PLANKTON STUDIES IN A MANGROVE ENVIRONMENT

III. BACTERIOLOGICAL ANALYSIS OF WATERS IN CANANÉIA

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SYNOPSIS

Waters of the mangrove swamp at Cananéia were analysed bacteriologically aiming the determination of the degree of bacterial contamination.

Aerobic counts varied significantly by season, tide and station with the highest of 13,000 and the lowest of 21/ml while coliform counts showed significant variation only by station with the highest of 1,600 and the lowest of 3 per ml.

Pseudomonas-Achromobacter group comprised the highest percentage of the total flora at the level of around 60%. The biochemical characteristic of the strains of this group are more saccharolytic than proteolytic, certainly due to the great number of diatoms present.

INTRODUCTION

As a link which joins the inorganic and organic phases of the chemical cycle in the sea, bacteria play an important role in the nutritional relationship of marine organisms, hence in the productivity of the water (Waksman 1934; ZoBell 1959a, b; Sverdrup *et al.* 1960). However, little is known on the matter at present, especially about mangrove swamp waters. On the other hand, since the inshore waters can be utilized for mollusc cultures, sewage pollution arises as an important problem to be studied from the view-point of ensuring quality and hygiene of the products (Fieger & Novak, 1961).

Previous papers (Teixeira & Kutner 1963; Teixeira, Tundisi & Kutner 1965) discussed in general some aspects of the bacterioplankton in Cananéia. The present paper deals, more in detail with the quantitative as well as qualitative analysis of the mangrove swamp waters.

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MATERIAL AND METHODS

Water samples were collected at Cananéia at three stations (Aroeira, Argolão and Ariri; for the exact position see article II of this series), four times, namely in January, April, July and October, 1962. Sampling of sub-surface layer was carried on with a 8-litre stainless steel plankton sampler. No sterilization was made but care was taken to handle it as clean as possible to avoid secondary contamination. Physical and chemical data of the samples at the collecting points are shown in Table I (see also article II of this series). Platings were made on the same day of sampling.

Total aerobic counts on meat-peptone-agar with 0.8% salt (Difco) and coliform counts on violet-red-bile-agar (Difco) were performed. Incubation time was 4 days for the former and 2 days for the latter, at room temperature of about 20-30°C through the year. In both cases, electric power was not available.

Further floral analysis of microorganisms was made on the samples by isolating strains from the counting plates of total aerobic counts and after a purification process, by observing the colony characteristics, and by mean of morphological studies, reactions to triple sugar iron agar (Difco), arrangement of flagella and resistance to 2.5 IU penicillin. Finally the microorganisms were identified as: *Enterobacteriaceae, Aeromonas, Pseudomonas/Vibrio, Achromobacter/Alkaligenes, Flavobacterium, Micrococcus, Bacillus, Corynebacterium* and *Actinomycetes*. Some strains of *Pseudomonas* and *Achromobacter* group were submitted to further biochemical tests (Watanabe, 1960).

The results were treated statistically by the F test, regarding the whole design as 2×4 arrangement (tides $\times$ seasons) of 3×2 two way layout (stations $\times$ depths).

RESULTS

The bacterial counts are given (Table II) as well as the results of F test applied on the data (Table III).

Total aerobic counts varied significantly due to tide and season at 1% level and by station at 5% level. Thus it can be said that: 1) the bacteria were more abundant at low than at high tide though this varies with the position; 2) seasonally they were at their highest in January-April and lowest in July-October, and 3) that smaller but still significant differences were found among the stations with lowest (Ariri) or highest counts (Aroeira) when we calculated the means by excluding the extremely high counts of ten thousands at low tide on April 3, sampled during heavy rain.

TABLE I — Physical and chemical data of the stations at sampling dates

STATION	TIDE	DEPTH m	S ‰				T °C				pH			
			20/1/62	5/4/62	19/7/62	4/10/62	20/1/62	5/4/62	19/7/62	4/10/62	20/1/62	5/4/62	19/7/62	4/10/62
Aroeira	low	0.0	3.42	1.58	12.15	8.26	25.12	26.30	18.98	22.12	—	—	—	7.55
		3.0	4.20	1.16	12.02	7.94	25.10	26.13	19.30	22.10	—	—	—	—
	high	0.0	3.60	1.84	8.13	5.68	25.80	26.70	19.09	22.40	—	7.20	—	7.80
		3.0	3.57	7.07	14.00	5.91	25.40	26.65	19.08	22.40	—	7.45	—	—
			21/1/62	2/4/62	16/7/62	1/10/62	21/1/62	2/4/62	16/7/62	1/10/62	21/1/62	2/4/62	—	1/10/62
Argolão	low	0,0	—	21.10	26.16	22.10	25.20	27.40	18.92	20.78	—	7.85	—	7.95
		10.0	21.49	27.43	27.38	22.86	25.10	27.30	19.00	18.85	—	8.20	—	—
	high	0.0	21.65	26.61	30.24	23.07	26.02	27.88	19.10	21.10	—	8.12	—	8.00
		10.0	27.61	30.59	30.94	27.71	24.78	27.18	19.10	20.53	—	8.15	—	—
			19/1/62	3/4/62	18/7/62	2/10/62	19/1/62	3/4/62	18/7/62	2/10/62	19/1/62	3/4/62	—	2/10/62
Ariri	low	0.0	23.35	23.08	28.20	24.79	24.65	27.70	18.95	21.18	—	7.80	—	8.10
		10.0	26.24	31.11	28.65	25.47	25.80	26.70	18.92	21.00	—	8.10	—	—
	high	0.0	24.51	27.36	29.14	25.64	25.18	26.40	19.31	21.20	—	7.98	—	7.90
		10.0	28.59	31.13	30.43	26.66	23.80	26.30	19.00	20.95	—	8.15	—	—

TABLE II — Total aerobic and coliform counts by tide, season, station and counts of inshore waters per ml of water

Date	Station	Sampling depth (m)	High tide		Low tide	
			Aerobic mean	Coli mean	Aerobic mean	Coli mean
20/1	Aroeira	0 3	3,600 12,000	96 1,600	7,400 9,200	160 240
21/1	Argolão	0 10	110 420	11 12	590 13,000	16 260
19/1	Ariri	0 10	100 150	15 8	320 600	26 8
5/4	Aroeira	0 3	1,200 250	40 20	1,600 1,100	400 80
2/4	Argolão	0 10	1,200 1,600	11 36	850 1,300	10 15
3/4	Ariri	0 10	1,100 570	81 6	74,000* 34,000*	18 80
19/7	Aroeira	0 3	860 960	13 21	720 710	13 52
16/7	Argolão	0 10	21 36	12 25	77 1,003	8 42
18/7	Ariri	0 10	130 230	12 73	170 320	10 15
4/10	Aroeira	0 3	240 330	52 69	540 350	120 230
1/10	Argolão	0 10	150 200	32 24	320 640	55 59
2/10	Ariri	0 10	96 150	3 8	400 380	23 7

* Unusually high due to heavy rain at the time of sampling.

TABLE III — Analysis of variance of the results shown in Table II. (The treatment was made by transferring into logarithmic numbers)

Variance due to	DF	Total aerobic			Coliform		
		SS	MS	F	SS	MS	F
Tide	1	2.75	2.75	9.49*	0.36	0.36	1.63
Season	3	8.38	2.79	9.62*	1.12	0.37	1.68
Station	2	2.58	1.29	4.45**	5.42	2.71	11.23
Depth	1	0.31	0.31	1.04	0.19	0.19	0.86
Error	40	11.56	0.29	—	8.79	0.22	—
Total	47	25.58	—	—	15.88	—	—

* Significant at 1% level.
** Significant at 5% level.

TABLE IV — Generic distribution of bacterial flora of surface water by season and by station shown as percentages of the total colonies tested

	Sampling month	January			April		July			October		
	Station	Aroei-ra	Argo-lão	Ariri	Aroei-ra	Argo-lão	Aroei-ra	Argo-lão	Ariri	Aroei-ra	Argo-lão	Mean
HIGH TIDE	Total nº of colonies	—	18	9	6	12	10	12	9	7	6	89
	Enterobacteriaceae	—	0	0	0	16.6	0	0	0	0	0	2.4
	Pseudomonas/Vibrio	—	55.5	66.6	49.9	33.3	10	33.3	22.2	85.6	16.6	41.4
	Aeromonas	—	5.5	11.1	16.6	8.3	0	0	0	0	0	4.4
	Achromobacter/Alkaligenes	—	27.7	0	0	41.6	0	24.9	11.1	0	19.9	19.0
	Flavobacterium	—	0	0	0	0	10	0	0	0	0	1.1
	Total gram negative	—	88.7	77.7	66.5	99.8	20	58.2	33.3	85.6	66.5	68.3
	Bacillus	—	11.1	11.1	33.3	0	10	33.3	0	0	0	11.2
	Micrococcus	—	0	0	0	0	40	0	11.1	0	0	5.6
	Corynebacterium	—	0	0	0	0	10	8.3	44.4	14.2	0	7.8
	Actinomycetes	—	0	11.1	0	0	10	0	11.1	0	0	3.3
	×	—	0	0	0	0	10	0	0	0	33.3	3.3
	Total gram positive	—	11.1	22.2	33.3	0	80	41.6	66.6	14.2	33.3	31.5
LOW TIDE	Total nº of colonies	11	—	12	7	11	18	19	14	9	10	111
	Enterobacteriaceae	0	—	16.6	28.5	18.1	11.0	0	7.1	0	0	8.1
	Pseudomonas/Vibrio	36.3	—	83.3	57.1	72.7	22.2	5.2	28.5	55.5	40	39.6
	Aeromonas	18.1	—	0	0	0	0	0	0	0	0	1.8
	Achromobacter/Alkaligenes	9.0	—	0	14.2	0	0	42.0	28.5	22.2	40	18.0
	Flavobacterium	9.0	—	0	0	0	33.3	0	0	0	0	6.3
	Total gram negative	72.4	—	99.9	99.8	90.8	66.5	47.2	64.1	77.7	80	73.8
	Bacillus	0	—	0	0	9.0	5.5	52.6	7.1	11.1	0	12.6
	Micrococcus	9.0	—	0	0	0	16.6	0	0	0	20	5.4
	Corynebacterium	0	—	0	0	0	11.0	0	21.3	11.1	0	5.4
	Actinomycetes	18.1	—	0	0	0	0	0	0	0	0	1.9
	×	0	—	0	0	0	0	0	7.1	0	0	0.9
	Total gram positive	27.1	—	0	0	9.0	33.1	52.6	35.5	22.2	20	26.2

× = Chlamydobacteriales yeast and nanoplanktonic organisms grown on the culture medium.
— = Impossible to analyse due to contamination.

Coliform counts showed significant variation only by station at 1% level, highest at Aroeira, followed by Argolão and Ariri.

Table IV gives the generic distribution of the bacterial flora of surface water. *Pseudomonas* and *Achromobacter* were the two of the highest in their percentages all through the year comprising about 40% and 20% respectively of the total flora, followed by *Corynebacterium* and *Micrococcus* with equal contribution of about 5%. No difference is noticed between low and high tide.

Comparing gram negative with gram positive proportion, mean percentage through the year showed 70% for the former and 30% for the latter, a little higher for the former at low tide. In July gram positives were higher in the total flora in both tides.

Morphologically the dominant *Pseudomonas-Achromobacter* group showed rod shape of varied type and size, with mono polar to peritrichous flagelli, generally with greyish, normal size, wet, entire translucent to opaque colonies.

In once case a strain showing the characteristics of *Chlamydobacteriales* was isolated. Some strains of Micrococci were larger in size with thickened wall, granules and variable gram, as observed by Kriss (1963).

As much as 21.4% of the total flora showed yellow to brownish colored colonies. Only three strains of green pigmented *Pseudomonas* were isolated. Sverdrup (1960) states that about 70% of marine bacteria are colored as opposed to 15% of the terrestrial forms. Thus the present samples are closely related to terrestrial forms and certainly come from the run-off of water from land to the mangrove. This explains the great number of bacteria present after heavy rains.

TABLE V — Some biochemical characteristics of the Pseudomonas-Achromobacter group isolated from the surface layer in October 1962, given as percentage of total number of strains tested

Station	Total nº of strains	Litmus milk peptonized	Gelatin liquified	Nitrate reduced	H_2S produced	Glucose fermented	Lactose fermented	Sucrose fermented	Dulcitol	Citrate used as sole carbon source	Methyl red test positive	Voges Proskauer test positive
Argolão	16	31.2	6.3	100.0	12.5	62.5	18.7	43.6	6.3	6.3	31.2	6.3
Ariri	14	21.4	14.3	92.8	7.1	42.8	14.3	42.8	0.0	14.3	21.4	7.1

The biochemical characteristics of the strains of the *Pseudomonas-Achromobacter* group are given in Table V. In general this group of water flora in the mangrove swamp is more saccharolytic, probably due more to the great number of diatoms present, than to proteolytic strains. Most of them are nitrate reducers, very weak in Dulcitol fermentation, in the utilization in citrate as the only carbon source and in the production of acetyl-methyl carbinol (V.P. test).

DISCUSSION

As Miniussi (1959) pointed out, Aroeira has the most intricate water movement, receiving flows from three directions. This mechanical turbulence of tidal movement would be responsible for the highest aerobic count at this station, bringing up the abundant microorganisms and organic particles from bottom mud into upper water layers, supplying favorable conditions for bacterial growth (Teixeira, Tundisi & Kutner 1965). On the other hand, the suppressing effects of sea-water on bacteria, as dilution, sedimentation, agglutination, osmotic pressure and biologically active organic matters (Sieburth 1962; Jones 1959) would decrease the contamination at Ariri and Argolão, that suffer a more direct influence of inflow from the sea.

The use of a not sterilized sampler in the present experiments is highly questionable, leading us to the confusing conclusion that there is no variation of bacterial counts in different depths, which disagrees with data obtained by other authors (Sverdrup, 1960).

Coliform counts had significant variation only by station. This is reasonable since the place of sewage discharge is the sole factor responsible for the contamination. However, the results obtained showed that the count at Aroeira is higher than at Argolão, which is closer to the town of Cananéia, the only large source of the coliforms in this region. This record does not agree with Rittenberg's (1956), which showed regularly higher count closer to the sewage outfall. Nevertheless, this again might be explained by the same reasons indicated for the aerobics or may be due to local water currents.

Although the reliability of the number of coliform is dubious due to the lower temperature of incubation caused by the difficulty in power supply, the counts are equal to Rittenberg's (1956) and the specific investigation of the isolates from the violet-red-bile agar proved most of them to be *citrobacter* spp. with some *Escherichia coli*. In few cases *Aeromonas* and *Pseudomonas*-like bacteria were identified. In this connection the determinations of *Escherichia coli* and *Enterococci*, parallel to coliform would become important to give a clearer picture of the pollution situation.

Based on the information obtained, the most suitable area in this region for oyster culture from the hygienic point of view, would be found somewhere in the "Mar de Cubatão", nearer to

Ariri. The salinity condition for the mollusc growth would also be optimum there (Lima & Vazzoler, 1962).

The large majority of marine bacteria are gram negative. The phenomenon of low percentage of gram negative in July, the season of low bacterial contamination draw our interest to find out the cause. Scarcity of nutrients due to low bloom of plankton or low water temperature alone cannot fully explain the selectivity of species in the bacterial flora. In this respect it would be interesting to investigate the antibiotic action of sea-water specific to gram positives (Saz *et al.*, 1963) in the plankton blooming season of January-April.

On the whole the present data, although treated statistically, are insufficient to lead to definite conclusions and must be regarded as a preliminary survey. The bacterial contamination in brackish water would be highly influenced by every day conditions of tide, weather, discharge of sewage, in addition to the factors analysed here. Therefore, more frequent samplings within shorter intervals are necessary to obtain a more precise and detailed picture of bacterial population of this mangrove region.

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RESUMO

As águas da região do mangue de Cananéia foram analisadas bacteriològicamente, com o objetivo de estabelecer seu grau de contaminação.

As contagens aeróbicas variaram significativamente nas várias épocas, locais e marés, com um máximo de 13.000 bactérias/ml e um mínimo de 21/ml, ao passo que as contagens de coliformes mostraram variação significativa sòmente segundo os diversos locais com um máximo de 1.600 e um mínimo de 3/ml.

O grupo *Pseudomonas/Achromobacter* abrangeu a mais alta percentagem da flora total com cêrca de 60%. As características bioquímicas das linhagens dêste grupo são mais sacarolíticas do que proteolíticas, certamente devido ao grande número de diatomáceas presentes.

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