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DISTRIBUTION OF PROTEOLYTIC, AMYLOLYTIC AND LIPOLYTIC BACTERIA IN THE KAŠTELA BAY

DISTRIBUCIJA PROTEOLITIČKIH, AMILOLITIČKIH I LIPOLITIČKIH BAKTERIJA U KAŠTELANSKOM ZALJEVU

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Quantitative presence of proteolytic, lipolytic and amylolytic bacteria and their percentage proportion in the total number of bacteria was studied in different seasons at four stations in the Kaštela Bay (Adratic Sea). The highest number of studied bacterial groups was recorded from station C in the area where the majority of municipal and industrial effluents are received and spread in the surface layer. Increased proportion of individual physiological bacterial groups may be a good indicator of pollution by defined organic compounds.

INTRODUCTION

The role of heterotrophic bacteria in marine ecosystems is very important owing to their biochemical activity, that is their ability to utilize and decompose dissolved organic matter. Any change in dissolved organic matter quantity in the sea affects the number of heterotrophic bacteria and their metabolic activity.

Bacteria included in heterotrophic group are physiologically very diverse concerning their biochemical activities and roles in the process of organic matter degradation.

This paper is a report of the study of three groups of heterotrophic bacteria, proteolytic, amylolytic and lipolytic, wich play an important role in the processes of decomposition of complex organic compounds present in municipal and industrial effluents.

The Kaštela Bay is a closed and shallow basin very liable to land influence, with a surface area of 61 km^2 and an average depth of 23 m. Its western and northwestern parts are shallowest and its central part deepest (Zore, 1955). It is characterized by great oscillations of its biological and chemical parameters. The freshwater inflows are numerous, coming from the Jadro River in its eastern part, Pantan stream in its western part and a number of permanent or temporary submarine springs.

Mean surface temperature ranges from 11.86°C in winter to 22.93°C in summer, with annual mean of 17.28°C, while surface salinity range is 33.78×10^{-3} (April) to 36.96×10^{-3} (October) (Zore-Armanda, 1980).

A number of industries is concentrated in this area (concrete, chemistry, iron works, food). Therefore the bay is under the constant influence of industrial effluents and loaded by different inorganic and organic compounds affecting the ecosystem of this area.

MATERIAL AND METHODS

From February 1982 to 1984, 12 samplings were performed at stations A, B, C, D (Fig. 1.), of which four were performed in spring and summer and two in autumn and winter respectively.

At stations A and B samples were collected from 0 and 10 m, at station C from 0, 10 and 20 m and at station D from 0, 10, 20 and 40 m.

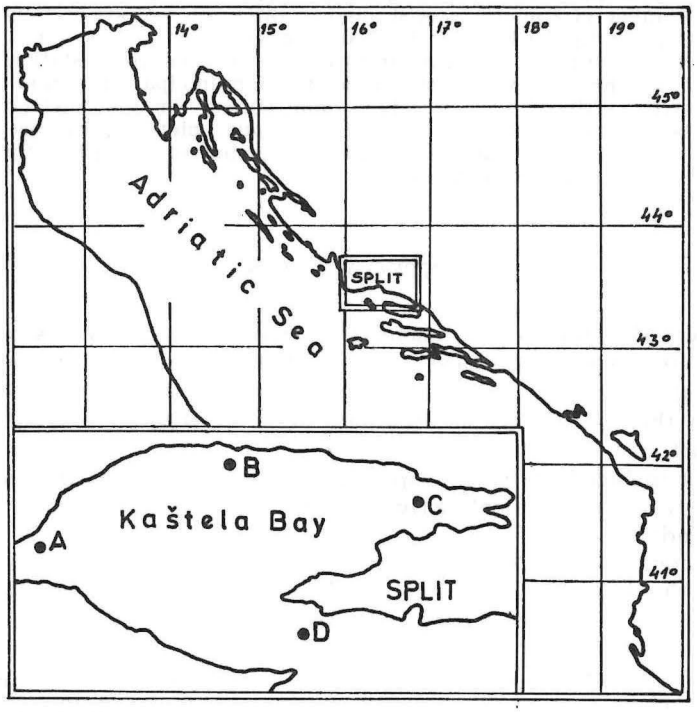

Fig. 1. Study area

ZoBell's medium 2216 (ZoBell, 1946) was used as a basic medium for the growth of proteolytic, amylolytic and lipolytic bacteria. Medium was enriched by defined substances for each group of studied bacteria.

Basic medium for proteolytic bacteria was enriched by 0.4% gelatine. Upon incubation, the grown up colonies were affected by Hg Cl_2 solution, the colonies round which a light zone was formed performed the liquefaction of gelatine.

Basic medium for amylolytic bacteria was enriched by 0.2% starch with Lugol solution as reagent. Light zones occurred round colonies which performed the hydrolysis of starch.

In lipolytic bacteria the medium was enriched by 1% Tween-80. Halo of insoluble oleic acid released by lipasis, was formed round the lipolytic bacteria colonies.

Incubation, performed at 20°C, took 7 days in all the groups. Total counts of bacteria were made by using the acridine orange direct count (AODC) technique (H o b b i e *et al.*, 1977).

RESULTS AND DISCUSSION

Mean values of the number of individual bacterial groups and their percentage proportion in the total number of bacteria are presented in Table 1.

Horizontal distribution

With respect to the obtained values for studied bacterial groups particularly pronounced horizontal gradient was established. Stations B and C were for almost an order of magnitude richer in all bacterial groups than stations A and D (Fig. 2). The highest mean values of both proteolytic and lipolytic bacteria were recorded from station C, while the mean annual value of amylolytic bacteria was higher at station B. The percentage proportions of studied bacterial groups in the total number of bacteria were higher at stations B and C too (Table 1).

Station C is in the Vranjic basin in the eastern part of the Kaštela Bay, which is a very specific and isolated entity. It is a shallow basin, with very poor circulation (Z o r e - A r m a n d a, 1980). In addition, food industry is concentrated there (meat, beer), and a major part of municipal wastes from the town of Split are discharged there.

All this led us to conclude that increased proportion of complex organic compounds (proteins, lipids, carbo-hydrates) in this part of the Kaštela Bay (station C) was favourable for the development of bacteria which decompose these compounds. Density of studied bacterial groups decreased with the distance from this area.

Seasonal distribution

No regular pattern of seasonal distribution of studied bacterial groups was established, with the exception for proteolytic bacteria, which showed maximum density in autumn and minimum in winter (Fig. 2). Seasonal variations are generally less marked for amylolytic and lipolytic bacterial groups. Therefore, it is rather difficult to discuss the occurrences of maximum and minimum densities.

Table 1. Percentage proportions of studied bacterial groups in the total number of bacteria

Station	Season	Total number of bacteria (AODC x 10^5 ml $^{-1}$)	Lipolytic bacteria (CFU x 10^2 ml $^{-1}$)	Lipolytic/ Total (%)	Proteolytic bacteria (CFU x 10^2 ml $^{-1}$)	Proteolytic/ Total (%)	Amilolytic bacteria (CFU x 10^2 ml $^{-1}$)	Amilolytic/ Total (%)
A	Spring	6.5	4.8	0.07	4.9	0.08	7.3	0.11
	Summer	6.6	1.8	0.03	3.7	0.06	2.4	0.04
	Autumn	8.1	4.6	0.06	5.0	0.06	2.9	0.04
	Winter	7.1	1.8	0.03	2.9	0.04	1.8	0.02
	Mean	7.1	3.3	0.05	4.1	0.06	3.6	0.05
B	Spring	7.4	3.5	0.05	31.1	0.42	22.2	0.30
	Summer	8.8	22.8	0.26	29.3	0.33	19.7	0.22
	Autumn	9.3	14.8	0.16	37.4	0.40	29.4	0.32
	Winter	8.5	4.5	0.05	21.2	0.23	12.8	0.15
	Mean	8.5	11.4	0.13	29.7	0.35	21.0	0.25
C	Spring	10.2	6.2	0.06	30.0	0.29	9.1	0.09
	Summer	11.9	15.1	0.13	22.4	0.19	7.6	0.06
	Autumn	11.6	29.2	0.25	85.0	0.73	39.1	0.34
	Winter	8,9	3.1	0.03	19.6	0.22	5.2	0.06
	Mean	10.7	13.4	0.13	39.2	0.37	15.3	0.14
D	Spring	4.8	1.0	0.02	5.8	0.12	7.3	0.15
	Summer	5.3	4.5	0.08	3.2	0.06	5.2	0.10
	Autumn	8.4	3.0	0.04	11.8	0.14	6.1	0.07
	Winter	6.4	2.8	0.04	2.2	0.03	3.9	0.06
	Mean	6.2	2.8	0.05	5.8	0.09	5.7	0.09

Vertical distribution

Certain regular pattern of vertical distribution of studied bacterial groups was established at all the stations, except at station A where they were most

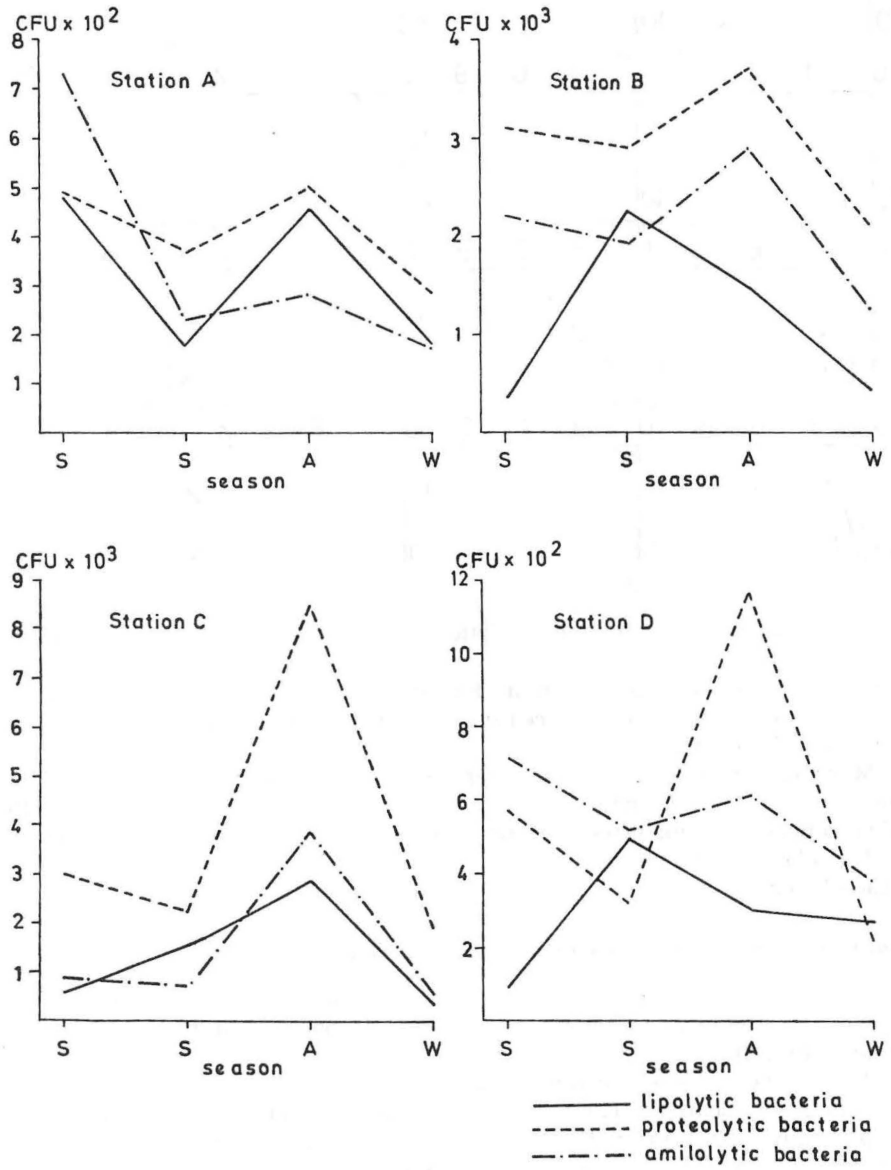

Fig. 2. Horizontal and seasonal distribution of studied bacterial groups

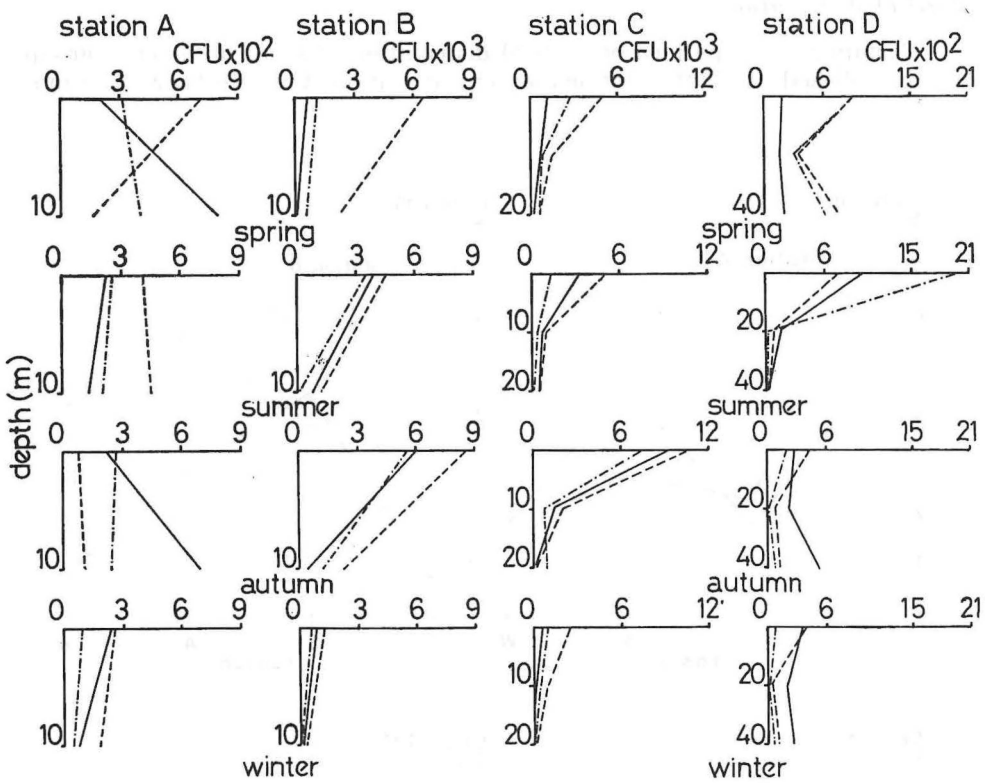

Fig. 3 Vertical distribution of Proteolytic (——), Amylolytic (— . . —) and Lipolytic

poorly represented (Fig. 3). Their density was greatest in the surface layer, and decreased with depth. Increased density above the bottom was recorded only from station D.

Marked surface maxima are very likely due to discharges of effluents which spread on the surface. In the paper by Pucher-Petković (1975), which reports on the effects of organic pollution on primary production in the Kaštela Bay, these effects were also found to be best expressed in the surface layer.

Quantitative relations between studied bacterial groups

Proteolytic bacteria occurred in greatest number throughout the study area, followed by amylolytic bacteria while lipolytic bacteria numbers were lowest (Table 1, Fig. 2).

The studies of specific physiological bacterial groups in the Adriatic Sea are relatively scarce. Krstulović (1980) studied the proportion of phospho-mineralizators in the total number of heterotrophic bacteria in the Kaštela Bay and at the open sea stations, and Cviić (1955) examined the physiological activity of heterotrophic bacteria in the middle Adriatic.

Increased density of studied bacterial groups in the Vranjic basin in relation to the rest of the Kaštela Bay, is very likely due to the present pollution,that is increased quantity of proteins and lipids as products of meat industry, and carbohydrates as products of beer industry. In this respect, studied bacterial groups may be the indicators of pollution by different kinds of organic compounds.

CONCLUSIONS

The highest numbers of proteolytic, amylolytic and lipolytic bacteria was recorded from station C, which is in the area which receives most of municipal and industrial effluents. Density of these bacterial groups decreased with the distance from this area. To conclude, increased proportion of individual physiological bacterial groups may be a good indicator of pollution by defined organic compounds.

No regular pattern of seasonal distribution of studied bacterial groups was established, with the exception for proteolytic bacterial group which showed maximum density in autumn and minimum in winter.

Vertical distribution of proteolytic, amylolytic and lipolytic bacteria showed certain regular pattern, manifested by the greatest density in the surface layers, most probably due to the discharges of effluents which spread in the surface.

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KRATKI SADRŽAJ

Kvantitativna zastupljenost proteolitičkih, amilolitičkih i lipolitičkih bakterija, kao i njihovo postotno učešće u ukupnom broju bakterija, istraživano je sezonski na 4 postaje u Kaštelanskom zaljevu.

Najveći broj proteolitičkih, amilolitičkih i lipolitičkih bakterija nađen je na postaji C koja je smještena u području gdje se ulijevaju otpadne vode grada i prehrambene industrije. Dalje je gustoća spomenutih grupa bakterija opadala s obzirom na udaljenost od ovog područja. Dakle, povećana prisutnost pojedinih fizioloških skupina bakterija može biti indikator zagađenja određenim organskim spojevima.

Nije utvrđena pravilnost u sezonskoj raspodjeli ispitivanih grupa bakterija, osim za grupu proteolitičkih bakterija čija je maksimalna gustoća utvrđena u jesenskom periodu, a minimalna u zimskom.

Vertikalna distribucija proteolitičkih, amilolitičkih i lipolitičkih bakterija pokazala je određenu pravilnost koja se manifestirala najvećom gustoćom u površinskim slojevima, najvjerojatnije kao posljedica površinskog ulijevanja i širenja otpadnih voda.