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SHORT COMMUNICATIONS

Zonal Distribution of Epiphytic Microorganisms on the Eelgrass *Zostera marina*

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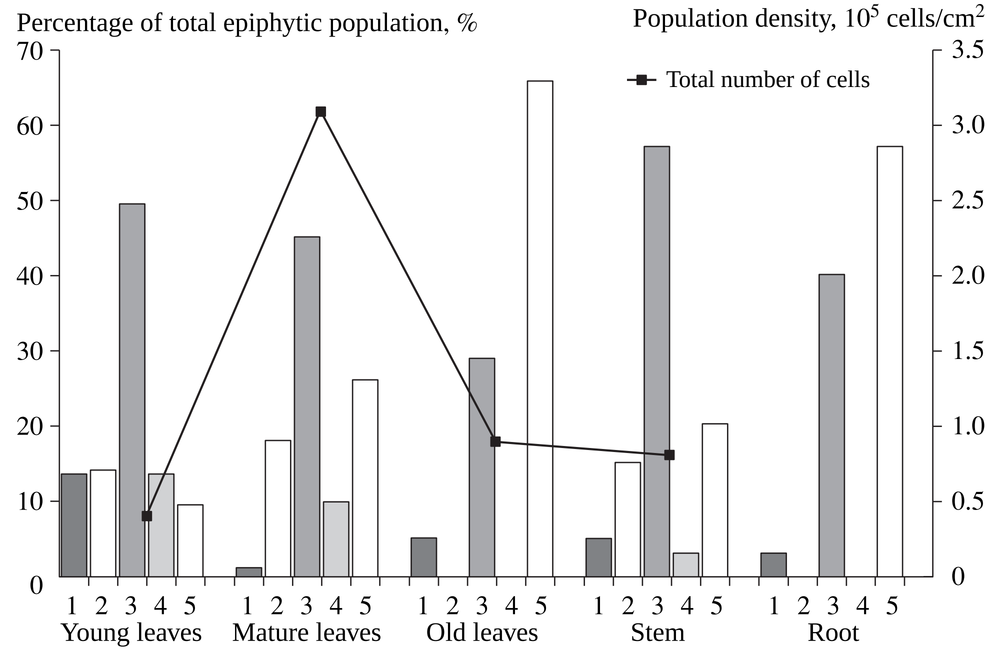
The structure and the functioning of marine bacterial communities colonizing submarine perennial grasses of the family *Zosteraceae* are largely determined by the complex trophic interrelations between the grasses and their epiphytes. The productivity and the biomass of seaweeds, some species of which are used as food or the sources of valuable biologically active compounds [1], are regulated by various biotic and abiotic factors [2]. Seaweeds are colonized by epiphytes, which are represented by microalgae, bacteria, microscopic fungi, and protozoans. When the population of epiphytic organisms appears too large for the seaweed leaves to obtain sufficient light, there may be disturbances in their carbon metabolism and a substantial decrease in the productivity of the marine communities [3, 4]. A symbiotrophic relationship between epiphytic bacteria and the eelgrass *Zostera marina* allows these bacteria to take organic carbon, phosphorus, and nitrogen from the eelgrass leaves [2, 5]. It should be noted that the investigations of the taxonomic diversity of epiphytic bacteria on *Zostera marina* are scarce and limited, to the best of our knowledge, to two studies reporting the isolation of bacteria of the genera *Flavobacterium*, *Pseudomonas*, and *Vibrio* from the surface of this eelgrass [6, 7].

The present work was undertaken to study the distribution of epiphytic bacteria of different taxa over the roots, stems, and young, mature, and old leaves of the eelgrass *Zostera marina*.

Experiments were performed with 166 bacterial strains isolated from the eelgrass surface by the replica plating method (1 cm^2). Grass samples were collected in the summer months of 1998–1999 in Trinity Bay of Peter the Great Bay of the Sea of Japan, where *Zostera marina* forms large beds. The taxonomic characteristics of the strains were determined by the standard methods [8, 9]. The phenotypic studies of bacteria included Gram staining; the determination of the type of flagellation, pigmentation, and metabolism; analysis for the presence of oxidase, catalase, urease, arginine dihydrolases, and lysine- and ornithine decarboxylases; estimation of the bacterial requirement for sodium ions; tests for the production of indole and H_2S; and tests for the hydrolysis of gelatin, casein, starch, Tween-80, chitin, and alginate. The strains were identified to a genus level using the type bacterial strains.

The results obtained in this work showed that, generally, the total population of epiphytic bacteria exceeded the bacterial population of seawater, amounting to 0.3–3.3 × 10^5 cells per cm^2 of the eelgrass surface. It should be noted that the epiphytic bacterial population of mature undamaged eelgrass leaves was an order of magnitude higher than that of young eelgrass leaves (see figure). This finding can be accounted for by the antibacterial activity of some substances (such as tannic and phenylcarbonic acids) secreted by the young eelgrass leaves, owing to which bacterial cells can hardly attach to them [10]. At the same time, the mature eelgrass leaves, which lack such substances, can be more easily colonized by epiphytic marine bacteria.

Among the epiphytic bacteria isolated, we identified actinobacteria, including those of the genus *Micrococcus/Halococcus*, firmicutes with a low G+C content of DNA (*Bacillus/Halobacillus*), the sensu lato cytophages *Cytophaga* and *Flavobacterium*, and the proteobacteria *Alteromonas, Pseudoalteromonas, Marinobacter, Marinobacterium, Fundibacter, Pseudomonas, Halomonas, Shewanella*, and *Vibrio* (see figure). Heterotrophic gram-negative bacteria were dominant (up to 80% of the total number of the isolates). The figure shows how the taxonomic composition of epiphytic bacteria varies depending on the morphological zone and the age of the eelgrass leaves. The members of the phylogenetic cluster *Flavobacterium–Cytophaga–Bacteroides–Flexibacter* were detected on all parts of the eelgrass, comprising from 30 to 55% of the total number of the isolates from particular eelgrass zones. Aerobic proteobacteria of the genera *Alteromonas, Pseudoalteromonas*, *Marinobacter*, and *Marinobacterium* dominated in the phylloplane of old leaves and in the rhizoplane (up to 65 and 56% of bacterial isolates, respectively). It should be noted that young leaves, in spite of being rarely colonized, exhibited the presence of all of the bacterial taxonomic groups studied, with actinobacteria dominating (12%) and proteobacteria being in the minority (8%). The facultatively anaerobic didermic bacteria of the genera *Shewanella*, *Vibrio*, and *Bacillus/Halobacillus* were isolated from the eelgrass



Distribution of epiphytic bacteria on the eelgrass *Zostera marina*: (1) actinobacteria; (2) *Bacillus/Halobacillus*; (3) *Flavobacterium* and *Cytophaga*; (4) *Vibrio* and *Shewanella*; (5) *Alteromonas, Pseudoalteromonas, Marinobacter, Marinobacterium, Halomonas, Fundibacter,* and other unidentified aerobic proteobacteria.

stem, young leaves, and mature leaves but not from the old leaves and roots.

Thus, the taxonomic investigation of the epiphytic bacteria colonizing the eelgrass *Zostera marina* allowed us to reveal, on a macrozone level, the distribution of these bacteria. For instance, the epiphytic bacteria of the genera *Flavobacterium* and *Cytophage* primarily colonized the stems and the young and mature leaves of the eelgrass. The old leaves, where the epiphytic bacterial population was low, were dominated by aerobic proteobacteria. Such changes in the epiphytic bacterial population of the eelgrass may be explained by the involvement of different extracellular products secreted by *Zostera marina* (proteins, polysaccharides, and carbonic acids) in the regulation of bacterial adsorption.

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REFERENCES

1. Loenko, Yu.N., Artyukov, A.A., Kozlovskaya, E.P., Miroshnichenko, V.A., and Elyakov, G.B., *Zosterin*, Vladivostok: Dal’nauka, 1997.
2. Kirchman, D.L., Mazzela, L., Alberte, R.S., and Mitchell, R., Epiphytic Bacterial Production on *Zostera marina*, *Mar. Ecol. Progr. Ser.*, 1984, vol. 15, pp. 117–123.
3. Neckles, H.A., Koepfler, E.T., Haas, L.W., Wetzel, R.L., and Orth, R.J., Dynamics of Epiphytic Photoautotrophs and Heterotrophs in *Zostera marina* (Eelgrass) Microcosms: Responses to Nutrient Enrichment and Grazing, *Estuaries*, 1994, vol. 17, no. 3, pp. 597–605.
4. Kharlamenko, V.I., Degradation of Organic Compounds in Coastal Ecosystems, *Cand. Sci. (Biol.) Dissertation*, Vladivostok, 1985.
5. Penhale, P.A. and Thayer, G.W., Uptake and Transfer of Carbon and Phosphorus by Eelgrass (*Zostera marina* L.) and Its Epiphytes, *J. Exp. Mar. Biol.*, 1980, vol. 42, pp. 113–123.
6. Harrison, P.G., Control of Microbial Growth and of Amphipod Grazing by Water-Soluble Compounds from Leaves of *Zostera marina*, *Mar. Biol.*, 1982, vol. 67, pp. 225–230.
7. Shien, W.Y., Simidu, U., and Maruyama, Y., New Marine Nitrogen-fixing Bacteria Isolated from an Eelgrass (*Zostera marina*) Bed, *Can. J. Microbiol.*, 1988, vol. 34, pp. 886–890.
8. *Methods for General and Molecular Bacteriology*, Gerhardt, Ph. *et al.*, Eds., Washington: ASM, 1994.
9. Ivanova, E.P., Kiprianova, E.A., Mikhailov, V.V., *et al.*, Characterization and Identification of Marine *Alteromonas nigrifaciens* Strains and Emendation of the Description, *Int. J. Syst. Bacteriol.*, 1996, vol. 46, no. 1, pp. 223–228.
10. Vlachos, V., Critchley, A.T., and von Holy, A., Differential Antibacterial Activity of Extracts from Selected Southern African Macroalgal Thalli, *Bot. Mar.*, 1999, vol. 42, pp. 165–173.