INSTITUT ZA OCEANOGRAFIJU I RIBARSTVO — SPLIT
FNR JUGOSLAVIJA

№ 6. BILJEŠKE 1953.

Attachement of bacteria to slides submerged in sea water

by

Vlaho Cviić
Institute of Oceanography and Fisheries, Split

ZoBell and Allen (1932 and 1935) have ascertained that in the fouling of different submerged surfaces, there is a sertain succession of organisms, and that the first film on such surfaces is laid by bacteria. — The rapidity of the creation of the first film, i. e. the swiftness of the attachment of bacteria depends on different oecological factors. The same authors have furthermore ascertained in examining the rapidity of the attachment of 74 marine bacterial species under laboratorial conditions, that only 32,87% of the bacteria stuck to the submerged slides. — The authors have called these species »attachement bacteria« and later on Henrici (1936) proposed the name of »periphitic bacteria«. — In connection with the preliminary works about the study of the problem of ships fouling, we have performed different experiments by immersing some slides into the sea, in order to get a picture about the quickness of the bacterial attachment on clean and smooth surfaces in the conditions of the Adriatic Sea. — These particulars will serve to us as an orientation in our work about the attachment of bacteria on coloured surfaces submerged into sea water.

To the laboratory-technicians Ojdana Marović and Peter Bilić I thank very much for the technical performance of this work.

In the experiments we have adopted the method of ZoBell and Allen (1933 and 1935) respectively of Henrici (1933). The same method has been used for soil bacteria by Winogradsky (1908) and

Conn (1932). Some microscope objective slides, well washed in acid and kali-bichromate and sterilized were put on a wooden frame (30 × 15 cm). For each experiment we put 28 so prepared slides and 3—4 pieces were taken off after 24,48 it c. hours for the purpose of calculating the number of the attached bacteria. The wooden frame was submerged into the sea 1,0—2,0 m. deep at about 4—5 m. from the shore. — The withdrawn slides were dried at the air and then dyed with genciana-violet. The counting of bacteria was done at the microscope by means of oil immersion. On each slide were counted 20 visible fields, and consequently for each test 60—80 visible fields. From the particulars obtained we calculated the arithmetical mean, as an average number of bacteria on the visible field. Occasionally particulars about the temperature were teken too, as well as the salinity, the currents and in some experiments the number of bacteria in the surrounding water.

In the first series of experiments the slides were dipped into the sea in the harbour of the Institute, where the water is stiller and richer in organic materials. The sea temperature varied in that period (from 18th. March till 10th. April 1952) from 11,72° C. and the average temperature during the same period was of 11,97° C. The salinity during that period varied from 36,25‰ to 37,38‰ and the average salinity was of 36,91‰. — On the table 1 is shown the rapidity of attachment of the bacteria on 1 cm². Five successive immersions of the slides were performed. The experiments lasted from 96 to 120 hours: they were interrupted after the attachment of the diatomeae and other micro-organisms became so dense that it hindered the counting of the bacteria. The number of bacteria on 1 cm² in the single experiments varied enough and the average variation amounted to 32.100 bacteria on 1 cm². The average number of bacteria for all the experiments (shown at the bottom of the table) shows a sudden and rather regular increase in the bacterial population.

In the second series of experiments the slides were plunged into the sea outside the harbour of the Institute, in a spot where the water is in constant flow. The sea temperature varied during that period (from 8th. April to 4th. May 1952) from 14,08° C. to 20,30° C. and the average temperature was of 16,79° C. The salinity during the same period varied from 30,14‰ to 36,57‰ and the average salinity was of 35,26‰. Four successive experiments were performed. — As it can be seen from table 2 the increase in the number of bacteria on 1 cm² for each experiment, as well as the increase of the average number of all the experiments, was weaker than in the previous experiments in the Institute harbour: the attachment went rather gradually and on the slides there were far fewer detrituses. — Here, too, it was proved that the variation in the number of bacteria on 1 cm² betwen the single amounts to 25.125 bacteria per 1 cm².

Tab. 1. *Rapidity of bacteria attachment on slides submerged in the sea in the Institute harbour*

Experiment	Date	after 24h number of bacteria	after 48h number of bacteria	after 72h number of bacteria	after 96h number of bacteria	after 120h number of bacteria
I	18-23. III. 1952.	114 425	213.925	248.750	357.500	—
II	23-28. III. 1952.	49.750	—	273.625	412.925	557.200
III	28.III-2. IV. 1952.	79.600	124.375	223.875	—	462.675
IV	2-6-IV. 1952.	84,575	194.025	218.900	367.450	487.550
V	6-10-IV. 1952.	59.700	—	189.130	288.250	—
Average		77.610	177.441	230.856	331.541	502.475

In the third series of experiments the slides were sunk into the sea outside the Institute harbour: the surveys were more rarely performed and besides the number of bacteria on the slides we counted also other organisms (diatomeae, peridineae, flagellata and the larvae of some crustacea). The sea temperature varied during that period (from May 5

Tab. 2. *Rapidity of the bacteria attachment on slides submerged in the sea outside the Institute harbour*

Experiment	Date	after 24h number of bacteria per cm^2	after 48h number of bacteria per cm^2	after 72h number of bacteria per cm^2	after 96h number of bacteria per cm^2	after 120h number of bacteria per cm^2	after 144h number of bacteria per cm^2	after 186h number of bacteria per cm^2
I	8-16-IV 1952.	29.780	94.525	164.175	208.850	—	373.025	457.700
II	16-21-IV 1952.	18.900	73.625	114.375	195.025	—	288.550	410.825
III	24-29-IV 1952.	21.875	82.575	94.425	223.875	313.425	—	—
IV	29-IV 4-V 1952.	44.775	—	134.275	279.100	333.225	—	—
Average		29.562	83.575	128.812	226.710	323.325	330.525	434,262

Tab. 3. *Rapidity of bacteria and other micro-organisms attachement on slides submerged in the sea outside the Institute harbour*

Experiment	Date	after 2 days		after 4 days		after 5 days		after 6 days		after 7 days		after 8 days		after 10 days		after 11 days	
		bacteria per cm^2	other micro-organisms	bacteria per cm^2	other micro-organisms	bacteria per cm^2	other micro-organisms	bacteria per cm^2	other micro-organisms	bacteria per cm^2	other micro-organisms	bacteria per cm^2	other micro-organisms	bacteria per cm^2	other micro-organisms	bacteria per cm^2	other micro-organisms
I	5-V. 1952.	99.500	131	195.026	161	—	—	237.625	143	—	—	941.275	481	1,109.425	595	—	--
II	16-V. 1952.	74.625	212	129.350	337	—	—	427.850	372	—	—	860.675	539	1,467.625	787	—	—
III	28-V. 1952.	39.800	118	—	—	338.300	127	—	—	537.300	382	—	—	975.600	390	1,303,950	520
IV	9-VI. 1952.	64.775	95	214.025	229	293.525	239	—	—	557.200	240	791.025	498	—	—	1,438 275	659
V	1-VII. 1952.	54.725	120	154.225	249	—	—	571.625	236	—	—	1,034.800	528	1,266.325	487	1,602.000	598
Average		66.685	135	173.156	244	310.912	183	426.033	250	547.250	311	906.943	511	1,204,743	565	1,448.074	592

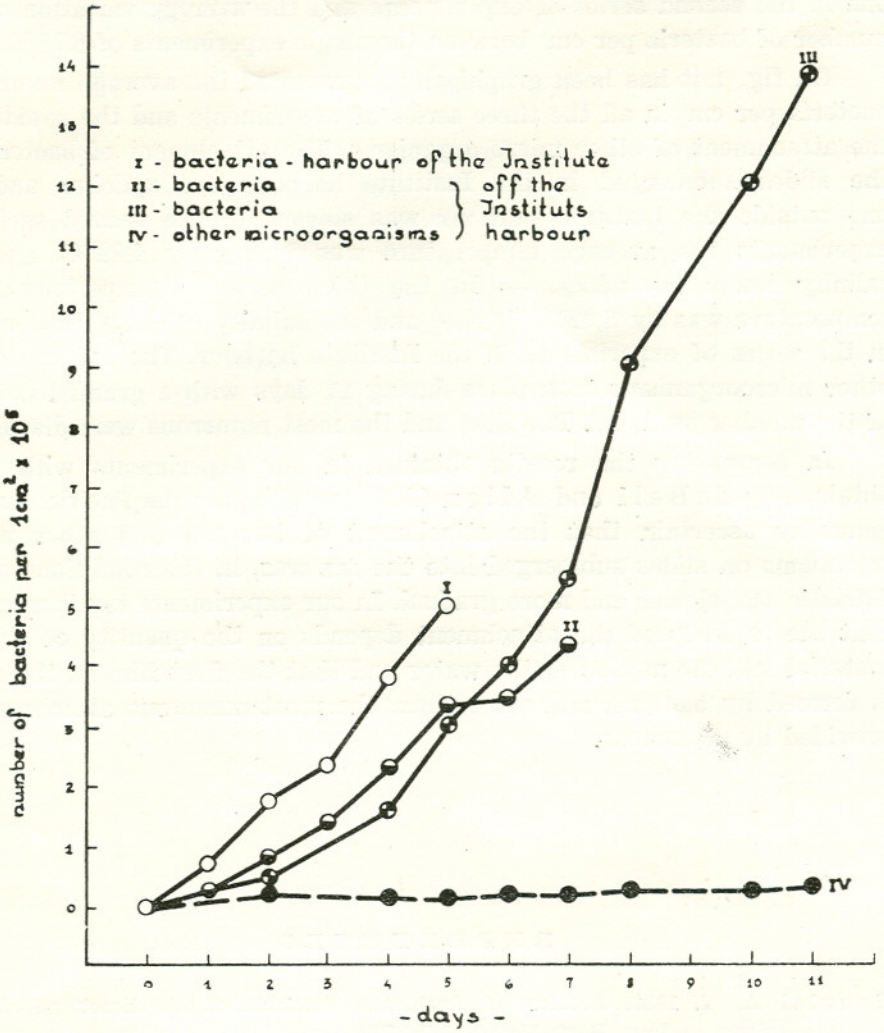

Fig. 1. The rapidity of attachement of bacteria and other microorganisms on slid.s submerged in the sea inside and outside of the Institute harbour

to July 1, 1952) from 16,20° C to 21,04° C and the average temperature was of 18,19° C. The salinity varied from 35,64‰ to 37,16‰, while the average salinity was of 36,77‰. — The sea current on the spot and at the time when the slides were submerged was very changeable (some times it changed even every hour) in two directions (N—S) and its strength varied from 0 to 1 mile per hour. The number of bacteria on 1 ccm in the surrounding sea water varied in that period from 81 to 803 and the average was of 344 bacteria per ccm. Five successive experiments were performed. On table 3 is shown the rapidity of the attachment of bacteria and other micro-organisms. The attachment of the bacteria went gradually, like the

one in the second series of experiments and the average variation of the number of bacteria per cm² between the single experiments of 85.889.

On fig. 1 it has been graphically represented the average number of bacteria per cm² in all the three series of experiments and the rapidity of the attachment of other micro-organisms. The attachment of bacteria on the slides submerged in the Institute harbour was quicker and the one outside the Institute harbour was slower. In the second series of experiments the average temperature was higher by 4,82° C. and the salinity lower by 1,65‰. — In the third series of experiments the temperature was by 8,22° C. higher and the salinity by 0,14‰ lower than in the series of experiments in the Institute harbour. The attachment of other microorganisms took place during 11 days with a gradual increase of the number on 1 cm². The first and the most numerous were diatomeae.

In comparing the results obtained in our experiments with those obtained by ZoBell and Allen (1935) working on the Pacific, we may generally ascertain that the attachment of bacteria and other micro-organisms on slides submerged into the sea was, in the conditions of the Adriatic sea, slower and more gradual. In our experiments too it appeared that the rapidity of the attachment depends on the quantity of organic material and the motion of the water and that the first film on the slides is formed by bacteria and after them the most numerous attachment is provided by diatomeae.

REFERENCES

Henrici A. T. 1933. Studies of freshwater bacteria. I. A direct microscopie technique. — Jour. Bact. No. 25, 277—286.

Henrici A. T. 1936. Studies of freshwater bacteria. III. Quantitative aspects of the microscopic method. Jour. Bact., No 32, 265—280.

ZoBell E. C. 1936. Peripytic habits of some marine bacteria — Proc. Soc. Exptl. Biol. Med.. No. 35, 270—273.

ZoBell E. C. 1946. Marine Microbiology. — Cronica Botanica, Waltham, Mass U. S. A.

ZoBell E. C., and E. C. Allen 1933. Attachement of marine bacteria to submerged slides. — Proc. Soc. Expt.l Biol. Med. No. 30., 1409—1411.

ZoBell E. C. and E. C. Allen 1935. The significance of marine bacteria in the fouling of submerged surfaces. — Jour. Bact. Vo. 29., No. 3., 239—251.

Visscher J. P. 1928. Nature and extent of fouling of ships bottoms. — Bureau of Fisheries. Bull. No. 43., 193—252.

Recived for publication November 26. 1952.

PRIČVRŠĆIVANJE BAKTERIJA NA STAKALCA URONJENA U MORE

Kratak sadržaj

U okviru istraživanja brzine obraštaja raznih površina, uronjenih u more, a na temelju izvršenih eksperimenata, daju se podaci o brzini pričvršćivanja bakterija i drugih mikroorganizama na stakalca uronjena u more. Na stakalca uronjena u luci Instituta bakterije se pričvršćuju znatno brže, nego na ona uronjena izvan luke Instituta. Od mikroorganizama prve se pričvršćuju bakterije, zatim drugi mikroorganizmi među kojima su prve i najbrojnije diatomeae.