Baltic Coastal Zone
No. 10

(61-72)
2006

Institute of Biology and Environmental Protection
Pomeranian Pedagogical University
Słupsk

SCANNING ELECTRON MICROSCOPY INVESTIGATION OF BACTERIAL COLONIZATION OF MARINE BEACH SAND GRAINS

Zbigniew Jan Mudryk[1], Beata Podgórska[2]

[1] *Department of Experimental Biology, Pomeranian Pedagogical Academy, Arciszewskiego 22, 76-200 Słupsk, Poland*
[2] *Department of Genetic and Marine Biotechnology, Institute of Oceanology, PAS św. Wojciecha 5, 81-347 Gdynia, Poland*

Abstract

Scanning electron microscopy was employed for the investigation of bacteria living on sand grains in a sandy marine beach in the Gulf of Gdańsk (southern Baltic Sea). Bacteria colonize the sand grains; individual topography and shape of the grains were decisive for the colonization. Grains of diverse topography characterised by a great irregularity of shape were preferred, and protected surface sites were favoured. Many of the attached bacteria were found to produce polymer secretions; entire colonies attached by means of polymer nets were observed. A significant morphological diversity of bacteria in the vertical profile of the beach was determined. Bacteria inhabiting the sand grains showed the ability to reproduces.

Key words: Baltic Sea, beach, sand grains, bacteria

INTRODUCTION

Marine sandy beches are the most dynamic of soft bottom habitats whose structure is determined by the wind, sand and water in the state of constant motion (McLachlan et al. 1996, Rodriguez et al. 2003, Rodil and Lastra 2004). Very often they are subject to considerable anthropopressure due to their recreational role (Węcławski et al. 2000).

Despite the extreme conditions these habitats are favourable for the development of microorganisms. This is mainly due to: 1) large surface area for the adsorption of nutrients and for microbial colonisation, 2) a high porosity resulting in an aerobic environment, 3) temperatures generally higher than in totally submerged environments, and 4) a constant resupply of nutrients provided by the action of waves and tides (Novitsky and MacSween 1989). As a result, sandy beaches and splash zones are abundantly inhabited by benthic microflora including mainly bacteria; some of them, when attached to the sand grains are generally known as epipsammon (Brown and McLachlan 1990). Bacteria as source food utilize mainly the metabolic products of phytobenthos, animal faeces, mainly those produced by meio- and macrofauna and dead remains of plants and animals (Koop and Griffiths 1982, Mudryk et al. 2001). According to Koop et al. (1982), Jędrzejczak (1999) and Ochieng and Erftemejer (1999) bacteria play a key role in decomposition and mineralization the beached organic matter and nutrient recycling into the nearshore ecosystem.

The objective of the present study was to determine the distribution of bacteria and their adsorption on the surface of the sand grains, and to determine the morphological diversity of bacteria in the vertical profile of the marine beach.

MATERIAL AND METHODS

The studies were carried out on the sandy beach near Sopot, located in the Gulf of Gdańsk (southern Baltic Sea) (54°27′N, 18°33′E) (Fig. 1). It represents a dissipative beach type with longshore bars and troughs, composed mainly of medium grain size quartz sand (Węcławski et al. 2000).

Sand samples were collected at four sites on non-tidal a beach transect (Urban-Malinga and Opaliński 1999). Sampling sites were located as follows: 1) in the sea, approximately 1-1.5 m from the waterline into the water, at a depth of about 1 m, 2) at the waterline, 3) on the beach, at distance 30 m from the waterline, 4) in the dune, about 60 m away from the shore (Fig. 1).

Surface (0-10 cm) sand samples were collected by Morduchaj-Boltowski core scoop. Sand samples were placed in sterile glass boxes, placed on ice and immediately transported to the laboratory. The time between sample collection and microscopic analyses usually did not exceed 2-3 hours.

The sand samples were treated according to the procedure described by Weise and Rheinheimer (1978) and Novitsky and MacSween (1989). Sand grains were fixed in a solution containing (%, v/v): 2.5 glutaraldehyde; filter-sterilized seawater, 85; and distilled water, 15. After fixation the grains were washed repeatedly with double-distilled sterile water in order to remove salt crystals. The grains were dehydrated in a graded ethanol series (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 96%, each change 5 min) and subsequently in HMDS (hydroxymexamethyldisilazane). After drying, the grains were attached to SEM stubs using double-sided conductive tape and sputter-coated with gold. The samples were examined using a Philips SEM – 515 microscope; the acceleration tension was 20 kV. About 500 sand grains were observed. Micrographs were recorded on Kodak Penatomic X film using a Pentax K 1000 35 mm camera.

Dimensions of bacterial cells were measured from scanning electron micrographs and statistical tests (standard deviation – SD, coefficient of variation – CV, coefficient of dispersion – CD) used in this analysis were based on Velji and Albright (1986). Significant differences between size bacterial cells and studied sites were tested by nonparametric ANOVA Kruskal-Wallis test using the STATISTICA (version 5.2) software package.

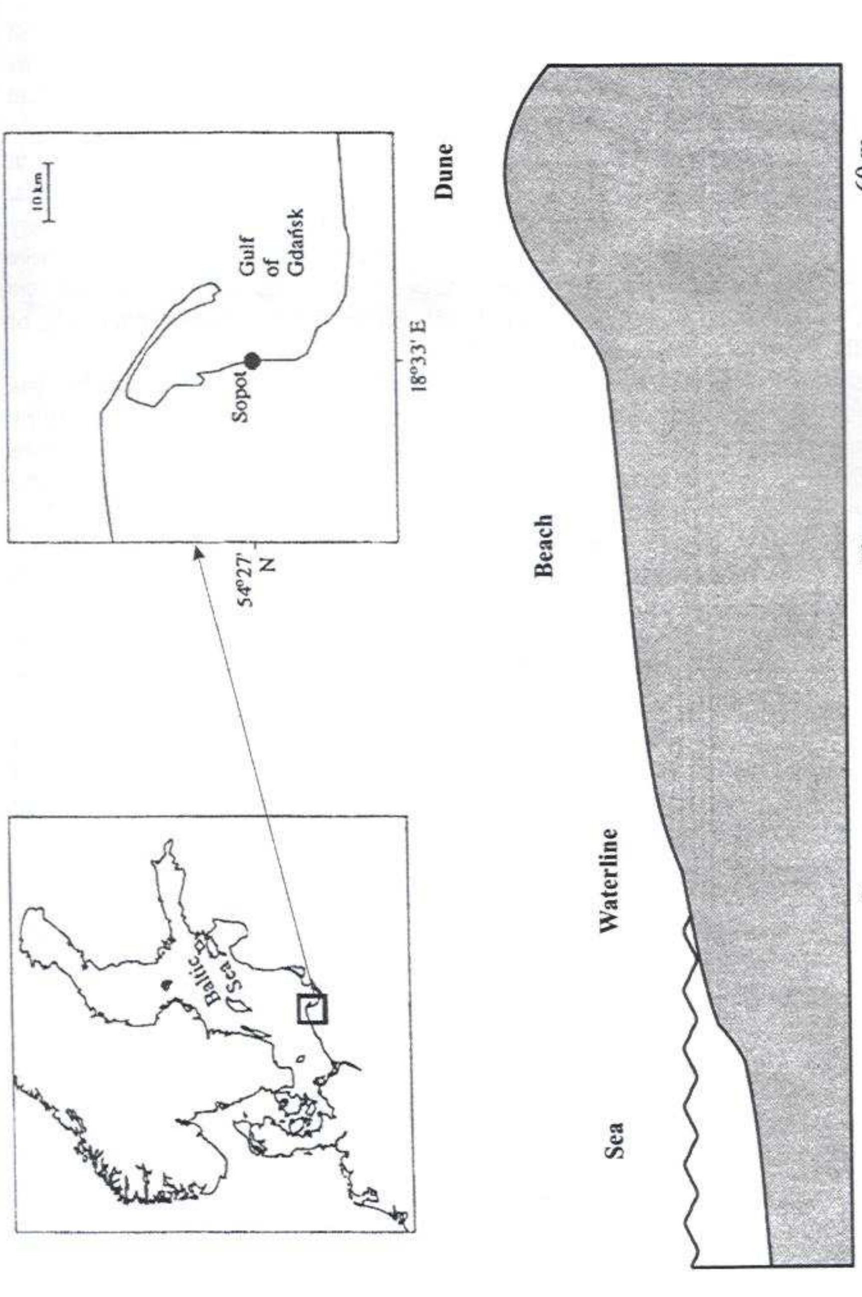

Fig. 1. Map of the study area and location of sampling sites on the sandy beach in Sopot

RESULTS

The topography of bacterial distribution on the sand grains collected from the Sopot beach is shown in Figure 2. The analysis of SEM photographs reveals that only a small part of the surface of the grains was colonized by bacteria. The colonization was with a preference for shielded and irregular surfaces. As a result the bacteria were most often found in crevices, fissures, cracks, and creases (Fig. 2 A, B), or in sand grain breakage sites (Fig. 2 C). Diatoms were also often present; as bacteria, they were confined to topographically protected locations (Fig. 2 D). Only very rarely were the bacteria observed on exposed surfaces or peaks of the grain surface (Fig. 2 E), and on the sharp edges were not observed at all. The sand grains were most often colonized by single bacterial cells, or by small colonies consisting of 5-20 cells, morphologically usually alike (Fig. 2 F).

The analysis of SEM photographs (Fig. 3) reveals that epipsammon-forming bacteria have a specific mechanism of adsorbing to the surface of the sand grains. Many bacterial cells formed characteristic filamentous structures (Fig. 3 A, B, C) by means of which they attached themselves firmly to the sand grains. This protected bacteria living in such a dynamic environment as a marine beach from abrasion resulting from the action of waves and wind. Such filaments were produced by various morphological forms of bacteria, coccal (Fig. 3 A, B) as well as cylindrical cells (Fig. 3 C). Bacteria living in microcolonies, in close vicinity of each other, produced filaments attaching them not only to sand grains, but also to each other, and forming net-shaped structures (Fig. 2 D). In certain cases, as a result of filament merging, bacterial cells formed a biofilm on the surface of the sand grains (Fig. 3 E). Colonial bacteria living in the biofilm formed separate communities strongly attached to the sand grains and well protected from the environmental stress. Not only bacterial cells were attached to the sand grains by polymer filaments; diatoms formed similar structures allowing a more successful colonization of sand grains (Fig. 3 F).

Analysis of Figure 4 and Table 1 reveals differences in morphological types and cell sizes of the epipsammon bacteria between the study sites. A Kruskal-Wallis test showed significance effect ($H = 32.60$, $p < 0.001$, $N = 84$) of sites on size of the bacterial cells. In the sample from site 1 located in the sea, bacterial cells formed

Table 1

The size (µm) of the bacterial cells inhabiting various parts of the studied beach

Zone	Mean	Range	SD	CD	CV (%)	N
Sea	0.19	0.07 - 0.73	0.08	0.03	44.1	71
Waterlinie	1.38	0.77 - 1.85	0.55	0.21	39.8	3
Beach	1.68	1.32 - 2.00	0.32	0.06	19.1	4
Dune	1.56	1.17 - 1.91	0.30	0.05	19.5	6

ANOVA Kruskal-Wallis test – $H(3, N = 84) = 32.60$, $p = 0.001$

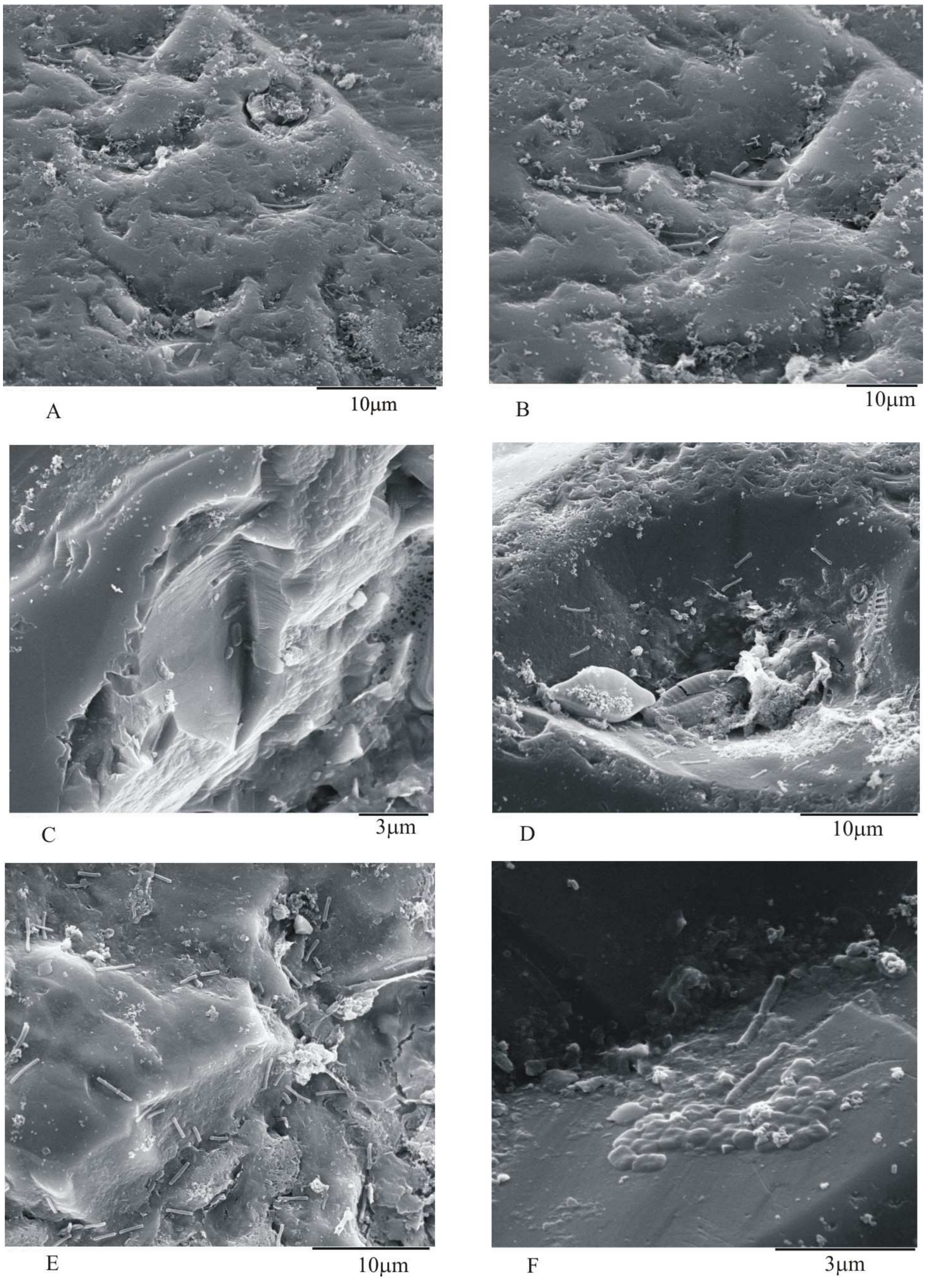

Fig. 2 Scanning electron micographs of quartz sand grains with location of bacteria
A. Total view distribution of bacteria on the surface of sand grain. Note that large surface areas are free of bacterial cells. **B.** Bacteria colonization cervices, fissures, cracks, pores and depressions areas of sand grain. **C.** Location of bacterial cells in grain breakage site. **D.** Bacteria and diatoms located in depth hollow on the grain surface. **E.** A few bacteria observed on exposed surfaces or peaks of the grain surface. **F.** Small colonies of bacteria attached on sand grain

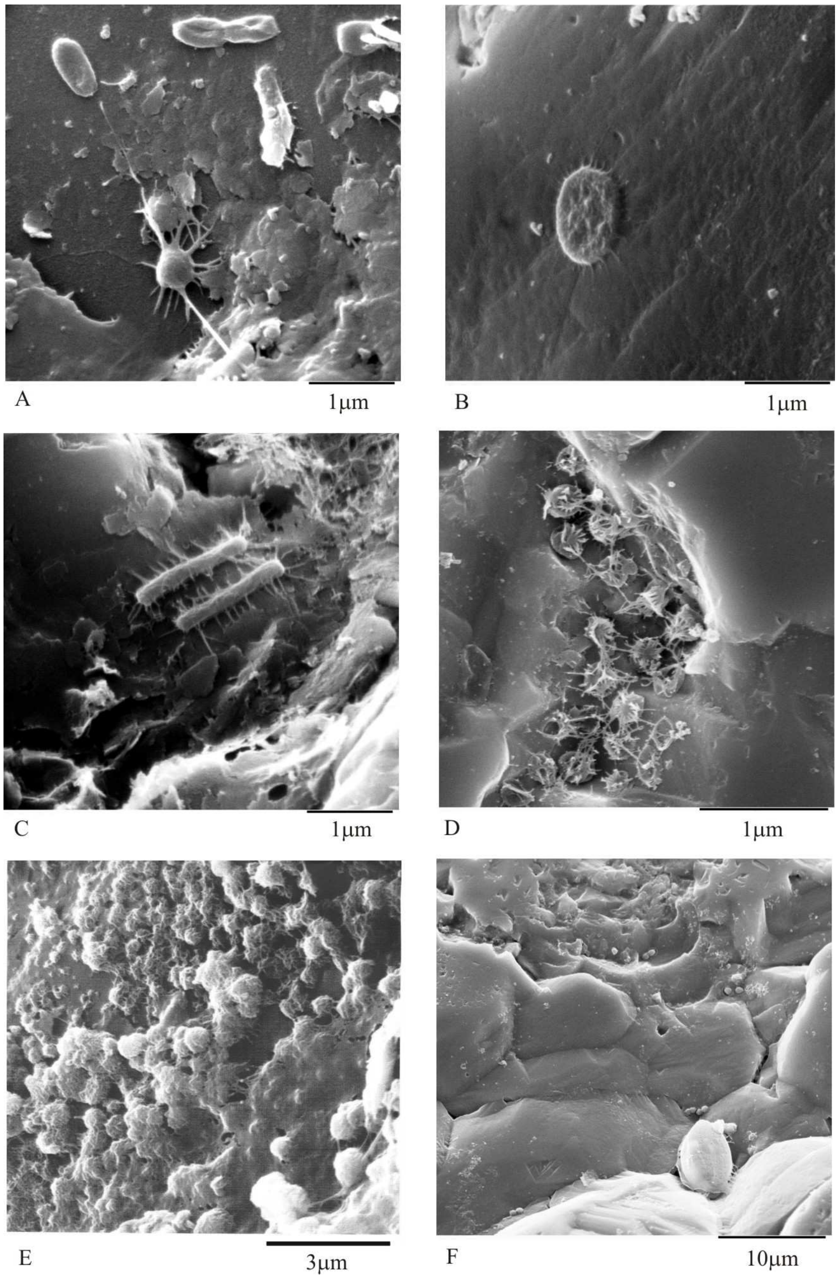

Fig. 3 Filamentous structures attaching bacteria to the sand grains
A. Coccal form bacteria produced long filamentous structures, **B.** Cocci bacteria with short filaments. **C.** Two rods bacteria attached to the grain surface by filaments. **D.** Bacteria microcolonies produced filaments net attaching them to sand grain and also to each other. **E.** Bacterial biofilm attached by filaments to the sand grain, **F.** Diatom formed filaments allowing colonization of sand grain.

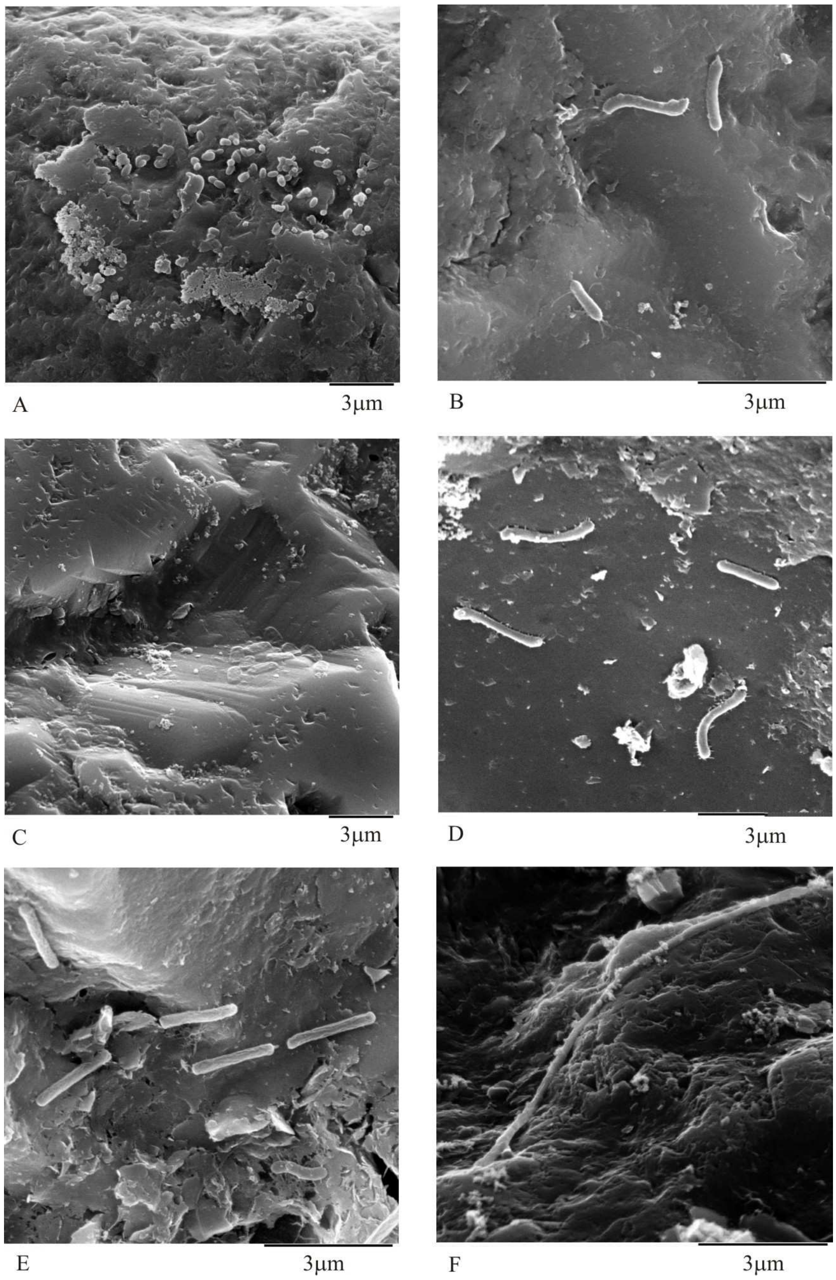

Fig. 4 Morphological diversity of epipsammon bacteria inhabiting various parts of the beach
A. Short cylindrical bacterial cells on the surface of sand grain isolated from sea. **B.** Cylindrical bacteria types inhabiting sand grain in waterline. **C.** Flat and disc-shaped cells bacteria closely adhering to the sand grain. **D.** Rod-shaped forms bacteria isolated from middle beach. **E.** Simple long cylindrical forms bacteria located on sand grain samples from dune. **F.** Bacterium with a long filament isolated from dune

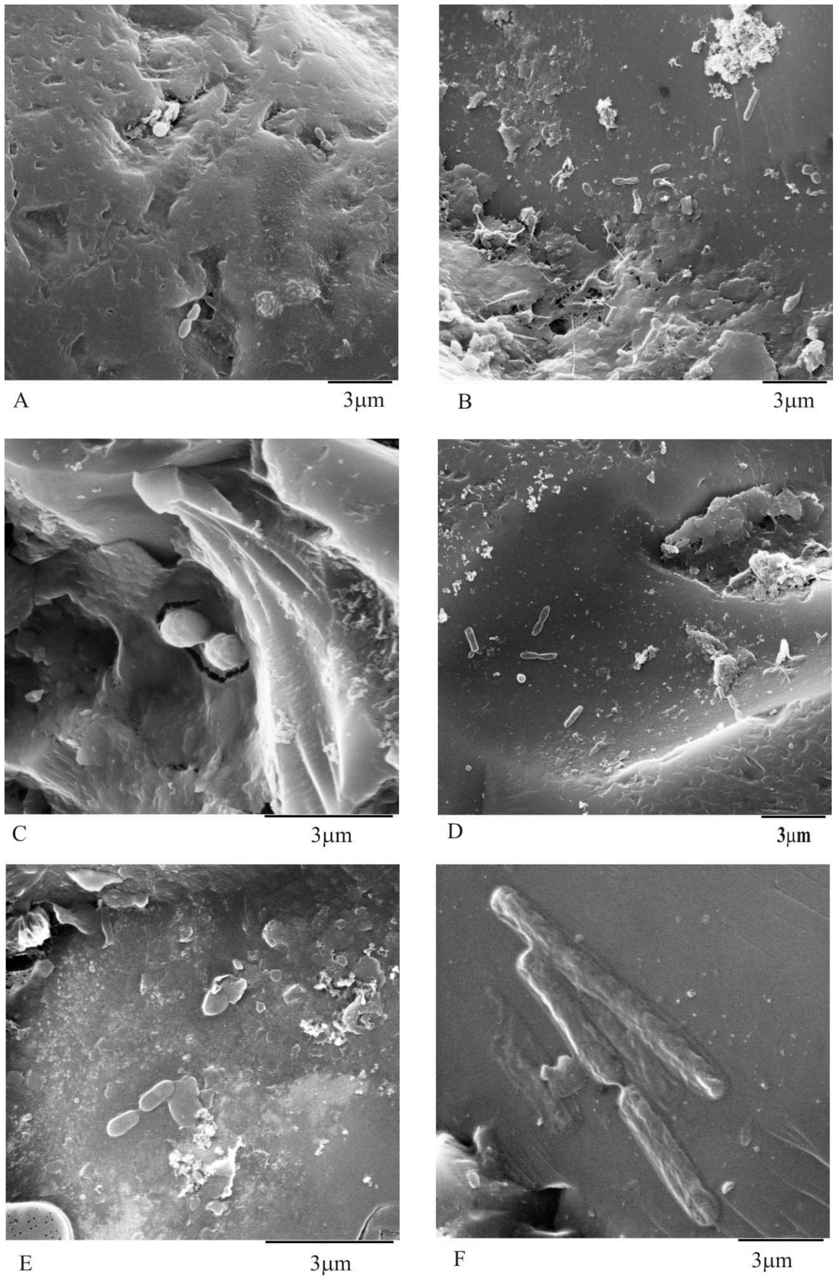

Fig. 5 Division of bacterial cells on sand grains
A. Examples of bacterial reproduction on surface sand grain. **B.** Cylindrical bacteria inhabiting the sand grain showed the ability to reproduces. Note that detrital material is concentrated between bacteria. **C.** Reproduction of coccus bacteria by transversal cell division. **D.** Initial stage of bacterial division. **E.** Sequel stage of cells division. **F.** Final stage bacterial reproduction – daughter cells separate from each other

short cylindrical forms of the mean size 0.19 ± 0.08 μm (mean ± SD) (Fig. 4 A). At site 2 located at the waterline, cylindrical bacteria were larger than in the sea (1.38 ± 0.55 μm) (Fig. 4 B). Flat, disc-shaped cells, closely adhering to the sand grains, and thus well protected from being detached from this substratum, were also observed (Fig. 4 C). On the beach (site 3), similar to the waterline, rod-shaped forms were present (Fig. 4 D). They were, however, longer (1.68 ± 0.32 μm) than those occurring at the waterline. In the dune (site 4) simple long (1.56 ± 0.30 μm) rods predominated (Fig. 4 E); filamentous forms were also noted (Fig. 4 F).

The ability to reproduce is an evidence of the biological activity of bacteria adsorbed to the sand grains. Figure 5 presents examples of bacterial reproduction by transversal cell division. Figures 5 A, B, C show various morphological forms (cylindrical and spherical) of bacteria during cell division, and figures 5 D, E, F show bacterial cells in various stages of this process. In the initial stage of division only a small narrowing can be seen (Fig. 5 D). In the succeeding stages the narrowing becomes bigger, and in the final stage the daughter cells separate from each other (Fig. 5 E, F).

DISCUSSION

In the sandy sediments of marine beaches subjected to extreme activity of waves and wind, over 90% of bacteria is fixed to the surface of the sand grains (Meyer-Reil et al. 1978). Scanning electron microscopy (SEM) characterized by a very high power of resolution allows for a close to perfect observation of bacteria living in a sand grain microhabitat. Employment of SEM makes it possible to study the topography of distribution, numbers, and morphology of the populations of bacteria adsorbed to the sand grains (Weise and Rheinheimer 1978, Novitsky and MacSween 1989). It shows that only a small part of the available sand grain surface is colonized by bacteria. The study carried out by Hargave (1972) and De Flaun and Mayer (1983) with the use of SEM showed that microbial colonization of sand grains was very small compared to the available surface. Bacteria populated as little as 0.01-5% of the available area, which is also confirmed by the observation of microphotographs of the sand grains collected at the Sopot beach. Weise and Rheinheimer (1978) and Yamamoto and Lopez (1985) draw attention to the fact that it is not the surface area that limits the sand grain colonization by bacteria, but their individual topography and their shape. Significantly more bacteria occur on the grains with diverse topography characterised by high degree of irregularity than on grains with smooth surfaces. It was determined that bacteria prefer surfaces with rich texture and tend to colonize cracks, crevices, fissures, pores, and depressions, while they avoid exposed surfaces (Meadows and Anderson 1966, Weise and Rheinheimer 1978, Novitsky and MacSween 1989). The analysis of the SEM photographs of the sand grains from the Sopot beach also reveals that it was those niches that were populated by bacterial single cells and colonies. Because of such localization on sand grains bacteria are able to withstand abrasion and removal stresses of high energy environment, due mainly to the waves and wind. It also protects the bacteria from being eaten by grazing macro- and meiofauna and from mechanical damage caused by direct contact with neighbouring sand grains (De Flaun and Mayer 1983).

Analysis of scanning electron micrographs of bacteria inhabiting sand grains on the Sopot beach reveals that those organisms usually occur as single cells or as small colonies amounting to 5-20 cells. These results fully correspond with the description of the microphotographs of bacteria inhabiting the sand grains on the beaches in the German part of Baltic Sea and North Sea coast (Weise and Rheinheimer 1978) and on two beaches in Nova Scotia (Canada) (Novitsky and MacSween 1989). Those authors point out however that the SEM data must be considered cautiously because the preparation techniques are rather harsh for bacteria. Especially the dehydration processes may destroy the structures by which bacterial cells in microcolonies are attached to each other. In scanning electron micrographs single cells may thus predominate, whereas in natural conditions they formed bacterial colonies.

The results of many studies (Yamamato and Lopez 1985, Defarge et al. 1996, Braitwithe and Gribble 1998) indicate that besides favourable localization, many bacteria form specific structures, which allow for the adsorption to the sand grains and for stable attachment to each other. Those structures are extracellular polysaccharide secretions (EPS). They facilitate adhesion and protect bacteria from the processes of erosion. SEM analysis of the bacteria from the Sopot beach reveals that many of those organisms were also able to synthesise EPS. The studies of Boyle and Reade (1983) and of Decho (1990) show that the EPS matrix plays a key role in the optimal functioning of bacterial cells living on the surface of the sand grains. It gives a very efficient protection from the amplitude of physical and chemical factors, such as pH, salinity, oxygen, temperature, and nutrient concentration. Because of a close vicinity of bacterial cells in the organic matrix, a genetic transfer between them is also facilitated (Loosdrecht et al. 1990). Additionally, it is a very good medium for bacteria to communicate with each other because of numerous canals and nets present (Robinson et al. 1984).

A significant morphological diversity of bacteria in the perpendicular profile of the Sopot beach was determined. Short cylindrical forms, often aggregated in small colonies, predominated in the samples collected in the sea; in the samples from at the waterline, beach and dune single long cylindrical and filamentous cells predominated. This diversification is possibly due to the different a physicochemical conditions (temperature, oxygen, pH, salinity, nutrients, organic matter) along the vertical profile resulting in diverse conditions for the bacteria to live in beach (Novitsky and MacSween 1989, Jędrzejczak 1999).

In summary, the data presented here indicate that individual bacterial cells inhabiting sandy beaches are able to withstand the abrasion and shear stress associated with a high energy environment. Novitsky and MacSween (1989) and Brown and McLachlan (1990) draw attention to the fact that bacteria colonizing sand grains are optimally adapt to the extreme environment of marine beaches, representing a major part of the global marine coast ecosystem.

REFERENCES

Boyle C. D., Reade A. E., 1983. Characterisation of two extracellular polysaccharides from marine bacteria. *Appl. Environ. Microbiol.,* 46, 392-399.

Braitwithe C. J., Gribble C. D., 1998. Phosphatic microbial biofilms gravels in a vadose environment. *J. Coast. Res.*, 14, 1422-1425.

Brown. A. C., McLachlan A., 1990. *Ecology of sandy shores.* Elsevier, Amsterdam.

Decho A. W., 1990. Microbial exopolymer secretions in ocean environments: their role in food webs and marine processes. *Oceanogr. Mar. Biol. Rev.,* 28, 75-153.

De Flaun M. F., Mayer L. M., 1983. Relationships between bacteria and grain surfaces in intertidal sediments. *Limnol. Oceanogr.,* 28, 873-881.

Defrage C., Trichet J., Jaunet A. M., Robert M., Tribble T., Sanspne F. J., 1996. Texture of microbial sediments revealed by cro-scanning electron microscopy. *J. Sedim. Res.,* 66, 935-947.

Hargrave B., 1972. Aerobic decomposition of sediments and detritus as a function of particle surface area and organic content. *Limnol. Oceanogr.,* 17, 583-596.

Jędrzejczak M. F., 1999. The degradation of stranded carrion on a Baltic Sea sandy beach. *Oceanol. Stud.* 3/4, 109-141.

Koop R. G., Griffiths C. L., 1982. The relative significance of bacteria, meio- and macrofauna on an exposed sandy beaches. *Mar. Biol.,* 66, 295-300.

Koop K., Newell R. C., Lucas M. I., 1982. Microbial regeneration of nutrients from the decomposition of macrophyte debris on the shore. *Mar. Ecol. Prog. Ser.,* 9, 91-96.

Loosderecht M. C., Lyklema J., Norde W., Zehnder A. J., 1990. Influence of interfaces on microbial activity. *Microbiol. Rev.,* 54, 75-87.

McLachlan A., De Ruyck A., Hacking N., 1996. Community structure on sandy beaches: patterns of richness and zonation in relation to tide range and latitude. *Revi. Chil. Hist. Nat.,* 69, 451-467.

Meadows P. S., Anderson J. P., 1966. Microorganisms attached to marine and freshwater sand grains. *Nature* 212, 1059-1060.

Meyer-Reil L., Dawson R., Liebezeit G., Tiedge H., 1978. Fluctuation interactions of bacterial activity in sandy beach sediments and overlying waters. *Mar. Biol*., 48, 161-171.

Mudryk Z., Podgórska B., Ameryk A., 2001. Bacteriological characterisation of a Baltic sandy beach in summer. *Ecohydrol. Ecohyrobiol.,* 1, 503-509.

Novitsky J. A., MacSween M. C., 1989. Microbiology of a high energy beach sediment: evidence for an active and growing community. *Mar. Biol. Prog. Ser.*, 52, 71-75.

Ochieng C. A., Erftemejer P. L. A., 1999. Accumulation of seagrass beach cast along the Kenyan coast: a quantitative assessment. *Aqua. Bot.,* 65, 221-238.

Robinson R. W., Akin D. E., Nordsted R. A., Thomas M. V., Aldrich. H. C., 1984. Light and electron microscopic examinations of methane producing biofilms from anaerobic fixed-bed reactors. *Appl. Environ. Microbiol.,* 48, 127-136.

Rodil I. F., Lastra M., 2004. Environmental factors affecting benthic macrofauna along a gradient of intermediate sandy beaches in northern Spain. *Estuar. Coast. Shelf Sci.,* 61, 37-44.

Rodriguez J. G., Lastra M., López J., 2003. Meiofauna distribution a gradient of sandy beaches in northern Spain. *Estuar. Coast. Shelf Sci.,* 58, 63-69.

Urban-Malinga B., Opaliński K., 1999. Vertical zonation of the total, biotic and abiotic oxygen consumption on a Baltic sandy beach. *Oceanol. Stud.,* 28, 85-96.

Velji M., Albright J., 1986. Microscopic enumeration of attached marine bacteria of seawater, marine sediment, faecal matter and kelp blade samples following pyrophosphate and ultrasound treatments. *Can. J. Microbiol.,* 32, 121-126.

Węcławski M., Urban-Malinga B., Kotwicki L., Opaliński K. W., Szymelfenig M., Dutkowski M., 2000. Sandy coastlines are there conflicts between recreation and natural values? *Oceanol. Stud.*, 2, 5-18.

Weise W., Rheinheimer G., 1978. Scanning electron microscopy and epifluorescence investigation of bacterial colonization of marine sand sediments. *Microbial. Ecol.*, 4, 175-188.

Yamamoto N., Lopez G., 1985. Bacterial abundance in relation to surface area and organic content of marine sediments. *J. Exp. Mar. Biol. Ecol.*, 90, 209-220.

BADANIA SKANINGOWE BAKTERII KOLONIZUJĄCYCH ZIARNA PIASKU PLAŻY MORSKIEJ

Streszczenie

W pracy przedstawiono wyniki badań dotyczących bakterii kolonizujących ziarna piasku plaży morskiej zlokalizowanej w rejonie Sopotu. Próby piasku pobierano z morza z odległości około 1,5 m od linii brzegowej (st. 1), z linii brzegowej (st. 2), środkowej części plaży (st. 3) oraz wydmy (st. 4). Przy użyciu mikroskopu skaningowego badano rozmieszczenie bakterii na ziarnach piasku, mechanizm ich adsorpcji do powierzchni ziaren piasku, zróżnicowanie morfologiczne i wymiary oraz ich zdolność do rozmnażania.

Badania te wykazały, że bakterie mają zdolność do selektywnego kolonizowania ziaren piasku preferując głównie osłonięte i nieregularne powierzchnie (szpary, szczeliny, wklęśnięcia, fałdy, rysy). Wiele komórek bakterii kolonizujących ziarna piasku tworzyło charakterystyczne struktury włókniste, za pomocą których organizmy te trwale wiązały się z podłożem. Wykazano istotne zróżnicowanie morfologiczne bakterii na poszczególnych stanowiskach badawczych. Wiele bakterii zaabsorbowanych do ziaren piasku charakteryzowało się zdolnością do rozmnażania.