Microb. Ecol. 5:245–251 (1980)

MICROBIAL ECOLOGY

Budding and Prosthecate Bacteria from Freshwater Habitats of Various Trophic States

J. T. Staley, K. C. Marshall, and V. B. D. Skerman

Department of Microbiology and Immunology, University of Washington, Seattle, Wa.;
School of Microbiology, University of New South Wales, Sydney, N.S.W.;
and Department of Microbiology, University of Queensland, St. Lucia, Queensland

Abstract. Budding and prosthecate bacteria were enumerated in spring and summer by viable counting procedures in several freshwater habitats in Australia including oligotrophic lakes, a mesotrophic lake, and eutrophic ponds. *Caulobacter* spp. were the most numerous type encountered. They were present in the highest concentrations (exceeding 1000/ml) in the mesotrophic lake during the summer. Their proportion to total viable heterotrophic bacteria was also highest (35.1 to 37.7) in this habitat. From 17 to 330/ml *Caulobacter* spp. were counted in the eutrophic habitats where their proportion to total viable numbers was less than 1.0%. In the oligotrophic lakes they varied from 5 to 23/ml and comprised greater than 5% of the total viable count. *Hyphomicrobium*-like bacteria were also numerous in the mesotrophic lake and in one oligotrophic lake during the summer sampling period. *Ancalomicrobium* spp. occurred in high concentrations (130/ml) in the mesotrophic lake. Budding bacteria of the *Planctomyces-Pasteuria* group were most numerous in the eutrophic habitats where as many as 240/ml were counted; their proportion to total heterotrophs remained relatively constant regardless of trophic state, however. A similar pattern was observed with *Prosthecobacter* spp.

Introduction

Henrici and Johnson (5) found stalked bacteria among the periphyton community of glass slides they had submerged in Lake Alexander, Minnesota, USA. Among the most common microcolonial forms they encountered on the slides was a group of polarly appendaged bacteria for which they proposed a new genus, *Caulobacter*.

Caulobacter spp. have been reported to be abundant (i.e., 10–10,000/ml) in the planktonic habitat of lakes of the Volga-Don river system (2). For their quantitative enumeration in plankton samples, Belyaev developed an extinction dilution–most probable number procedure using a dilute medium. This procedure was adapted for use in enumeration of other budding and prosthecate bacteria as well as *Caulobacter* spp. in a

0095-3628/80/0005/0245 $01.40

eutrophic stream in Michigan, USA (11). In that quarterly study, *Caulobacter* spp. were the most numerous of the prosthecate and budding bacteria, usually ranging between 100 and 1000/ml. *Hyphomicrobium-Hyphomonas* spp. were abundant during the December sampling period. *Prosthecomicrobium* and *Ancalomicrobium* were observed too, but occurred in lower concentrations.

The principal objective of this investigation was to determine if there were any relationships between the trophic state of freshwater habitats and the numbers of budding and appendaged bacteria. Several Australian freshwater habitats ranging from oligotrophic to eutrophic were selected for the study.

Materials and Methods

Freshwater Habitats

Lake Woronora, Cataract Reservoir, and Prospect Reservoir are three impoundments under the jurisdiction of the Metropolitan Water, Sewage, and Drainage Board of Sydney, New South Wales, Australia. Lake Woronora was impounded in 1941, has a maximum depth of 61 m, and a volume of 71,790 megaliters; during the study the epilimnetic waters had a total phosphorus concentration of 4.0–7.0 mg/m^3. Cataract Reservoir was impounded in 1907. It has a maximum depth of 66 m, a volume of 94,300 megaliters, and the total phosphorus content ranged from 3.0–4.0 mg/m^3. On the basis of their phosphorus concentrations, the two preceding lakes are considered oligotrophic (Ian Smalls, personal communication). Prospect Reservoir was the first reservoir built by the city of Sydney. It was completed in 1888, has a total volume of 8870 megaliters and a maximum depth of 24 m. Although it receives runoff water from its own watershed, the major source of water is piped to it from outlying reservoirs. Total phosphorus in Prospect Reservoir is 8.5–9.0 mg/m^3, a range typical of mesotrophic habitats.

Two eutrophic freshwater ponds also were included in the study. One, less than 1 m deep, was located in Centennial Park, Sydney, whereas the other, about 1–2 m deep called University Lake, was located on the campus of the University of Queensland, St. Lucia.

Sample Collection

The first set of samples was collected in spring (November 1, 1977); the second one was obtained in mid- to late summer (February 2 to March 21, 1978).

The samples were collected manually using sterile 100-ml screw cap bottles. Lake samples were obtained by boat at a depth of about 0.5 m from the surface in the pelagic zone. Pond samples were obtained near shore from a depth midway between the surface and the sediment.

Enumeration

Samples were processed as soon after collection as possible. This was usually immediately after arriving on shore (i.e., within 15 min of collection); in one case (Prospect Reservoir, Feb. 15, 1978) the sample was iced and returned to the laboratory before inoculation.

For the lowest dilution, five 10-ml portions of each freshwater sample were aseptically pipetted into five sterile tubes, each of which contained 1.0 mg Bacto Peptone (Difco). For the next dilution, 1.0 ml portions of the sample were each inoculated into five tubes containing approximately 9.0 ml of dilute peptone broth, DPB [0.01% Bacto Peptone, 20.0 ml/l Hutner's modified salts solution (1), and 10 ml/l vitamin solution (10)]. For the next dilution, 0.1 ml portions of the freshwater samples were pipetted into five tubes of DPB. For subsequent 10-fold dilutions, lake water samples were diluted 1 in 10 in 9.0 ml sterile saline blanks, which were shaken well, and from which 0.1 ml was pipetted into each of five replicate DPB tubes. In this manner a series of 10-fold dilutions was prepared ranging up to the 10^{-9} dilution for eutrophic habitats.

Tubes were incubated at ambient temperature (Ca. 20–30°C) and light and were examined initially after 4 days' incubation. Macroscopic visual examination for turbidity was used as the criterion to determine total viable numbers. Tubes showing growth were marked with a "+." The entire set was examined on a daily basis thereafter until the maximum number of turbid tubes was attained. Total viable numbers were determined from standard 5-tube most probable number tables (9).

Determination of viable numbers of budding and prosthecate bacteria required microscopic examination of each tube individually. This was initiated after 4 to 7 days' incubation beginning with the tubes containing the lowest dilution. A sterile loop was used to remove a portion of the surface pellicle from a tube. With this a wet mount was prepared for examination under oil immersion using a phase microscope. When a morphological type distinctive for a genus or a group of organisms was observed in a particular dilution tube, it was recorded positive for that genus or group. Sets of tubes were examined on at least two successive weekly intervals.

Results and Discussion

As would be expected, total viable numbers of heterotrophic bacteria growing in the test medium employed paralleled the trophic states of the lakes. Oligotrophic Lake Woronora and Cataract Reservoir had between 79 and 330 total viable bacteria per ml whereas Prospect Reservoir, the mesotrophic habitat, contained between 1300 and 4900/ml, and the eutrophic ponds had in excees of 79,000/ml (Table 1).

The dilute peptone procedure used here for the viable enumeration of budding and appendaged bacteria may not provide the highest possible counts of viable heterotrophic bacteria. It was not intended for that purpose. It does provide, however, an indication of the relative concentrations of heterotrophic bacteria from these various habitats, and more importantly, it is the preferred medium for the enrichment and isolation of a number of the heterotrophic budding and appendaged bacteria. Indeed, most isolates of the genera *Caulobacter, Asticcacaulis, Prosthecomicrobium, Ancalomicrobium,* and *Prosthecobacter* have been obtained from enrichment cultures using this medium (7, 8, 13).

Caulobacter spp. were sometimes inhibited in the lower dilution blanks (2) presumably due to competition with other species. We occasionally noted this also, not only with *Caulobacter* but also with the *Hyphomicrobium-Hyphomonas* group and with *Ancalomicrobium*. Cells of these organisms usually could be located in the lower dilutions, but their proportion was often low compared to that in higher dilutions. As with other enrichment cultures this is a selective procedure in which succession occurs, resulting in the dominance of organisms at different times. This problem was partially overcome by examination of tubes on at least two separate occasions. In addition, all tubes in the series were examined regardless of whether lower dilutions were positive or negative. Errors due to succession of types and competition would result in an underestimation of true numbers. Accordingly, numbers provided in Table 1 are minimum estimates of the total numbers of budding and prosthecate bacteria as well as total heterotrophic bacteria.

Caulobacter spp. were the most common member of the prosthecate group encountered in the freshwater habitats. Their highest numbers were recorded in the mesotrophic lake, where 490/ml were found in the spring and 1720/ml in the summer. Their proportion relative to total viable heterotrophic counts was also highest in Prospect Reservoir. The lowest viable counts of *Caulobacter* spp. were found in the oligotrophic habitats in the spring (4.9 to 7.6/ml) and were not much higher in midsummer (23/ml). Their proportion relative to total viable counts was also lower than in the mesotrophic

Table 1. Incidence of budding and prosthecate bacteria in Australian lakes [viable # bacteria/ml (MPN)]

	Oligotrophic lakes						Mesotrophic lake				Eutrophic ponds					
	Lake Woronora				Cataract Reservoir		Prospect Reservoir				Centennial Pond				University Lake	
	Nov. 1, '77		Feb. 2, '78		Nov. 1, '77		Nov. 1, '77		Feb. 2, '78		Nov. 1, '77		Feb. 2, '78		Mar. 21, '78	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total viable counts[a]	79		330		172		1,300		4,900		79,000		490,000		130,000	
Caulobacter spp.	4.9	(6.2)	23	(7.0)	13	(7.6)	490	(37.7)	1,720	(35.1)	330	(0.417)	260	(0.053)	17	(0.013)
Prosthecobacter spp.	0.46	(0.6)	N.D.[b]		0.13	(0.075)	0.46	(0.035)	22	(0.448)	9	(0.011)	90	(0.018)	0.23	(0.01)
Pasteuria-Planctomyces spp.	0.02	(0.025)	0.23	(0.069)	0.08	(0.046)	0.33	(0.025)	1.7	(0.034)	7.9	(0.01)	240	(0.048)	33	(0.025)
Ancalomicrobium spp.	0.08	(0.10)	N.D.		0.08	(0.046)	130	(10.0)	130	(2.65)	0.09	(<0.01)	0.02	(<0.01)	1.4	(<0.01)
Prosthecomicrobium spp.	+[c]	(<0.01)	+	(<0.01)	+	(<0.01)	2	(0.153)	+	(<0.01)	0.14	(<0.01)	2.6	(<0.01)	24	(0.018)
Hyphomicrobium-Hyphomonas spp.	0.27	(0.34)	50[d]	(15.15)	0.05	(0.029)	0.79	(0.06)	1,720[d]	(35.1)	0.46	(<0.01)	3.5	(<0.01)	0.05	(<0.01)

[a] Determined by turbidity in dilute peptone broth.
[b] Not detected.
[c] Observed in at least one tube.
[d] This type grew on dilute peptone agar and produced a red pigment.

habitat. The numbers in the eutrophic habitats varied from 17/ml in University Lake to 330/ml in Centennial Pond. The lowest proportion of *Caulobacter* to total viable heterotrophs was found in these eutrophic habitats.

The numbers of *Caulobacter* spp. found in this study are comparable to numbers found by others in freshwater habitats. In the study by Belyaev (2) the highest numbers of *Caulobacter* spp. were found in the lakes of highest transparency, although all of the lakes studied appeared to be mesotrophic. In the clearest lakes as many as 10,000 *Caulobacter* per ml were reported, whereas in lakes of lower transparency as few as 10/ml were found. *Caulobacter* spp. were found in concentrations from 10 to over 1000/ml in the eutrophic stream, but as in the eutrophic habitats in this study their proportion relative to total viable heterotrophs was less than 1% (11). Unpublished analysis of Lake Washington samples indicates that this mesotrophic habitat has a high proportion of *Caulobacter* spp. during midsummer months when their viable numbers exceed 1000/ml.

Asticcacaulis spp. were only rarely encountered in any of the samples. The nonmotile fusiform caulobacter, *Prosthecobacter fusiformis* (3, 12), was found in almost all of the samples but only in low numbers relative to *Caulobacter*.

Budding bacteria of the *Pasteuria-Planctomyces* group also were enumerated. Their numbers have been combined, because it was frequently difficult to distinguish one genus from another. Obviously if stalked forms were present in a sample, one could conclude that *Planctomyces* spp. were present; however, since nonstalked daughter cells are produced by *Planctomyces* spp., it would not be possible to conclude that a sample containing *Planctomyces* did or did not have *Pasteuria*. Conversely, if a stalk is not detected using phase microscopy, it cannot be concluded that there are no *Planctomyces* present in the sample because some species produce stalks too fine to be visualized by oil immersion phase microscopy (1). *Pasteuria-Planctomyces* organisms were found in all of the samples but only in low concentrations relative to *Caulobacter* spp. The highest viable counts of this group were found in the more eutropic habitats, but their proportion relative to total viable bacteria was quite uniform in all habitats.

The multiple appendaged prosthecate genera, *Prosthecomicrobium* and *Ancalomicrobium*, were more difficult to enumerate in DPB. Because they lack holdfast structures, only rarely were they found aggregated with other cells in the surface pellicle. More often they were found free-floating within the medium. Thus unless their densities were quite high in a given tube they may have been overlooked. This could account for the low numbers of *Prosthecomicrobium* and *Ancalomicrobium* in most of the samples. A large number of *Ancalomicrobium* spp. (130/ml) was found in Prospect Reservoir during both sampling periods.

The final group enumerated in these freshwater habitats was the *Hyphomicrobium-Hyphomonas* group. Organisms in these genera may appear identical on examination by phase microscopy and thus cannot be differentiated microscopically. They were found to be particularly abundant in the midsummer samples taken from the mesotrophic and oligotrophic lakes. Isolates of these organisms from the highest dilution tubes produced pink to orange colonies. They did not grow on media tested anaerobically for the growth of *Rhodomicrobium* (4, 15). Likewise, they did not grow on media containing methylamine as carbon and nitrogen source. However, growth was observed on dilute peptone agar.

One new appendaged organism was also observed in the investigation. It appeared in high dilutions of the Prospect Reservoir samples during the late summer period. It is a

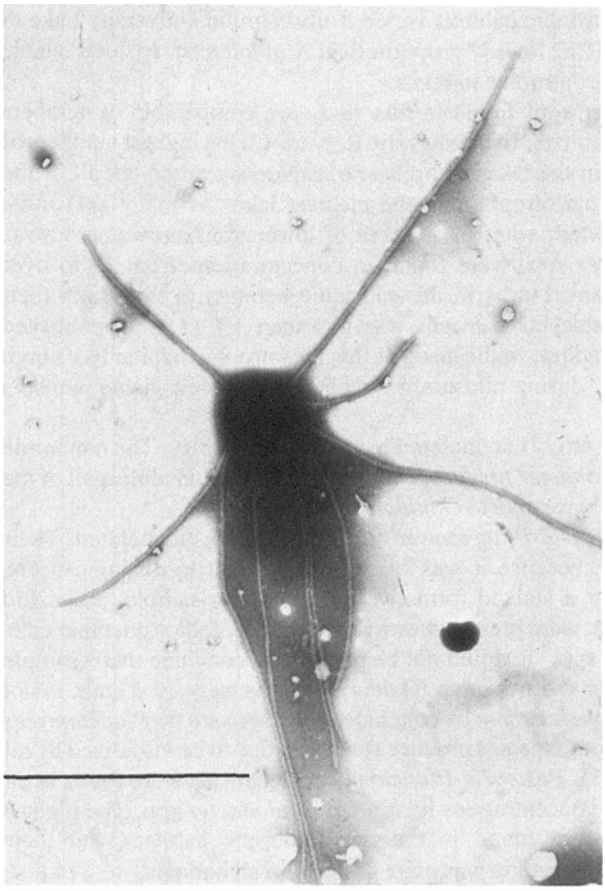

Fig. 1. Negative-stained multiple appendaged microorganism from Prospect Reservoir enrichment tubes. Bar represents 5 μm.

multiply appendaged bacterium with several long and thin (0.1 μm) prosthecae (Fig. 1). Buds were formed directly from the mother appendaged cell. Attempts to isolate it on dilute peptone agar, *Hyphomicrobium* medium (6), and a variety of other media were uniformly unsuccessful, although it could be transferred in mixed culture from one DPB tube to another.

Budding and prosthecate bacteria also have been reported to be abundant in at least one man-made "eutrophic" habitat, namely, pulp mill oxidation ponds. When direct electron microscopic enumeration procedures were used, budding and prosthecate bacteria comprised up to 10% of the total count which was usually in excess of 10^7 bacteria per ml. *Ancalomicrobium*, *Prosthecomicrobium*, *Hyphomicrobium-Hyphomonas*, *Caulobacter*, and *Prosthecobacter* were observed in both sulfite and kraft mill oxidation lagoons (14).

Thus the pattern of environmental distribution of these organisms, especially *Caulobacter* spp., is slowly emerging. There is little doubt they are universally distributed in typical freshwater habitats. Our results on their distribution indicate they may occur in significant concentrations in oligotrophic lakes even though their numbers

and proportion were reduced compared to mesotrophic habitats. This suggests nutrients may be limiting in oligotrophic lakes. Such a view is consistent with finding them in both higher concentrations and higher proportions to total viable counts in mesotrophic habitats. They appear to grow well in some eutrophic habitats, though their low proportion to total viable heterotrophic bacteria suggests they may not have the competitive advantage they possess in habitats with lower concentrations of nutrients.

Based on the results of this study, three avenues of research seem worthwhile pursuing. From a field research standpoint, it would be interesting to determine the seasonal distribution pattern of these bacteria in a mesotrophic lake. Careful physical, chemical, and biological analyses might enable a determination of the factor(s) which result(s) in the seasonal abundance and distribution of these organisms. It would also be of interest to determine whether the holdfast-containing representatives occur free-living or attached to particulate materials in the habitat. Finally, quantitative enumeration of these bacteria in marine habitats is warranted.

Acknowledgments. We thank the Sydney Metropolitan Water, Sewage, and Drainage Board for granting permission and use of facilities and equipment for conducting the investigation. We are grateful to Dr. Ian Smalls for his help in collecting samples and providing information on the limnology of the city's reservoirs. The electron micrograph was kindly provided by Tony McGregor.

This work was supported in part by a National Science Foundation U.S.-Australia Cooperative Science Senior Scientist Fellowship and a University of Queensland Senior Scientist Travel Fellowship.

References

1. Bauld, J., and J. T. Staley: *Planctomyces maris* sp. nov., a marine isolate of the *Planctomyces-Blastocaulis* group of stalked, budding bacteria. J. Gen. Macrobiol. **97,** 45-55 (1976)
2. Belyaev, S. S.: Distribution of the caulobacter group of bacteria in the Volga-Don reservoirs (English Translation). Microbiology **36,** 130-133 (1968)
3. deBont, J. A. M., J. T. Staley, and H. S. Pankratz: Isolation and description of a non-motile, fusiform, stalked bacterium, a representative of a new genus. Antonie van Leeuwenhoek **36,** 397-407 (1970)
4. Duchow, E., and H. C. Douglas: *Rhodomicrobium vannielii,* a new heterotrophic bacterium. J. Bacteriol. **48,** 409-416 (1949)
5. Henrici, A. T., and D. E. Johnson: Studies of freshwater bacteria. II. Stalked bacteria, a new order of schizomycetes. J. Bacteriol. **30,** 61-93 (1935)
6. Hirsch, P.: *Hyphomicrobium.* In R. E. Buchanan and N. E. Gibbons (eds.): Bergey's Manual of Determinative Bacteriology. Williams & Wilkens Co., Baltimore, (1974)
7. Houwink, A. L.: *Caulobacter* versus *Bacillus* spec. div. Nature **168** 654-655 (1951)
8. Poindexter, J. L. S.: Biological properties and classification of the *Caulobacter* group. Bacteriol. Rev. **28,** 231-295 (1964)
9. Standard Methods for the Examination of Water and Wastewater, 13th Ed. American Public Health Association, New York (1971)
10. Staley, J. T.: *Prosthecomicrobium* and *Ancalomicrobium:* new prosthecate freshwater bacteria. J. Bacteriol. **95,** 1921-1942 (1968)
11. Staley, J. T.: Incidence of prosthecate bacteria in a polluted stream. Appl. Microbiol. **22,** 496-502 (1971)
12. Staley, J. T., J. A. M. de Bont, and K. deJonge: *Prosthecobacter fusiformis* nov. gen. et sp., the fusiform caulobacter. Antonie van Leeuwenhoek **42,** 333-342 (1976)
13. Staley, J. T.: Budding and prosthecate bacteria. In A. I. Laskin and H. A. Lechevalier (eds.): Handbook of Microbiology, Vol. I, Bacteria. Chemical Rubber Co., Cleveland (1977)
14. Stanley, P. M., E. J. Ordal, and J. T. Staley: High numbers of prosthecate bacteria in pulp mill waste aeration lagoons. Appl. Environ. Microbiol. **37,** 1007-1011 (1979)
15. Whittenbury, R., and C. S. Dow: Morphogenesis and differentiation in *Rhodomicrobium vannielii* and other budding and prosthecate bacteria. Bacteriol. Rev. *41*:754-808 (1977)