Acta Adriat. 21 (1): 203—218 (1980)

YU ISSN: 0001—5113
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QUANTITATIVE AND QUALITATIVE INVESTIGATIONS OF ORGANIC PHOSPHORUS DECOMPOSING BACTERIA IN THE CENTRAL ADRIATIC

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The distribution and some properties of organic phosphorus decomposing bacteria were studied seasonally at three stations of the Central Adriatic during 1975. Their distribution and relation to other heterotrophic bacteria were analyzed by standard plate method on four media (Beef-pepton-agar, Tyler, Menkina and the medium with mud extract). Organic phosphorus decomposing bacteria were found in all the collected samples and their percent to other heterotrophic bacteria was observed to vary in different water and mud samples. Mean percent values in water environment ranged from 8. 1 to 14.8%, and in the sediments from 20.6 to 35.4%.

INTRODUCTION

Phosphorus is one of the most important elements for the growth of many aquatic organisms. Together with nitrogen it is an important factor on wich depend thc biological properties of the sea, productivity in the first place.

Numerous studies have been made on the cycle of phosphorus in the sea and lakes in order to throw some light on the productivity of communities in aquatic environments (Rigler, 1956; Watt and Hayes, 1963; Riley and Chester, 1971).

In aquatic environments phoshorus is distributed in the form of dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP) and particulate phosphorus (PP). Aquatic bacteria are commonly held responsible for assimilating DIP and DOP and mineralizing DOP and PP. Thus, there is no doubt about the role of bacteria as decomposers of organic matters in those environments.

Taking into account the great importance of this bacteria group, the target of our investigations was to study their quantitative composition in relation to other heterotrophic bacteria and their distribution in the central Adriatic.

MATERIAL AND METHODS

Material for these investigations was collected from three permanent stations of the central Adriatic: from the coastal area at the Bay of Kaštela station, from the channel area between islands at the Pelegrin station, and from the offshore area at the Stončica station (Fig. 1).

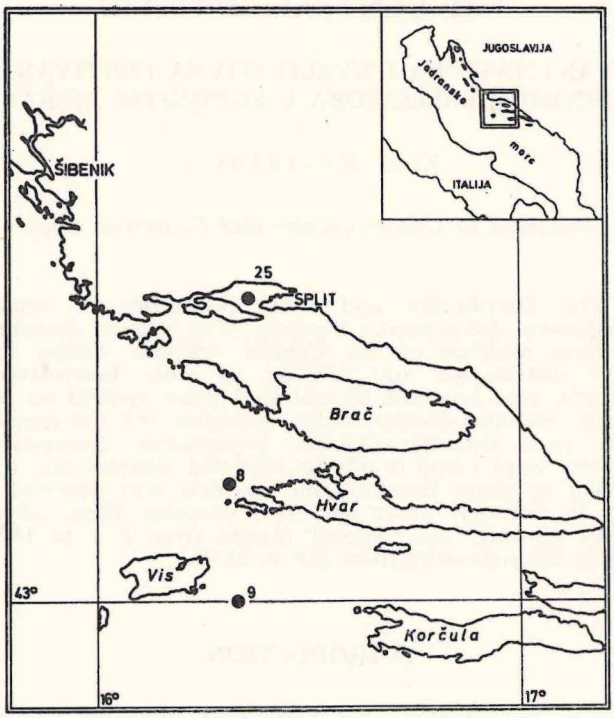

Fig. 1. Area of investigation
Stations: No. 25 — Kaštela Bay
No. 8 — Pelegrin
No. 9 — Stončica

The Kaštela Bay station in the shallow and enclosed area is characterized by the considerable oscillations of biological and physical-chemical parameters due to the direct influence of the adjacent land shallow waters (42 m).

Pelegrin station is close to the cape of Pelegrin on the island of Hvar. Its depth is 78 m. The station is under the influence of both the coast and the open sea, thus that the temperature and salinity vary here less than in the Bay of Kaštela, and more than in the open sea.

Stončica station is south-eastern from the cape of Stončica on th island of Vis. Depth is 107 m. It is under the influence of the open sea, thus the fluctuations in hydrographic parameters are usually less felt than at the above mentioned stations.

Samples were collected seasonally: in January, April, July and Octobeı 1975. The samples were collected from three various depths of water column and from sediments. In the Kaštela Bay the samples were collected from 0,20 and 35 m, at Pelegrin from 0,35 and 70 m and at Stončica from 0,50 and 100 m. Inoculation was performed by the standard plate method on four different media.

1. BPA (beef-pepton-agar medium)

Beef extract 2.2 g; peptone 5.7 g:, glucose 1.0 g; $FePO_4$ 0.3 g; agar 15 g; sea water (aged) 660 ml; distilled water 340 ml; pH 7.6.

2. Tyler mdium

Yeast extract 50 mg; Na-acetate 50 mg; agar 10 g; sea water (aged) 660 ml; distilled water 340 ml; pH 7.6.

3. Menkina medium

NH_4SO_4 0.5 g; $MgSO_4 \times 7$ H_2O 0.3 g; KC1 0.3 g; $MnSO_4 \times 5$ H_2O trace; $FeSO_4 \times 7$ H_2O trace; glucose 10 g; $CaCO_3$ 5 g; sea water 660 ml; distilled water 340 ml; pH 7.6.

4. Medium with mud extract

Glucose 5 g; yeast extract 0.5 g; $CaCO_3$ 5 g; agar 15 g; mud extract (Ocevski, 1966) 500 ml; distilled water 500 ml; pH 7.6.

Enumeration of heterotrophic bacteria and organic phosphorus decomposing bacteria was performed simultaneously on all the media. In fact five plates of each medium were inoculated for each sample. Three of them were used for enumeration of hetrotrophic bacteria and other two were used for the approvement and enumeration of organic phosphorus decomposing bacteria. The production of bacterial phosphatase on BPA and Tyler media was proved by nutrient media wich contained Na-phenolphtnalein phosphate (Krasilnikov and Kotelev, 1957). Lecitine was added to Menkina medium (Rodina, 1956) and the colonies which produce the phosphatases are surrounded by the ringlike transparent zone. This is aplicable too, to the mud extract medium.

Bacterial colonies wich produced bacterial phosphatases weree isolated and testeed by »Mercko test« for alkaline phosphatases and by »Mercko test« for acid phosphatases, modificed by Norris and Ribbons (1971).

RESULTS AND DISCUSSION

1. Growth of organic phosphorus decomposing bacteria and heterotrophic bacteria on different media

The relation between heterotrphic bacteria and organic phosphorus decomposing bacteria was studied in various samples of sea water and sediments. Bacteria wich decompose organic phosphorus compounds were found in all the investigated samples. They showed the relationship with total namber of heterotrophic bacteria. Samples characterized by larger number of heterotrophic bacteria showed simultaneously larger number of organic phoshorus decomposing bacteria, and opposite. Investigating the presence and distribution of this bacterial group in the sediments near Porto Novo Ayakanu and Chondromohan (1971) obtained similar results. Hayashi (1972) also came to the similar conclusions investigating their distribution in the sea calumn and sediments on some of the Pacific ocean stations.

In order to study their relation as the best possible, both heterotrophic bacteria and organic phoshorus decomposing bacteria were counted simultaneously on four different media. Thus we were able choose for further investigations the one which showed the best growth of both heterotrophic bacteria and organic phosphorus decomposing bacteria.

Menkina medium where growth of organic phosphorus decomposing bacteria which contained phosphodiesterases were investigated, showed a small number of heterotrophic bacetria and organic phosphorus decomposing bacteria. Similar investigations on the same medium were carried out by Ocevski (1969) in Ohrid lake and Gak (1959) in various aquatic environments. They obtained better results with these medium. This may indicate that the composition of this medium is more suitable for similar investigations in fresh waters.

Organic phosphorus decomposing bacteria which contain phosphomono — esterases were investigated on standard BPT medium, Tyler medium and the medium with mud extract. As expected, the least number of colonies developed on Tyler medium whose composition is the poorest one. In addition, one morphological type of colony mainly developed on this medium what is indicative of the selectivtiy. Far better results were expected from the medium with mud extract since it contained the substances which are closer to the substances of marine environment. In fact, in some cases organic phosphorus decomposing bacteria developed in larger number on this medium than on BPA medium. At the same time the number of other heterotrophic bacteria was almoust always far less. Therefore, the percent of colonies wich decompose organic phosphates was, in most cases, larger on this medium. It is probably due to the fact that on this medium organic phosphorus decomposing bacteria, were determined by the zone of $CaCO_3$ decomposing, which doesn't show for sure organic phosphorus decomposing bacteria, but also includes the possibility that the transparent zones appeared under the influence of acid metabolites. Taking into account the number and morfological differences of colonies, heterotrophic and organic phosphorus decomposing bacteria, the BPA medium appeared to be the most suitable for this kind of investigations (Tables 1,2 and 3). The results brought out in the text, will therefore, refer to those obtained on this medium (Tables 9,10 and 11).

2. Distribution of heterotrophic bacteria and organic phosphorus decomposing bacteria

The coastal station the Bay of Kaštela which is therefore under the strongest influcence of the coast, appeared to have the reachest bacterial population both in aquatic environment and in sediments. This area belongs, according to Buljan (1964), to the most productive zone, called D, owing to its quantity of nutrient salts, its depth and primary production. In the

Table 1. Mean values of total heterotrophic bacteria and organic phosphorus decomposing bacteria on different media in the Kaštela Bay

Medium	Depth	H*/ml, g/wet/	PD**/ml, g/wet/	PD/H %
BPA	0	708.7	51.5	7.2
	20	344.0	37.2	10.8
	35	870.7	63.0	7.2
	sed.	100275.0	35550.0	35.4
Tyler	0	126.0	2.5	1.9
	20	142.2	26.7	18.7
	35	410.5	20.0	4.8
	sed.	70700.0	15150.0	21.4
Menkina	0	427.0	37.5	8.7
	20	143.7	3.0	2.0
	35	253.2	10.7	4.2
	sed.	43300.0	4000.0	9.2
Mud ext.	0	504.2	36.7	7.3
	20	122.7	8.7	7.1
	35	532.0	3.0	0.5
	sed.	53800.0	23875.0	44.3

H^x — Heterotrophic bacteria
PD^{xx} — Organic phosphorus decomposing bacteria

Table 2. Mean values of total heterotrophic bacteria and organic phosphorus decomposing bacteria on different media at Pelegrin

Medium	Depth	H/ml, g/wet/	PD/ml, g/wet/	PD/H %
BPA	0	333.7	43.5	13.0
	35	260.2	34.5	13.2
	70	493.7	83.5	16.9
	sed.	57250.0	20075.0	35.0
Tyler	0	122.0	19.5	15.9
	35	38.5	7.2	18.7
	70	192,5	35.7	18.5
	sed.	56200.0	15175.0	27.0
Menkina	0	126.5	12.7	10.0
	35	155.2	2.2	1.4
	70	185.5	8.2	4.4
	sed.	45275.0	1250.0	2.7
Mud ext.	0	68.2	21.5	31.5
	35	86.5	4.7	5.4
	70	81.7	14.5	17.7
	sed.	57450.0	23700.0	41.2

period of investigations the heterotrophic bacteria density averaged 620 colonies/ml at all the levels (surface, intermediate and interface) of the sea water, and 100,275 colonies/g in wet sediments. The proportion of the organic phosphorus decomposing bacteria in the total number of heterotrophic bacteria in the sea water counted only 8.1 percent and in the sediments 35.4 percent.

Table 3. Mean values of total heterotrophic bacteria and organic phosphorus decomposing bacteria on different media at Stončica

Medium	Depth	H/ml, g/wet/	PD/ml, g/wet/	PD/H %
	0	258.0	22.2	8.6
B P A	50	300.7	16.7	5.5
	100	402.5	37.2	9.2
	sed.	48375.0	9975.0	20.6
	0	41.2	6.7	16.2
Tyler	50	79.7	5.7	11.4
	100	179.0	14.5	8.1
	sed.	32000.0	2825.0	8.8
	0	63.7	5.1	8.0
Menkina	50	58.2	5.5	9.4
	100	170.2	19.5	11.4
	sed.	58150.0	1750.0	3.0
	0	72.5	8.7	12.0
Mud ext.	50	67.7	9.7	14.3
	100	168.2	39.0	23.1
	sed.	51500.0	21225.0	41.2

Quantitative values for heterotrophic and organic phosphorus decomposing bacteria at the channel station of Pelegrin varied within the range of values obtained in the Kaštela Bay and Stončica. Heterotrophic bacteria density mean in the period of examinations was 363 colonies/ml for all three levels of water, out of wich 14.8 percent refered to the organic phosphorus decomposing bacteria. In wet sediment, density mean was 57,250 colonies/g, out of wich 34.9 percent refered to the organic phoshorus decomposing bacteria.

The offshore station of Stončica was in the period of investigations the poorest one, both in heterotrophic and organic phoshorus decomposing bacteria. This area belongs to the less productive zone with far less quantity of nutrient salts and with lower production. Out of the mean value for the heterotrophic bacteria (313 colonies/ml), 8.1 percent referred to organic phoshorus decomposing bacteria, and in wet sediments out of the mean value of 48,375 colonies/g, 20.6 percent produced phosphatases (Table 4).

Table 4. Mean values of hetrotrophic bacteria and organic phosphorus decomposing bacteria (colonies/ml, g)

	Sea column			Sediment		
	H	PD	PD/H %	H	PD	PD/H %
The Kaštela Bay	620	51	8.1	100,275	35,550	35.4
Pelegrin	363	54	14.8	57,250	20,015	34.9
Stončica	313	25	8.1	48,375	9,975	20.6

As it may be seen, the percentage of organic phosphorus decomposing bacteria in sediments considerably exceeds that of other heterotrophic bacteria in aquatic environment. This is probably due to greater quantities of phosphate in the sediments.

In the Kaštela Bay vertical distribution showed a regular pattern with minima in intermidiate layer, maxima in winter and summer in the surface and in spring and autumn in the interface one (Figs. 2 and 3). No regular pattern of vertical distribution of heterotrophic and organic phosphorus decomposing bacteria was noted at Pelegrin. At Stončica the largest number of organic phosphorus decomposing bacteria was, as a rule, recorded from the interface layer in all the months of investigations, and the least number appeared in the intermediate layer (Fig. 4). This is not in agreement with some of earlier studies (C v i i ć, 1955).

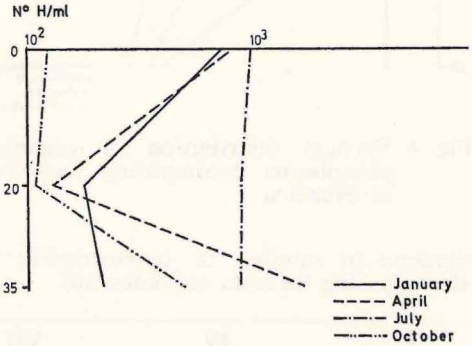

Fig. 2. Vertical distribution of heterotrophic bacteria in the Kaštela Bay

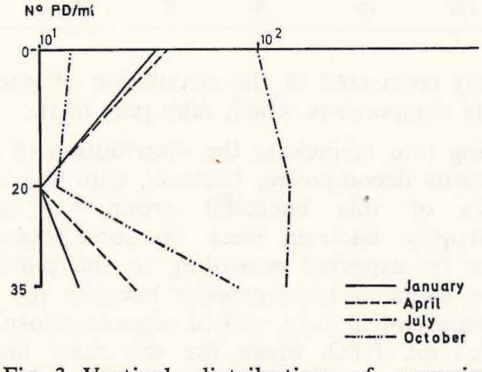

Fig. 3. Vertical distribution of organic pohsphorus decomposing bacteria in the Kaštela Bay

Seasonal fluctuations of organic phosphorus decomposing bacteria as well as of heterotrophic bacteria showed the pronounced summer maxima at all the stations, and the pronounced minimum at Stončica and Pelegrin in April and in the Kaštela Bay in January (Table 5). Thus, the seasonal fluctuations occur, but since the minima and maxima do not coincide on all the stations there is probably no regular seasonal fluctuation due to the temperature. Distribution of these bacterial groups can not be examined separately from the distribution of phosphates, phyto- and zooplankton

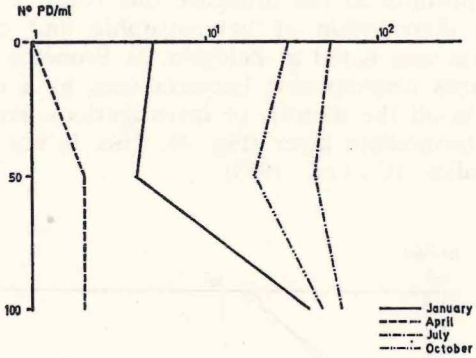

Fig. 4. Vertical distribution of organic phosphorus decomposing bacteria at Stončica

Table 5. Seasonal variations in number of heterotrophic bateria and organic phosphorus decomposing bacteria (colonies/ml)

	I		IV		VII		X	
Station	H	PD	H	PD	H	PD	H	PD
Kaštela B.	390	16	956	22	978	128	251	35
Pelegrin	529	93	139	4	552	105	230	14
Stončica	117	16	8	2	940	52	216	32

snice they are closely connected in the circulation of phosphorus. However, they are not the only components which take part in it.

We tried to bring into connexion the distribution of both heterotrophic and organic phosphorus decomposing bacteria, with the content of phosphates. Density values of this bacterial group, as well as the total number of heterotrophic bacteria were on some occasions considerably greather than might be expected according to the phosphate contents. In the Kaštela Bay the mean of heterotrophic bacteria for all three layers of water were 978 colonies/ml in July, and of organic phosphorus decomposing bacteria 129 colonies/ml. P-PO_4 mean for the same layers, was 0.069 μg at/1. Means of both heterotrophic (390 colonies/ml) and organic phosphorus decomposing bacteria (16 colonies/ml) were considerably less in January while the mean of phosphates was considerably higer (0.123 μg at/1). The same was recorded from Stončica. Maximum number of heterotrophic bacteria (940 colonies/ml) and of organic phosphorus decomposing bacteria (52 colonies/ml) was recorded in July when the mean of phosphates for three examined levels was only 0.059 μg at/1. In April, mean of P-PO_4 was 0.118 μg at/1, and means of heterotrophic bacteria (8 colonies/ml) and of organic phosphorus decomposing bacteria (1.6 colonies/ml) were extremly low. Johanes (1968) explains this by the fact that the presence of a large number of bacteria is not always the indicator of a rapid regeneration of phosphates. Very often it could be the proof of just oposite, that is to say of a rapid assimilation of dissolved nutrients. In addition, since it is known that phyto- and zooplankton play an important role in the phosphorus circulation. The presence of phosphates should not be observed separately from these two groups. At the some time, these planctonic components influence the third one, i. e. the bacterioplanktonic component. In his competition for nutrient matters phytoplankton influences bacterioplankton by metabolic products which can positively or negatively influence the density of bacterial population. On the other hand, dying out of phyto- and zooplankton enriches bacterial residence (sea water) by nutrient substrata, and finally zooplankton takes bacteria as food. All this indicates that the relation of organic phosphorus decomposing bacteria and heterotrophic bacteria are inversely related to phyto- and zooplankton.

From the results obtained during the eleven-year period of investigations (Karlovac et all, 1974) it is evident that two strongly pronounced phytoplankton maxima occur in the Kaštela Bay, of wich first one during the whole autumn, and the second one at the end of winter and at a begining of spring. During the period of this first maximum, the values of both the heterotrophic and of organic phosphorus decomposing bacteria were very low. During the period of the second phytoplanktonic maximum a small quontity of organic phosphorus decomposing bacteria was recorded. This was not the case with heterotrophic bacteria. Maximum number of both heterotrophic and organic phosphorus decomposing bacteria was recorded in July, the month known for the minimum of phytoplankton. Inverse relation of these bacterial groups to phytoplankton is more evident at Stončica and Pelegrin.

The relation of organic phosphorus decomposing bacteria and heterotrophic bacteria to zooplankton give the similar image, irrespective of whether the data of the earlier long-term observations were used (Vučetić, 1975) or the more recent ones from the period coinciding with the period of our investigations (unpublished data Vučetić). Its density seems to be one of the factors influencing dynamics variations in the number of bacterial population. This was clearly manifested in the channel and offshore areas where, with zooplankton maximum in April the lowest numerical values of the above mentioned bacterial groups were recorded. Thus, in July together with the maximum values of heterotrophic and organic phosphorus decomposing bacteria there were recorded the low values of zooplankton. This interdependence was not recorded from the Kaštela Bay. This was due to the fact that higer quantity of organic matter was available to heterotrophic bacteria in this area than at Pelegrin and Stončica. Therefore the influence of zooplankton wich fed on bacteria was less felt. In the investigations of ecological productivity more attention should be given to interrelations of planktonic communities, since than inffluence of abiogene factors. This kind of investigations, however, should be carried out over a longer period.

3. Some characteristics of organic phosphorus decomposing bacteria

From the total number of samples collected from the Kaštela Bay, 96 colonies, wich showed phosphatase activity, were isolated and analysed on microscope. The number of pure colonies amonuted 41 i. e. 42.7 percent of the total number of the colonies examined. All the colonies contained mainly motile Gram-negative rods. Their size ranged from 0.3—2 μm in width and from 1—7 μm in lenght. There was recorded very small quantity of the bacteria of coccal shape. They occurred in mixed colonies, if only.

All pure colonies were tested both with »Mercko test« for alkaline phosphatases, and with »Mercko test« for acid phosphatases. 80.5 percent colonies possesed phosphatases wich were active in alkaline environment, and 19.5 percent colonies showed phosphatase activity both in alkaline and acid environment. No colonies proved phosphatase activity in acid environment only (Table 6).

Table 6. Some characteristics of organic phosphorus decomposing bacteria isolated from the Kaštela Bay

Strain	Form	Motility	Gram	Size/μm/	Alk. ph.	Acid ph.
K— 1	rod	+	—	0.5 × 4.0	+	—
K— 2	rod	+	—	0.3 × 2.5	+	—
K— 3	rod	+	—	1.0 × 2.0	+	+
K— 4	rod	+	—	1.0 × 5.0	+	—
K— 5	rod	+	—	0.3 × 1.0	+	—
K— 6	rod	+	—	1.0 × 2.0	+	+
K— 7	rod	+	—	0.5 × 5.0	+	+
K— 8	rod	+	—	0.3 × 2.5	+	+
K— 9	rod	+	—	0.5 × 1.5	+	—
K—10	rod	+	—	0.5 × 4.0	+	—
K—11	rod	+	—	0.3 × 1.5	+	+
K—12	rod	+	—	0.5 × 2.0	+	—
K—13	rod	+	—	1.0 × 2.0	+	+
K—14	rod	+	—	0.5 × 3.0	+	—
K—15	rod	+	—	0.5 × 4.0	+	+
K—16	rod	+	—	1.0 × 7.0	+	—
K—17	rod	+	—	0.5 × 1.5	+	—
K—18	rod	+	—	1.0 × 2.0	+	+
K—19	rod	+	—	1.0 × 4.0	+	—
K—20	rod	+	—	0.5 × 3.0	+	—
K—21	rod	+	—	0.5 × 3.0	+	—
K—22	rod	+	—	0.5 × 5.0	+	—
K—23	rod	+	—	0.3 × 1.5	+	—
K—24	rod	+	—	1.0 × 3.0	+	—
K—25	rod	+	—	1.0 × 5.0	+	—
K—26	rod	+	—	0.3 × 1.5	+	—
K—27	rod	+	—	1.0 × 2.0	+	—
K—28	rod	+	—	1.0 × 3.0	+	—
K—29	rod	—	—	1.5 × 4.0	+	—
K—30	rod	+	—	0.3 × 2.0	+	—
K—31	rod	+	—	0.5 × 3.0	+	—
K—32	rod	+	—	1.0 × 2.0	+	—
K—33	rod	—	—	1.5 × 4.0	+	—
K—34	rod	—	—	1.5 × 5.0	+	—
K—35	rod	—	—	1.5 × 3.5	+	—
K—36	rod	+	—	0.3 × 1.5	+	—
K—37	rod	—	—	2.0 × 7.0	+	—
K—38	rod	+	—	1.0 × 5.0	+	—
K—39	rod	+	—	1.0 × 3.0	+	—
K—40	rod	+	—	1.0 × 2.0	+	—
K—41	rod	+	—	1.0 × 6.0	+	—

From the samples collected at Pelegrin, 68 colonies were isolated and analysed on microscope. 28 of them were pure colonies, i. e. 41.1 percent of all the colonies examined. Like in the Kaštela Bay, all pure colonies contained mainly motile Gram-negative rods. Their size ranged from 0.3—1 μm in width and from 2—7 μm in lenght. »Mercko test« showed that in 85.4 percent colonies possesed alkaline phosphatases, and 14.6 percent possesed both alkaline and acid phosphatases. No colonies, proved phosphatase activity in acid environment only (Table 7).

Table 7. Some characteristics of organic phosphorus decomposing bacteria isolated from Pelegrin

Strain	Form	Motility	Gram	Size/μm/	Alk. ph.	Acid ph.
P— 1	rod	+	—	1.0 × 3.0	+	—
P— 2	rod	—	—	1.0 × 2.5	+	+
P— 3	rod	+	—	1.0 × 4.5	+	+
P— 4	rod	+	—	0.5 × 3.0	+	—
P— 5	rod	+	—	0.5 × 2.0	+	—
P— 6	rod	+	—	1.0 × 2.5	+	—
P— 7	rod	+	—	1.0 × 2.0	+	—
P— 8	rod	+	—	0.3 × 2.5	+	—
P— 9	rod	+	—	0.5 × 3.0	+	—
P—10	rod	+	—	0.5 × 4.0	+	—
P—11	rod	+	—	0.5 × 4.5	+	—
P—12	rod	+	—	0.3 × 2.0	+	—
P—13	rod	+	—	0.5 × 2.0	+	+
P—14	rod	+	—	0.3 × 2.0	+	+
P—15	rod	+	—	0.5 × 4.0	+	—
P—16	rod	+	—	0.5 × 3.0	+	—
P—17	rod	+	—	1.0 × 4.0	+	—
P—18	rod	+	—	1.0 × 2.0	+	—
P—19	rod	+	—	0.3 × 3.0	+	—
P—20	rod	+	—	0.5 × 2.0	+	+
P—21	rod	+	—	1.0 × 2.5	+	+
P—22	rod	+	—	1.0 × 2.0	+	—
P—23	rod	+	—	0,5 × 4.0	+	—
P—24	rod	+	—	0.3 × 3.0	+	—
P—25	rod	—	—	1.5 × 6.0	+	—
P—26	yeast	—	—	4.0 × 7.0	+	—
P—72	rod	+	—	0.5 × 2.0	+	—
P—28	rod	+	—	0.5 × 3.0	+	+

75 colonies wich were isolated at Stončica and analysed on microscope showed phosphatase activity. 30 colonies were pure, i. e. 46.1 percent. 16.6 percent possesed phosphatases wich were active both in alkaline and acid enviroment, and the other 83.4 percent of them were active in alkaline environment only. Like in the Kaštela Bay and Stončica stations there was no colonie with acid phosphatase. The shape and size of cells were not different from those taken in the Kaštela Bay and Pelegrin (Tabla 8).

Table 8. Some characteristics of organic phosphorus decomposing bacteria isolated at Stončica

Strain	Form	Motility	Gram	Size/μm/	Alk. ph.	Acid ph.
S— 1	rod	+	—	1.0 × 2.0	+	—
S— 2	rod	+	—	0.5 × 1.0	+	—
S— 3	rod	+	—	0.3 × 1.5	+	—
S— 4	rod	+	—	1.0 × 2.5	+	—
S— 5	rod	+	—	0.5 × 2.0	+	—
S— 6	rod	+	—	1.0 × 2.5	+	+
S— 7	rod	+	—	0.3 × 2.0	+	—
S— 8	rod	+	—	0.5 × 4.0	+	—
S— 9	rod	+	—	0.5 × 2.0	+	+
S—10	rod	+	—	1.0 × 7.0	+	—
S—11	rod	+	—	1.5 × 5.0	+	—
S—12	rod	+	—	1.0 × 4.0	+	—
S—13	rod	+	—	0.3 × 2.5	+	—
S—14	rod	+	—	0.5 × 6.0	+	—
S—15	rod	+	—	0.5 × 4.0	+	+
S—16	rod	+	—	0.3 × 1.0	+	+
S—17	rod	+	—	0.5 × 2.5	+	—
S—18	rod	+	—	0.5 × 1.0	+	+
S—19	rod	+	—	0.3 × 1.5	+	—
S—20	rod	+	—	0.5 × 3.0	+	—
S—21	rod	+	—	1.0 × 4.0	+	—
S—22	rod	+	—	1.5 × 4.0	+	—
S—23	rod	+	—	0.5 × 2.0	+	+
S—24	rod	+	—	0.3 × 2.0	+	—
S—25	rod	+	—	0.3 × 1.5	+	—
S—26	rod	—	—	1.0 × 8.0	+	—
S—27	rod	—	—	1.5 × 4.0	+	—
S—28	rod	+	—	1.0 × 5.0	+	—
S—29	rod	+	—	1.0 × 2.0	+	—
S—30	rod	+	—	0.5 × 3.0	+	—

Table 9. Number of colonies of heterotrophic bacteria and organic phosphorus decomposing bacteria at the Kaštela Bay

Month	Depth /m/	BPA H/ml	BPA PD/ml	BPA %	Tyler H/ml	Tyler PD/ml	Tyler %	Menkina H/ml	Menkina PD/ml	Menkina %	Mud extract H/ml	Mud extract PD/ml	Mud extract %
I	0	763	34	4.4	43	0	0	1,219	147	12.0	239	132	55.2
	20	181	0	0	8	0	0	92	12	13.0	25	7	28.0
	35	228	15	6.5	13	0	0	107	32	29.9	27	7	25.9
	Sed.	125,000	3,000	2.4	71,000	0	0	72,000	2,000	2.7	39,000	26,000	66.6
IV	0	886	38	4.3	376	0	0	236	0	0	1,632	10	0.6
	20	135	0	0	138	0	0	138	0	0	160	2	1.2
	35	1,846	27	1.4	1,296	25	1.9	541	4	0.7	1,720	5	0.3
	Sed.	77,000	1,200	2.5	20,800	1,600	7.6	11,200	0	0	33,200	500	1.5
VII	0	1,062	120	11.2	62	10	16.1	186	0	0	106	5	4.7
	20	950	137	14.4	391	107	26.9	295	0	0	246	16	6.5
	35	921	130	14.1	180	55	30.5	220	0	0	143	0	0
	Sed.	192,000	125,000	65.1	161,000	45,000	27.9	73,000	6,000	8.2	100,000	58,000	25.0
X	0	124	14	11.2	23	0	0	67	3	4.4	40	0	0
	20	110	12	10.9	26	0	0	50	0	0	60	10	16.6
	35	520	80	15.3	153	0	0	145	7	4.8	238	0	0
	Sed.	37,000	13,000	35.1	30,000	14,000	46.6	17,000	8,000	47.0	43,000	11,000	25.5

Table 10. Number of colonies of heterotrophic bacteria and organic phosphorus decomposing bacteria at Pelegrin

Month	Depth /m/	BPA H/ml	BPA PD/ml	BPA %	Tyler H/ml	Tyler PD/ml	Tyler %	Menkina H/ml	Menkina PD/ml	Menkina %	Mud extract H/ml	Mud extract PD/ml	Mud extract %
I	0	986	147	14.9	357	46	12.8	384	44	11.4	157	72	45.8
	35	291	42	14.4	82	22	26.8	182	9	4.9	47	8	17.0
	70	310	90	29.0	143	38	26.5	200	5	2.5	53	37	69.8
	Sed.	55,000	2,000	3.6	39,000	0	0	17,000	0	0	10,000	6,000	60.0
IV	0	55	3	5.4	6	0	0	7	0	0	32	2	6.2
	35	317	7	2.2	9	0	0	110	0	0	220	5	2.2
	70	45	2	4.4	1	0	0	6	0	0	16	2	12.5
	Sed.	6,000	1,000	21.6	6,800	1,700	25.0	2,100	0	0	42,800	1,800	4.2
VII	0	100	15	15.0	22	0	0	52	0	0	17	2	11.7
	35	347	84	24.2	27	7	25.9	139	0	0	29	0	0
	70	1,210	215	17.7	330	77	23.3	346	16	4.6	53	0	0
	Sed.	150,000	70,000	46.6	170,000	56,000	32.9	151,000	2,000	1.3	170,000	84,000	49.4
X	0	194	9	4.6	103	32	31,0	63	7	11.1	67	10	14.9
	35	86	5	5.8	36	0	0	19	0	0	50	6	12.0
	70	410	27	6.5	296	28	9.4	190	12	6.3	205	19	9.2
	Sed.	18,000	7,000	38.8	9,000	3,000	33.3	11,000	3,000	27.2	7,000	3,000	48.0

Table 11. Number of colonies of heterotrophic bacteria and organic phosphorus decomposing bacteria at Stončica

Month	Depth /m/	BPA H/ml	BPA PD/ml	BPA %	Tyler H/ml	Tyler PD/ml	Tyler %	Menkina H/ml	Menkina PD/ml	Menkina %	Mud extract H/ml	Mud extract PD/ml	Mud extract %
I	0	62	5	8.0	7	0	0	40	14	35.0	55	23	41.8
	50	51	4	7.8	5	0	0	47	10	21.2	34	12	35.2
	100	239	40	16.7	100	5	5.0	260	57	21.9	162	115	70.9
	Sed.	13,000	1,000	7.6	16,000	0	0	12,000	3,000	25.0	10,000	2,000	20.0
IV	0	2	1	50.0	0	0	0	0	0	0	6	2	33.3
	50	11	2	18.1	0	0	0	2	0	0	7	4	57.1
	100	11	2	18.1	2	0	0	4	0	0	11	7	63.6
	Sed.	9,500	900	9.4	2,000	300	15.0	1,600	0	0	8,200	4,900	59.7
VII	0	820	53	6.4	103	27	26.2	173	5	2.8	43	6	13.9
	50	1,020	42	4.1	280	23	8.2	160	7	4.3	177	18	10.1
	100	980	60	6.1	297	31	10.4	254	0	0	240	0	0
	Sed.	160,000	36,000	22.5	103,000	11,000	10.6	210,000	3,000	1.4	174,000	76,000	43,6
X	0	148	30	20.2	55	0	0	42	7	16.6	86	4	4.6
	50	121	19	15.7	34	0	0	24	5	20.8	53	5	9.4
	100	380	47	12.3	317	27	8.5	163	21	12.8	260	34	13.0
	Sed.	11,000	2,000	18.1	7,000	0	0	9,000	1,000	11.1	12,000	2,000	16.5

CONCLUSIONS

1. BPA medium appeared to be the most suitable medium for quantitative investigations of heterotrophic bacteria and organic phosphorus decomposing bacteria, as well as for study of their interrelations.

2. Organic phosphorus decomposing bacteria were recorded from all the examined samples. They showed to be proportional related to the number and distribution of other heterotrophic bacteria. The percent of organic phosphorus decomposing bacteria to heterotrophic bacteria in sediments exceeded considerably of that in the sea water at all the investigated stations.

3. Organic phosphorus decomposing bacteria were Gram-negative rods, mainly motile. Most of them possesed alkaline phosphatases, the rest of them possesed both alkaline and acid phosphatases. No bacteria possesed acid phospatases only.

ACKNOWLEDGEMENTS

I am greatly indepted to Dr Buljan who put at my disposal his unpublished data about the phosphates and to Dr T. Vučetić for her unpublished data about the zooplankton for 1975.

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Received: May 22, 1979.

KVANTITATIVNA I KVALITATIVNA ISPITIVANJA FOSFOMINERALIZATORA U SREDNJEM JADRANU

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KRATAK SADRŽAJ

Kvantitativna i kvalitativna istraživanja bakterija koje razgrađuju organske spojeve fosfora, te njihova distribucija u odnosu na ostale heterotrofne bakterije vršena su na tri postaje: Kaštelanski zaljev, Pelegrin i Stončica. Uzorkovanje je vršeno sezonski 1975. godine s tri dubine (površinski, srednji i pridneni sloj) i iz sedimenta.

U svrhu što boljeg studiranja njihovog odnosa, i heterotrofi i fosfomineralizatori su brojeni istovremeno na četiri različite podloge (MPA, Tylerova, Podloga s ekstraktom mulja i Menkina), kako bi za daljnja istraživanja odabrali jednu koja pokazuje najbolje rezultate s obzirom na broj i morfološku raznolikost kolonija i heterotrofa i fosfomineralizatora. Najpogodnijom za takva istraživanja pokazala se MPA podloga.

Fosformineralizatori su pronađeni u svim ispitivanim uzorcima i nalaze se u određenoj proporcionalnoj povezanosti s brojem i distribucijom heterotrofa.

Prosječna vrijednost za gustoću heterotrofnih bakterija u Kaštelanskom zaljevu za sva tri nivoa vode u ispitivanom razdoblju iznosila je 620 kolonija/ml, u vlažnom sedimentu 100275 kolonija/g. Procentualno učešće fosfomineralizatora u ukupnom broju heterotrofa iznosilo je u morskoj vodi 8,1%, u sedimentu 35,4%.

Na postajama Pelegrin i Stončica zapažena je također znatno veća zastupljenost fosfomineralizatora u sedimentima nego u vodenoj sredini. U sedimentu postaje Pelegrin procentualna zastupljenost fosfomineralizatora iznosila je 35% od 57 250 kolonija heterotrofa/g, u vodenoj sredini 14,8% od 363 kolonije heterotrofa/ml.

Na pučinskoj postaji Stončica, koja je u istraživanom periodu bila najsiromašnija heterotrofnim bakterijama (313 kolonija/ml u morskoj vodi i 48 375 kolonija/g u sedimentu), 8,1% heterotrofa je razgrađivalo organske fosfate u vodenoj sredini, 20,6% u sedimentu.

Kvalitativna analiza pokazala je da su fosfomineralizatori Gram negativne bakterije štapičastog oblika i uglavnom pokretljive. Većina ih posjeduje samo alkalne fosfataze, manji broj i alkalne i kisele, dok kod ni jednog od ispitanih sojeva nije dokazano posjedovanje samo kiselih fosfataza.