

# A Bacteriological Study on the Sea Waters and Oyster in Puk Man, Korea

Jong-Duck Choi and Woo-Geon Jeong\*

Department of Marine Food Science and Technology, Gyungsang National University, and Institute of Marine  
Science, Tong-yeong, 650-160, Korea

\*Department of Aquaculture, Gyungsang National University, Tong-yeong, 650-160, Korea

= 국문요약 =

## 북만의 해수 및 굴의 세균학적 특성

최종덕 · 정우건\*

경상대학교 수산가공학과, \*양식학과

북만 해수의 물리 화학적 및 미생물학적 특성과 북만에서 양식되고 있는 굴에 대한 세균학적 수질을 조사하여 수출용 패류생산지정 해역수질에 합당한가를 파악함과 동시에 지표세균의 조성, 병원성 세균 등을 조사한 결과를 요약하면 다음과 같다.

조사 기간중 북만 해수의 수온은 6.8℃ - 23.0℃, 투명도는 2.7 - 5.9 m, COD 1.55 - 2.12 mg/l, DO 6.3 - 10.1 mg/l, 용존질소 1.63 - 20.87 µg-at/l, 인산염 0.16 - 1.66 µg-at/l, Chlorophyll. a는 1.04 - 6.78 mg/m<sup>3</sup> 범위였으며 염분농도는 31.57 - 34.47‰였다.

북만 해수의 세균학적 수질은 통영항, 인평포, 범송포 및 북신만 안쪽의 일부 해역을 제외하고는 수출용 패류의 생산해역의 수질기준에 합당하였다.

대장균군의 최확수는 양식을 하지 않는 조사지점 1과 2를 제외하고 해수 100 ml 당 < 3.0 - 4,600의 범위였으며 230을 초과하는 시료의 비율은 10.7%였고, 분변계 대장균의 최확수는 < 3.0 - 1,100의 범위였으며 43을 초과하는 시료의 비율은 9.5%로 한계치 10%를 약간 상회하였다.

대장균군의 분류결과 *Escherichia coli*가 약 52%나 되어 오염원의 주류가 분변오염임을 알 수 있었다.

살모넬라, 시겔라, 콜레라균 등 수인성 병원세균은 검출되지 않았다.

병원성 비브리오균은 여름철인 6-8월 사이에는 시료의 9 - 22%에서 양성으로 나타났다.

굴 내에 생균수는  $2.0 \times 10^2$  -  $1.6 \times 10^3$  범위였고 대장균군의 최확수는 < 18 - 16,000, 중앙치는 176, 분변계 대장균은 < 18 - 2,400, 중앙치는 4.2로 조사되었다.

Key words : Oyster, Coliform group, Bacteriological criteria, *Vibrio vulnificus*

## INTRODUCTION

Puk Man is a coastal area located in the northwestern area of Tongyeong City in

Kyeongsangnam-Do with Pupsong-po and Puksin bay to the northeast, and coastal area of Saryangdo, Koseong and Charan bay to the west. On the coast in Puk Man locate fish, oyster and sea squirt growing area, etc., enclosed culture, and on the

nearby coastal area are culture farms of shellfish such as oyster, mussel, etc. and cageculture.

Sea area in Puksin bay to the north-east of Puk Man is a semi-enclosed bay with features of slow flow of sea waters retardation of exchange of sea waters, and showed over-nourishment in red, green or brown color when microbes inhabiting in this area reach to certain environment which is formed by mass inflow of municipal wastewater and organic materials inside the bay (Cho, 1993). In addition, this sea area has been reported as a over-nourishment area where mass death of aquaculture is generated, thus decomposition of microbes and false order are generated (Lee *et al.*, 1986), and of lost functions of aquaculture growing area (Choi, 1995). It has been concern of damage to aquaculture in the vicinity of this area due to deterioration of oceanic environment connected with Puksin bay resulted from generation of the red tides and low-oxygen water mass.

Enhanced sanitation management of required for shellfish such as oyster, mussels, ark shell and little-neck clams, which they inhabit in the coastal sea area which is highly subject to inflow of land water, wastewater from factories and other contaminants. It is significantly important to sanitary management of sea waters where shellfish inhabit in the viewpoint that shellfish produced in polluted sea areas cause enteropathy (Jensen, 1996; Sherwood, 1952), that shellfish swallow floating microbes and virus harmful to human bodies as well as polluted materials, which are easily accumulated in the shellfish, and that entire flesh of the shellfish including organs except shell is edible.

Though there are number of physical and chemical studies on culture farms and coastal sea waters in Korea (Choi, 1991; Choi *et al.*, 1980; Song and Park, 1991; Lee, 1993; McGinitie, 1941; Choi *et al.*, 1974; Yoo *et al.*, 1980; Kim *et al.*, 1969), it is hard to locate microbiological studies relevant to sanitation of shellfish at shellfish growing area.

Therefore, this study investigated physical and chemical oceanic environment, sanitary indicative

bacteria, waterborne infectious disease microbes, pathogenic vibrios, and number of coliform groups and viable cell counts in oysters in Puk Man connected with Puksin bay where the red tides frequent, and reviewed the results to determine if the water quality in this sea area is suitable for farming shellfish to export to marketplaces in America and EU.

## MATERIALS AND METHODS

### 1. Sea area of investigation and locations of water sampling

The sea area of the investigation locates in the northwestern area of Tongyeong City in Kyeongsangnam-Do. The author analyzed physiochemical properties of the sea waters and the sediments at nine water sampling stations in this area as Fig. 1 between October, 1994 and April, 1996, and performed bacteriological experiments on oysters farms here.

### 2. Methods

Temperature of the sea water, salinity, pH, transparency and level of dissolved oxygen were measured on-site, chemical oxygen demand and nitrite nitrogen, nitrate nitrogen, ammonium nitrogen, phosphate were measured pursuant to the guideline

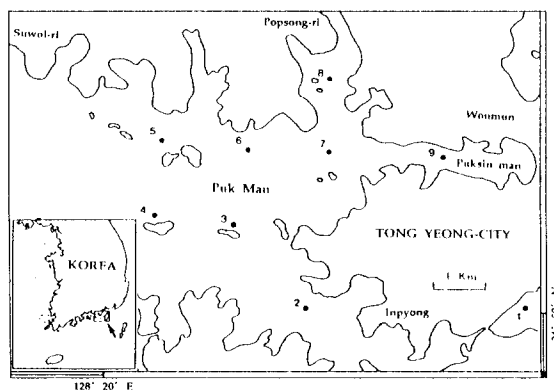


Fig. 1. The sampling stations in Pukman, Kyeongsangnam-Do, Korea.

of oceanic pollution and red tide investigation from the National Fisheries Research Institute. Pathogenic bacteriological analysis were pursuant to the bacteriological analytical manual from FDA in America (1992), and coliform group and fecal coliform were pursuant to the recommended procedures for the bacteriological examination of sea waters and shellfish in America (1962). Ignition loss of sediments was analyzed by dry ashing method, the chemical oxygen demand by  $KMnO_4$  method, and hydrogen sulfide by the gravimetric method respectively.

## RESULT AND DISCUSSION

### 1. Characteristics of sea waters and sediments in Puk Man

The measurement results of the average water temperature, salinity, transparency, DO, COD, nitrite nitrogen, nitrate nitrogen, ammonium nitrogen and phosphate are as per Table 1, and those of ignition loss of sediments, COD and hydrogen sulfide are as per the Table 2.

The water temperature slightly varied in months between 6.8 and 23.0°C, and those between July and October were over 20.0°C, which is sufficient condition for growing of pathogenic vibrios. Throughout the investigation period, the transparency was 2.7 to 5.9 m, COD 1.55 to 2.12 mg/l, DO 6.3 to 10.1 mg/l; nitrite nitrogen 0.05 to 2.56  $\mu\text{g-at/l}$ , nitrate nitrogen 0.43 to 11.20  $\mu\text{g-at/l}$ , ammonium nitrogen 1.15 to 7.11  $\mu\text{g-at/l}$ , phosphate 0.16 to 1.66  $\mu\text{g-at/l}$ , chl.-a 1.04 to 6.78  $\text{mg/m}^3$ , and salinity 31.57 to 34.47‰.

Table 1. Mean values of water quality in Puk Man, Kyeongsangnam-Do, Korea.

Date	Temp. (°C)	Trans. (m)	COD (mg/l)	DO (mg/l)	Sal. (‰)	Nutrients( $\mu\text{g-at/l}$ )				Chl. a ( $\text{mg/m}^3$ )	
						$\text{NO}_2\text{-N}$	$\text{NO}_3\text{-N}$	$\text{NH}_4\text{-N}$	$\text{PO}_4\text{-P}$		
1994	Oct.	20.8	2.5	1.81	8.0	32.66	0.49	4.14	5.82	0.48	3.56
	Nov.	17.0	3.8	1.57	9.0	33.28	0.47	3.47	1.15	0.26	2.14
	Dec.	11.4	4.5	2.12	8.7	33.80	0.63	8.70	5.68	0.72	-
1995	Jan.	7.6	5.7	2.12	9.1	34.34	0.39	4.59	1.97	0.53	5.01
	Feb.	8.1	5.9	2.05	10.1	34.09	0.25	2.73	3.05	0.31	1.32
	Mar.	10.2	3.4	1.98	9.2	34.05	0.25	3.70	3.57	0.38	1.04
	Apr.	13.6	4.1	1.40	8.5	33.44	0.08	0.43	1.57	0.42	1.44
	Jun.	19.4	3.4	1.71	7.7	33.88	0.05	1.49	4.54	0.23	4.40
	Aug.	23.0	2.7	2.11	6.0	33.25	0.22	1.65	1.18	0.32	5.53
	Sep.	21.1	3.2	2.15	6.8	31.57	0.21	1.18	1.57	0.31	2.71
	Oct.	20.8	5.3	1.93	6.3	31.71	0.35	1.09	2.41	0.16	5.09
	Nov.	14.6	5.3	2.09	8.7	33.19	2.56	9.60	7.11	1.66	1.73
Dec.	10.8	5.3	1.90	10.7	34.47	0.93	11.20	3.01	0.91	1.84	
1996	Jan.	7.6	4.2	2.11	8.6	33.85	0.41	3.38	3.63	0.54	6.78
	Feb.	6.8	4.6	1.80	9.2	34.20	0.61	3.65	3.21	0.48	1.80
	Apr.	10.8	3.3	1.55	9.2	34.18	0.29	1.13	3.92	0.31	2.44

Trans : Transparency, COD : Chemical Oxygen Demand

DO : Dissolved Oxygen, Chl. a : Chlorophyll a

**Table 2.** Mean value of environmental factors in the sediment of Puk Man, Kyeongsangnam-Do, Korea.

Date		COD (mg/l)	H <sub>2</sub> S (mg/l)	Ignition loss (%)
1994	Oct.	26.39	0.34	7.9
1995	Jan.	27.27	0.40	8.7
	Nov.	27.17	0.47	8.6
1996	Feb.	27.15	0.43	9.2
Over all		27.00	0.41	8.6

Chemical oxygen demand of sediments in the area showed high concentration of 26.39 to 27.27 mg/g exceeding 20.0 mg/g of the orcenic nutritional criteria (Fishery Resources Protection Association of Japan, 1973), and the ignition loss also showed relative high value between 7.9% and 9.2%.

## 2. Bacteriological water quality

### 1) Pollution status of sanitary indicative bacteria

The test results of coliform and fecal coliform groups in the sea waters in Puk Man are classified and contained in Table 3.

In the entire sea area the central value of pollution level of coliform group in 100 ml of MPN(most probable number) from sea waters was below 3.0 and the maximum value was 4,600, while central value of fecal coliform was below 3.0 and the maximum value was 1,100. From 168 samples excluding those from investigation station 1 (Tongyeong harbor) and 2 (Inpyeongpo) without shellfish growing area, 18 samples (10.7%) and 16 samples (9.5%) respectively exceeded standard number of coliform group of 230/100 ml and fecal coliform of 43/100 ml for approval of shellfish production sea areas for export. Though the values didn't exceed the central value (70) of the MPN, the samples slightly over 10% exceeded the microbiological water quality standard in the approved sea area. Upon classification of the samples into investigation stations, the area between Changku and Changkudo in the vicinity of the center of Puk Man showed fair microbiological

**Table 3.** MPN's of total coliform and fecal coliform of sea water in Puk Man, Kyeongsangnam-Do, Korea (1994~1996).

Station	Coliform group MPN/100 ml			Fecal coliform MPN/100 ml			No. of Samples
	Median	Range	%, >230	Median	Range	%, >43	
1	490	93~4,600	83.3	75	23~1,100	87.5	24
2	49	24~2,400	25.0	<3.0	<3.0~460	16.7	24
3	<3.0	<3.0~75	8.3	<3.0	<3.0~240	12.5	24
4	<3.0	<3.0~43	-	<3.0	<3.0~23	-	24
5	<3.0	<3.0~23	-	<3.0	<3.0~23	-	24
6	<3.0	<3.0~23	-	<3.0	<3.0~23	-	24
7	<3.0	<3.0~460	8.3	<3.0	<3.0~150	8.3	24
8	43	21~2,400	25.0	<3.0	<3.0~460	20.8	24
9	150	93~2,400	29.2	23	<3.0~1,100	25.0	24
over all	<3.0	<3.0~4,600	20.3	<3.0	<3.0~1,100	19.0	216

\* Stations are same as indicated in Fig. 1

water quality. On the contrary, station 1 at Tongyeong harbor and station 2 in front of Inpyeongpo exceeded central values of coliform group (230) and fecal coliform (70) respectively. And sampling station 8 and 9 showed the central value of the MPN for coliform group and fecal coliform smaller than 70, however, were identified improper for approved sea areas, since 13 samples (27%) of 48 samples exceeded 230 per 100 ml in the MPN of coliform group and 43 (22.9%) of 48 did the standard threshold of 10%.

**2) Composition of coliform bacteria**

Understanding composition of the pollution indicative bacteria detected from sea waters in Puk Man enables to estimate pollution feasibility of pathogenic bacteria such as *Salmonella* sp., *Shigella* sp., etc.

Therefore, the results of classification by the IMViC test of 98 colonies which were suspected as coliforms after streak culture on EMB agar plates from the positive test tubes during the coliform group confirm test are as per Table 4.

Out of the 98 colonies, *Escherichia coli* group recorded 52.2% (52 strains), the highest value, *Klebsiella aerogenes* group 9.2% (9 strains), and

*Citrobacter freundii* group 3.1% (3 strains). Meanwhile, the fact that *Escherichia coli*, the typical bacteria derived from organs of warm-blooded animals occupied 45.9% indicates significant effects of fecal contamination by human beings and livestock.

**3) Detection of pathogenic bacteria**

A number of studies indicates frequent detection of pathogenic vibrios in coastal sea waters in Korea particularly in summer seasons (Chang *et al.*, 1986; Chang *et al.*, 1996; Kim *et al.*, 1997a; 1997b). Accordingly, the author investigated the distribution of pathogenic vibrios and other pathogenic bacteria in the summer season (June, July and August) in 1995, and the results are as per Fig. 2.

*Salmonella* sp, *Shigella* sp. (data not shown) and *Vibrio cholerae* were not detected from sea waters in Puk Man. However, *V. parahaemolyticus*, *V. vulnificus* and *V. minicus* were detected in the range of 9 to 22%, and, particularly, *V. vulnificus* of septicaemia vibrios reached 15% in August, which invites attention.

**3. Viable cell counts and coliform group in oysters**

The monthly number of viable cell counts and

**Table 4.** Classification of Coliform bacteria isolated from sea water at Puk Man by IMViC reaction.

Coliform types	Composition (%)	No. of strains/ No. of tested strains
<i>Escherichia coli</i> group		
variety i	45.9	45/98
variety ii	5.1	5/98
variety iii	2.0	2/98
<i>Citrobacter freundii</i> group		
variety i	3.1	3/98
<i>Klebsiella aerogenes</i> group		
variety i	5.1	5/98
variety ii	2.0	2/98
variety iii	1.0	1/98
etc.	35.8	35/98

coliform group in oysters cultured in Puk Man as per Table 5.

MPN of coliform group showed minimum < 18, maximum 16,000 and average 176, while fecal coliform was <18 to 2,400 and average 4.2. The values were low in January, February and March, and high in June, July and August. From these results, the author estimated relationship with temperature of sea water. The monthly changes in number of viable cell counts in oysters of the culture farms in Puk Man are as per Fig. 3.

January was the month of least changes in number of live microbes with value of  $2.0 \times 10^2$ , while June and July were those of higher changes with value of  $1.6 \times 10^3$ . These results also indicate relationship with temperature of sea water.

### SUMMARY

A bacteriological study on the sea waters and oyster in Puk Man was conducted to evaluate

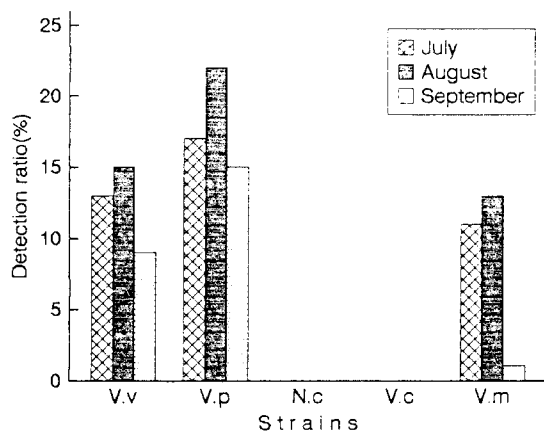


Fig. 2. Detection ratio of pathogenic vibrios from sea waters in Puk Man, Kyeongsangnam-Do, Korea (1995).

V.v : *Vibrio vulnificus*

V.c : *Vibrio cholerae*

V.p : *Vibrio parahaemolyticus*

V.m : *Vibrio mimicus*

N.c : NAG vibrio

sanitary conditions of the bay and compliance of waters with the recommended bacteriological criteria for the designated area of shellfish cultivation.

The samples were collected at 9 sampling stations established once a month from October 1994 to April 1996.

During the study period, temperature ranged from 6.8 to 23.0°C, transparency from 2.7 to 5.9 m, chemical oxygen demand from 1.55 to 2.12 mg/l, dissolved oxygen demand from 6.3 to 10.1 mg/l, dissolved nitrogen from 1.63 to 20.87, phosphate from 0.16 to 1.66 µg-at/l, chlorophyll a from 1.04 to 6.78 mg/m<sup>3</sup>, respectively.

The coliform group, fecal coliform, classification of coliform group with IMViC reactions and pathogenic vibrios were analyzed. MPNs of coliform group and fecal coliform in sea waters were ranged from < 18 - 4,600/100 ml and < 18 - 1,100/100 ml, respectively. MPNs of coliform group and fecal coliform of oysters were ranged from < 18 - 16,000/100 ml and < 10 - 2,400/100 ml, respectively. The viable cell counts in oysters ranged from  $2.0 \times 10^2$  to  $1.6 \times 10^3$ .

The bacteriological criteria of sea waters in shellfish growing in sampling areas should be less than 70 per 100 ml of sea waters for median value of coliform MPN, and below 10% the samples which contain more than 230 for coliform MPN or more than 43 for fecal coliform MPN. The sea waters

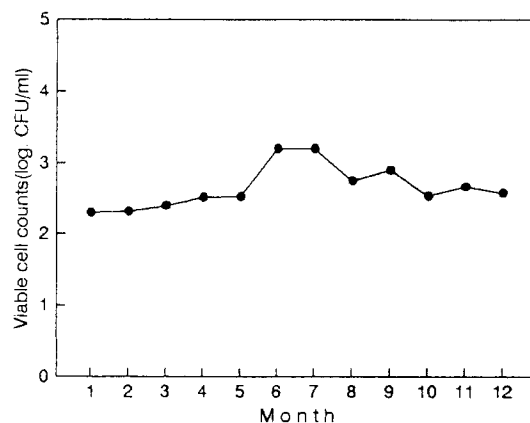


Fig. 3. Monthly variation of viable cell counts (cell forming unit) in Puk Man, Korea (1995).

**Table 5.** Bacteriological examination of oyster in Puk Man, Gyung-Nam, Korea(1995)

Data	Coliform group		Fecal coliform		Plate count ( $\times 10^2$ /ml)	No. of samples
	MPN/100ml		MPN/100 ml			
	Range	Median	Range	Median		
Jan.	<18-230	62	<18-45	<18	2.0	9
Feb.	20-170	61	<18-68	<18	2.1	9
Mar.	<18- 78	20	<18-45	<18	2.5	9
Apr.	<18-1,300	140	<18-230	<18	3.3	8
May.	68-490	212	<18-45	<18	3.4	8
Jun.	3,500-16,000	5,400	45-2,400	330	15.9	8
Jul.	230-9,200	1,300	<18-2,400	78	15.5	8
Aug.	130-3,500	490	18-220	45	5.6	8
Sep.	230-3,500	515	20-1,300	269	7.9	6
Oct.	<18-170	62	<18-20	<18	3.5	8
Nov.	<18-1,300	73	<18-20	<18	4.7	8
Dec.	330-790	490	20-330	45	3.8	9
Over all	<18-16,000	176	<18-2,400	<18	5.9	98

from 7 sampling stations were complied water coliform criteria recommended for designated shellfish growing areas.

During the study period, infectious bacteria such as *Vibrio cholerae*, *Salmonella* sp. and *Shigella* sp. were not detected from the samples, but detection ratios of *Vibrio parahaemolyticus* and *Vibrio vulnificus* were 9 - 15% in summer months.

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