La mer 24: 117-124, 1986
Société franco-japonaise d'océanographie, Tokyo

The bacterial adhesion to copepods in coastal waters in different parts of the world*

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Abstract: The incidence of copepods (*Acartia* spp.) with bacteria (ICWB) was examined for samples obtained from 7 different coastal areas; Kuberg in northern Norway, Conception Bay in Canada, Woods Hole and San Francisco Bay in the United States, Vera Cruz in Mexico, the Caribbean Sea and Melanesia. Values of ICWB varied with areas and months and they were generally less than 10 % as previously reported in other coastal areas. However, an extremely high value, 83.8 %, was obtained from Woods Hole copepods. Slime was observed on copepods from Woods Hole, San Francisco Bay and Vera Cruz, suggesting the presence of bacteria capable of producing polysaccharides outside the cell wall.

1. Introduction

SEM examinations of copepods collected from aquatic environments in Bangladesh (COLWELL *et al.*, 1980), three Nebraska lakes (HOLLAND and HERGENRADER, 1981) and Tokyo Bay waters (NAGASAWA *et al.*, 1985) visually revealed bacterial attachment to copepods. NAGASAWA and NEMOTO (1986) reported that bacterial colonization of copepods is a widespread phenomenon in marine coastal areas. They examined copepods from Tokyo Bay (Japan), Po estuary (Italy), Miramare (Italy), Bay of Naples (Italy) and Helgoland (Germany). Furthermore, NAGASAWA and NEMOTO (in press) demonstrated the incidence of copepods associated with bacteria in Tokyo, Sagami, Suruga and Otsuchi Bays in Japan as well as the yearly change in the incidence of such copepods in Tokyo Bay. The present study provides further information on the incidence of copepod-bacteria associations taking place in coastal waters in different parts of the world.

2. Materials and methods

Plankton samples obtained from 7 different localities (Fig. 1) were used in this study to determine whether bacterial colonization of copepods is a global phenomenon in marine coastal areas. The seven areas include 1) Kuberg in northern Norway (DAVIS, 1977), 2) Conception Bay in Canada (DAVIS, 1982), 3) Woods Hole and 4) San Francisco Bay in the United States, 5) Vera Cruz in Mexico, 6) the Caribbean Sea and 7) Melanesia. Samples obtained from 4), 5), 6) and 7) belong to the Smithsonian Oceanographic Sorting Center; samples from the Caribbean Sea were obtained during the Caribe I cruise of the R/V Alpha Helix while samples from Melanesia were collected during the Moro Expedition by the R/V Alpha Helix. Samples used in this study were composed of 10 from 1), 3 from 2), 1 from 3), 5 from 4), 2 from 5), 5 from 6) and 5 from 7) (Fig. 2, Table 1).

Adults of *Acartia* spp. were removed from the preserved plankton samples; specimens obtained from 1) and 2) were *A. longiremis* according to DAVIS (1977, 1982), whereas those from 3) were *A. tonsa* (Onbé, personal communication). Species of *Acartia* obtained from other areas (4, 5, 6 and 7) were not identified. These adult copepods were examined in a JSM-35 scanning electron microscope following the preparation procedure described by NAGASAWA *et al.* (1985). The number of specimens examined per sample varied with the seven areas (Fig. 2, Table 1). Incidence of copepods with bacteria (ICWB) is defined as the percentage of number of copepods infested to number of those examined for each sample. Although some copepods had large numbers of bacteria and other had a small number of bacteria, only the percentages of

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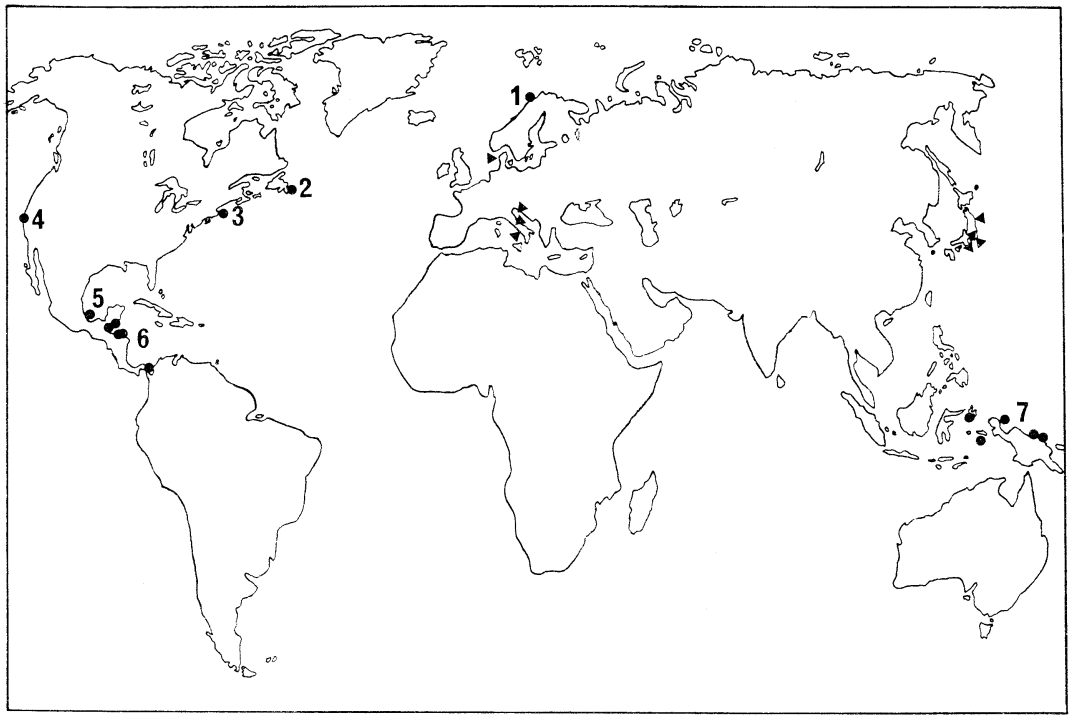

Fig. 1. Map showing 7 areas (●) where samples examined in this study were obtained and another areas (▲) previously investigated by NAGASAWA and NEMOTO (1986, in press). 1, Kuberg; 2, Conception Bay; 3, Woods Hole; 4, San Francisco Bay; 5, Vera Cruz; 6, Caribbean Sea; 7, Melanesia.

copepods with and without bacteria will be discussed here. The term "slime", as described by ROTH (1977), is used in the present study: a network of carbohydrate fibers not distinctly associated with any one bacterium, but apparently serving a community function in holding an aquatic slime layer to a surface.

3. Results

Attachment of bacteria to copepods was usually selective. Bacteria were found more frequently on the ventro-lateral side of the copepod than on the dorsal side, except animals obtained from Woods Hole. Body parts where bacterial attachment occurred were not always identical for animals from different areas. Generally a small number of bacteria were attached to individual copepods from Kuberg, Conception Bay, Vera Cruz and the Caribbean Sea. In contrast, large numbers of bacteria were attached to individuals obtained from Woods Hole and San Francisco Bay. Melanesia samples included specimens with

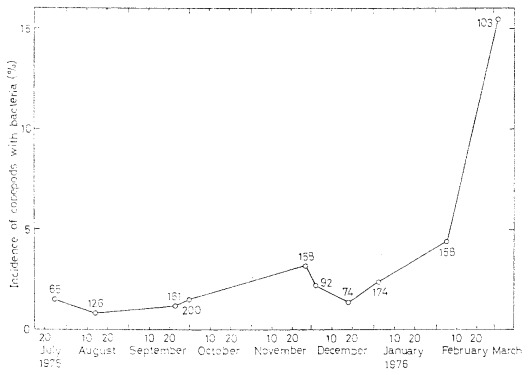

Fig. 2. Change in the incidence of copepods with bacteria in Kuberg during the period from July 25, 1975 through March 2, 1976. Figures show the number of copepods examined.

small to large numbers of bacteria. Most bacteria attached to copepods were rods; they were short, long, thick, slender or indented rods (Fig. 3). Along with these rods, beaded bacteria were observed (Fig. 3i).

Table 1. Data on sampling records of copepod collections and incidence of copepods with bacteria. Figures in parentheses indicate numbers of copepods examined. For data on Kuberg copepods see Fig. 2.

Area	Station	Date	Incidence of copepods with bacteria (%)	Remarks
Conception Bay	B	Feb. 1, 1978	0(59)	*Acartia longiremis*
	B	Mar. 24, 1978	0(75)	
	C	Mar. 24, 1978	1.0(203)	
Woods Hole	1	Sept. 1, 1984	83.9(117)	*Acartia tonsa*
San Francisco Bay	19	Sept. 16, 1980	4.2(189)	*Acartia* sp. 15.0°C, 28.8‰S
	24	Dec. 16, 1980	0(160)	11.6 29.3
	30	Dec. 16, 1980	0(156)	11.2 29.2
	32	Dec. 16, 1980	0(261)	10.9 28.7
	168	Nov. 12, 1980	0(123)	16.1 28.8
Vera Cruz	M3. 1	Nov. 19, 1981	11.3(124)	*Acartia* sp. 28°C, 12‰S
	M9. 1	Sept. 18, 1982	2.7(226)	32 13
Caribbean Sea	PN-51-60	June 29, 1977	0(127)	*Acartia* sp. Sasardi Islands, Panama
	PN-51-260	July 15, 1977	6.2(113)	Glover's Reef, Belize
	PN-52-260	July 15, 1977	5.0(119)	Glover's Reef, Belize
	PN-55-260	July 15, 1977	3.8(160)	Turneffe Islands, Belize
	PN-65-260	July 17, 1977	0(162)	Southern Lagoon, Belize
Melanesia	68-PN-80	June 21, 1979	0(144)	*Acartia* sp. Muschu Bay, Papua New Guinea
	73-PN-80	June 22, 1979	1.5(134)	Wewak Harbor, Papua New Guinea
	89-PN-80	June 30, 1979	2.0(147)	Korrida Bay, Indonesia
	129-PN-80	July 7, 1979	2.4(84)	Ambon Harbor, Indonesia
	145-PN-80	July 14, 1979	1.1(87)	Dodinga Bay, Indonesia

1) Kuberg

Bacteria were found near the base of antennules, antennae and legs, around the labrum, in the depressed parts of the lateral side and on the back. Large colonies of bacteria were rare while attachment of a small number of bacteria was common (Fig. 3a). Each of 10 samples included some copepods (*A. longiremis*) with bacteria. Incidence of copepods with bacteria (ICWB) ranged from 0.8 to 15.5 % during the period from July 25, 1975 through March 2, 1976 (Fig. 2). Incidence was not significantly different in 9 samples from July through February, but the difference in ICWB was significant between these 9 and a sample taken in March.

Temperature and salinity records were available at depths of 0 and 50 m for August, September, January, February and March. The temperature ranged from 1.9 to 8.6°C at the surface and from 2.7 to 7.5°C at 50 m. Variation of salinity was small; 31.2 to 33.0‰ at the surface and 32.5 to 33.4‰ at 50 m.

2) Conception Bay

Bacterial attachment was rare. Bacteria were observed near the base of antennules and on the ventral side. Most often a small number of bacteria were found (Fig. 3b), although colonies occurred occasionally (Fig. 3c). Among three samples examined, only one contained copepods (*A. longiremis*) with bacteria. The value of ICWB was small, 1.0 %.

Temperatures at Stn. B ranged from −1 to 0°C from surface down in February, while it was uniform at −1°C in March. Salinities were between 31 and 32‰. The average bacterial concentration ranged from 10^4 to 10^5 per ml (acridine orange counts) in Conception Bay waters (D. Deibel, personal communication).

3) Woods Hole

The heavy colonization of bacteria (large numbers of dense colonies) was observed on the ventral side as well as on the dorso-lateral side. Slime was very noticeable (Fig. 3d). Large

numbers of bacteria were also found in the joints of segments (Fig. 3e), near the base of antennules, antennae and legs, around the labrum and on the setae of legs. Bacteria often covered most of the whole body. Frequent occurrence of such bacterial adhesion was peculiar to copepod (*A. tonsa*) from Woods Hole. ICWB was extremely high, 83.8 %. No temperature and salinity records were available.

4) San Francisco Bay

Bacteria were found near the base of antennules, antennae, and swimming legs, in the joints of segments and on the legs. Slime was sometimes found together with bacteria (Fig. 3f). Colonies of bacteria were often observed (Fig. 3g), while copepods with a small number of bacteria were rare (Fig. 3f). Only one sample among five included copepods with bacteria. Values of ICWB ranged from 0 to 4.2 %. Data on temperatures and salinities are listed in Table 1.

5) Vera Cruz

Bacterial adhesion was observed near the base of antennules and antennae, around the labrum and on the setae of legs. In most cases a small number of bacteria were attached to copepods (Fig. 3h); large numbers of bacteria were rare (Fig. 3i). A small amount of slime was observed (Fig. 3h). Both samples included copepods with bacteria. ICWB ranged from 2.7 to 11.3 %. Temperature and salinity records are listed in Table 1.

6) Caribbean Sea

Bacterial adhesion was observed only near the base of antennules, antennae and mandible. Only a small number of bacteria were colonized (Fig. 3j). Among 5 samples examined, three included copepods with bacteria. Incidence of bacterial adhesion to copepods ranged from 0 to 6.2 %. Data on temperatures and salinities are not available.

7) Melanesia

Bacteria were found near the base of antennules, antennae, and legs, around the labrum and in the joints of segments. The number of bacteria found ranged from small to large numbers (Figs. 3k and l). Among 5 samples, four included copepods with bacteria. ICWB ranged from 0 to 2.4 %. Temperature and salinity records are not available.

4. Discussion

Values of ICWB varied with areas and months; generally they were less than 10 % as in Bay of Naples, Po estuary and Miramare (NAGASAWA and NEMOTO, 1986), Tokyo Bay, Sagami Bay and Otsuchi Bay (NAGASAWA and NEMOTO, in press). Surprisingly, an extremely high value was obtained from Woods Hole copepods, 83.8 %, which is more than twice the ICWB obtained from Helgoland and from Suruga Bay. Data reported here on ICWB (Table 1, Fig. 2) as well as values reported from elsewhere (NAGASAWA and NEMOTO, 1986, in press) indicate a wide distribution of copepods with associated bacteria.

NAGASAWA and NEMOTO (1986, in press) suggest that ICWB may depend on the number and species of bacteria occurring in the sea rather than on hydrographic elements such as temperature, salinity and chemical oxygen demand. Data available on bacterial numbers in the areas shown in Fig. 1 are very few. In addition, methods used to count bacteria are not identical; bacterial numbers are based either on plate counts (viable bacterial counts) or acridine orange counts. Near Helgoland bacterial numbers by plate counts were 10^1 to 10^3 per ml in the water (HICKEL and GUNKEL, 1968). Bacterial counts by acridine orange ranged from 10^4 to 10^5 per ml in Conception Bay waters. However, bacterial concentration may be lower than these values if it is estimated by plate counts. Compared with values of ICWB between Helgoland and Conception Bay, they were much higher in the former than in the latter. Viable bacterial counts gradually decreased (10^4–10^5, 10^3–10^4, 10^2–10^3 per ml) from the inner part to the outer part of Tokyo Bay (NAGASAWA and NEMOTO, in press). Despite such change of bacterial numbers, values of ICWB (4.5, 7.7, 3.7 %) were not significantly different. Although Suruga Bay waters had the lowest concentration of bacteria, 10^1 per ml among waters examined by NAGASAWA and NEMOTO (in press), Suruga Bay copepods had the highest value of ICWB, 39 %. This suggests that there is no obvious correlation between bacterial counts and values of ICWB. The present study includes insufficient data on environmental factors and bacterial concentrations, but this drawback may be negli-

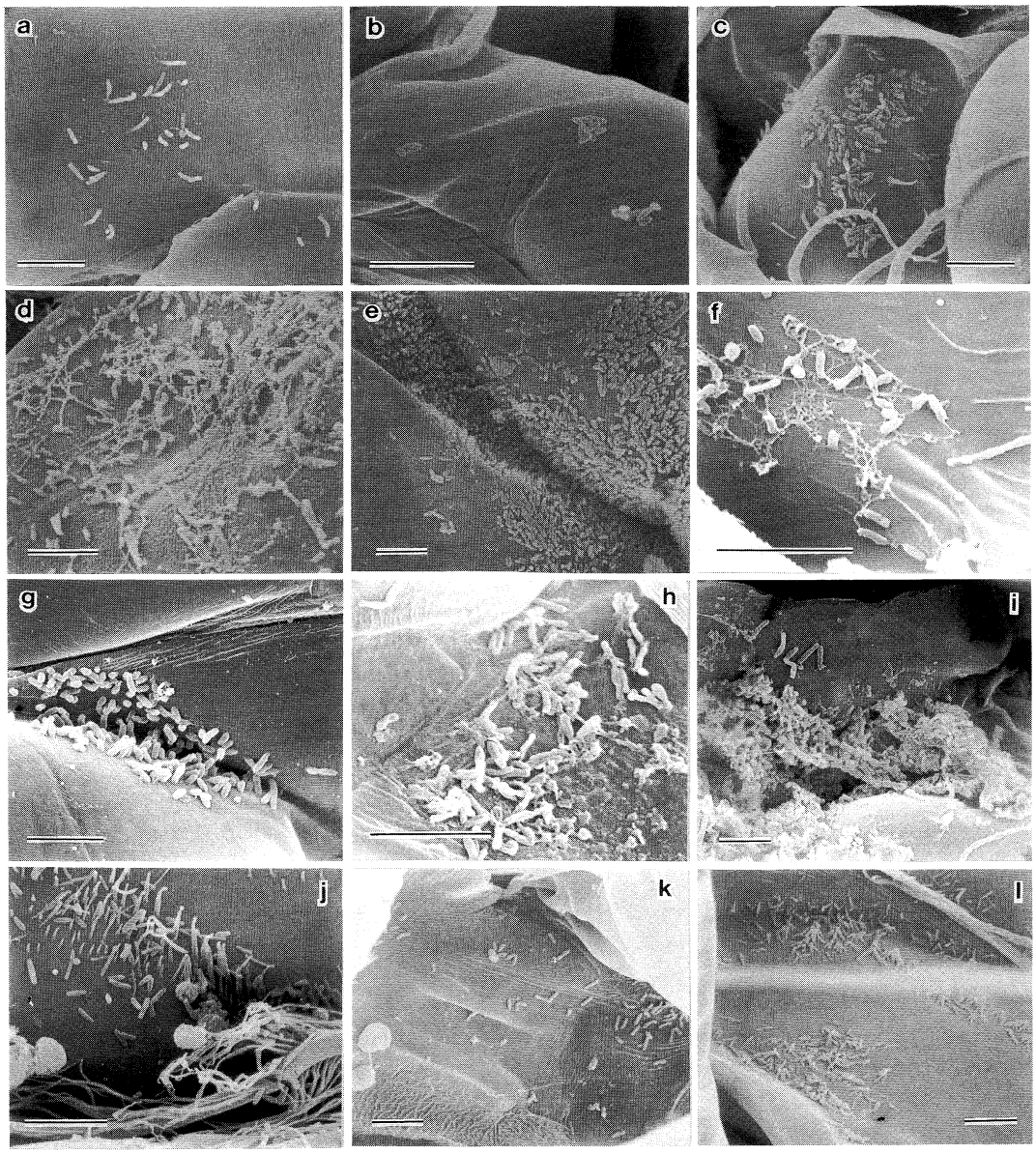

Fig. 3. Scanning electron micrographs of bacterial attachment to copepods obtained from Kuberg (a), Conception Bay (b and c), Woods Hole (d and e), San Francisco Bay (f and g), Vera Cruz (h and i), Caribbean Sea (j) and Melanesia (k and l). Scale bars indicate 5 μm. a, Short and long rods are scattered around the labrum of *Acartia longiremis*. b, A small number of short rods are attached near the antennule of *A. longiremis*. c, A colony of short rods is present near the labrum of *A. longiremis*. d, Short rods are present together with large amounts of slime. e, A high concentration of short rods is observed in the joints of segments. f, Short rods, a long one and slime are present near the antenna. g, A large number of short rods are attached to the joints of segments. h, Indented rods are colonized near the base of antennule along with a small amount of slime. i, Short rods are densely colonized, whereas long rods and beaded bacteria are also attached. j, Bacteria are scattered on the ventral side. k, A small number of bacteria are attached near the labrum. l, A few colonies of bacteria are observed at the base of antennule.

gible. The tendency for bacteria to become attached to copepods may depend on the physiological activity and developmental stages of host animals rather than on bacterial numbers in the water.

In many cases adhesion of bacteria is due to the presence of extracellular polymers, which may comprise polysaccharide and/or protein (FLETCHER, 1980). In general, attachment by extracellular polymeric adhesives involves three stages: reversible adhesion, irreversible adhesion and microcolony formation. After an initial stage of reversible adhesion, firm attachment or irreversible adhesion (FLETCHER, 1980) occurs and is usually followed by a third stage. During this stage attached cells multiply and are joined by additional attaching cells. This then leads to the formation of microcolonies. Bacteria colonized on copepods in this study correspond to an advanced third stage of attachment. Difference in the number of bacteria or their colonies may depend on hours or days elapsed after start of bacterial attachment to the copepod.

Many procaryotes synthesize organic polymers which are deposited outside the cell wall, as a loose, more or less amorphous layer called a capsule, or slime layer. Slime was observed on copepods from Woods Hole, San Francisco Bay and Vera Cruz (Fig. 3); in particular Woods Hole samples had slime which densely covered some parts of the copepod surface. Such marked occurrence of slime is due to bacteria capable of producing polysaccharides outside the cell wall, suggesting that such unique bacteria are present in Woods Hole, San Francisco Bay and Vera Cruz. These exopolymers often form much more widely dispersed accumulation, in part detached from the cells that produce them as observed in Fig. 3 and samples from Shinhamako (NAGASAWA, unpublished data). This slime layer is clearly not essential to cellular function; many bacteria do not produce it (STANIER *et al.*, 1976). The colonization of bacteria producing organic polymers as extracellular slime is rather uncommon and is unlike bacterial colonization previously reported by NAGASAWA *et al.* (1985), and NAGASAWA and NEMOTO (1986, in press).

Acknowledgements

I express my sincere thanks to Prof. T. NEMOTO, Ocean Research Institute, University of Tokyo, for his interest in this study. I thank Dr. C.C. DAVIS, Memorial University of Newfoundland, and Dr. F.D. FERRARI, Smithsonian Institution, for kindly providing me with the samples of copepods obtained from Kuberg, Conception Bay, San Francisco Bay, Vera Cruz, Caribbean Sea and Melanesia and for their careful reviews of the manuscript. I also wish to thank Dr. J. CHENEY, Woods Hole Oceanographic Institution, who helped me with collecting samples there. My special thanks are due to Dr. D. DEIBEL, Memorial University of Newfoundland, who offered laboratory space at Marine Sciences Research Laboratory while I was there to remove specimens of copepod from the samples possessed by Dr. C.C. DAVIS. I would like to express my sincere gratitude for efforts Drs. C. C. DAVIS and F.D. FERRARI made to obtain data on temperature and salinity; data from San Francisco Bay and Vera Cruz were provided by the courtesy of Drs. J. ORSI and B.S. CLEMENTINA, respectively.

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世界各地の沿岸におけるかいあし類への細菌付着

永　沢　祥　子

要旨: 細菌が付着する *Acartia* 属かいあし類の出現率（ICWB）を7つの沿岸海域，すなわちノルウェー北部のクーベルク，カナダのコンセプション湾，米国のウッズホールとサンフランシスコ湾，メキシコのヴェラクルツ，カリブ海およびメラネシアから得た試料について調べた。ICWB の値は海域と月により変化した。その値は以前他の沿岸海域で報告されたように一般に 10% より小さかった。しかし，きわめて高い値 83.8% がウッズホールのかいあし類から得られた。ウッズホール，サンフランシスコ湾，ヴェラクルツのかいあし類には細菌とともに粘質物が観察された。これは細胞壁の外部にポリサッカライドを産生する細菌が存在することを示唆する。