻類上의 酵母相을 2個月間에 걸쳐 實施하였다. 48個의 分離種中 9種이 이 地域에서 分離되었는데 深水 地域에 널리 分布한 *Rhodotorula*, *Torulopsis* 그리고 *Debaryomyces*가 이 地域의 共通種이었으며 温帶深水帶에 優點種을 이루고 있는 *Torulopsis candida*가 個體數에 있어서 가장 많았다. 그리고 酵母의 集團密度와 海藻類의 分布와는 相關 關係가 存在한 것 같았다.

1975年 5月과 7月中 2種의 海藻類上에 酵母가 棲息했으며 海藻에서 分離한것 中 가장 個體數가 많았던 것은 *Rhodotorula glutinis* var. *glutinis*이었으며 이 菌種이 Chlorophyta와 Phaeophyta에 優勢하게 棲息한 것과 酵母의 生育에 水温의 役割에 關하여 考察하였다.

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