Percentage of Gelatinolytic Bacteria among Heterotrophic Bacteria as Indicator of Water Quality

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Received 9 June 2003
Revised version 29 October 2003

ABSTRACT. The relationship between the physiological group of gelatinolytic bacteria and the abundance of heterotrophic bacteria in freshwater ecosystems was described, based on analysis of 1082 different freshwater samples collected in Croatia. Percentages of gelatinolytic bacteria among the population of heterotrophic bacteria showed a significant negative correlation with the abundance of heterotrophic bacteria. The relation between the physiological group (gelatinolytic bacteria) and heterotrophic bacteria can be considered to be an indicator of the pollution degree of freshwaters. A high relative content of gelatinolytic bacteria (>76 %) always indicates the colony-forming units of heterotrophic bacteria <1000/mL, which corresponds to the high water quality; gelatinolytic bacteria <11 % indicate polluted waters. Isolated strains of aerobically grown gelatinolytic bacteria were Gram-negative rod-shaped or Gram-positive endospore-forming rod-shaped cells.

Microbial populations, according to their composition, characterize various types of surface waters. Especially in polluted waters, the relations between individual physiological groups of bacteria can reflect changes which take place under the influence of polluting waste materials (Rodina 1972; Metwali 2003; Wahlström and Danilov 2003).

The gelatinolytic bacteria (GB) are a diverse group of bacteria, unified by their ability to degrade gelatin by extracellular gelatinase activity. The ability to hydrolyze gelatin is a well established bacterial classification characteristic (Holt *et al.* 1994). Besides this, the physiological group of GB can take part in the cycling of nutrients in the water ecosystem (Rodina 1972). It was observed that the greatest part of the population of all viable heterotrophic bacteria (HB) consists of the physiological group of GB in the less polluted parts (estimated by oxygen concentration, KMnO₄ consumption, biochemical oxygen demand and numbers of fecal coliform bacteria) of the rivers since in more polluted parts the contribution of GB is low (Stilinović 1979a,b; Stilinović and Futač 1983).

The aim of our study was to establish the relationship between the physiological group of GB and the abundance of HB in different freshwater ecosystems.

MATERIALS AND METHODS

Environmental freshwater samples (*n* = 1082) collected in Croatia from six water ecosystems were tested: karstic Plitvice Lakes (LP), natural Vransko Lake on the Island Cres (serves as an accumulation of drinking water; LV), artificial water accumulation Butoniga (AB), interstitial water samples taken around Zagreb (IW), rivers Dobra, Mrežnica, Korana and Krka (under low anthropogenic influence; RL), river Sava (under high anthropogenic influence; RH).

Water samples were collected at different depths (0.2–5.0 m) and different locations of the described ecosystems, using 5-L Niskin bottles. Subsamples for microbiological analyses were kept in sterile 250-mL bottles at 4 °C during transport to the laboratory.

*Bacterial cultivation.* Abundance of viable aerobically grown HB and GB was determined as colony forming units (CFU) on the tryptic-glucose-yeast agar (TGYA; *Biolife*) supplemented with gelatin (TGYA-G; in g/L distilled water): agar 15, gelatin (bacteriological grade) 15, tryptone 5, yeast extract 2.5, glucose 1; pH adjusted prior to autoclaving (121 °C, 20 min) to 7 ± 0.1 with 1 mol/L NaOH or 1 mol/L HCl. Serial dilutions (10⁻¹ to 10⁻⁸) of 1 mL sample were prepared. Dilutions (0.1 mL) were plated by a spread

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plate method (APHA 1995) in duplicate onto agar plates and incubated in the dark at 22 ± 0.1 °C. As the number of developed colonies after 2 d of incubation did not increase by incubating up to 3 d the counted number of all developed colonies after 2 d of incubation was taken as final CFU/mL of HB. After incubation, the plates were overlaid with a 0.46 mol/L HgCl₂ solution (Rodina 1972), colonies that produced clear zones caused by gelatin liquefaction were counted, and CFU/mL of GB was calculated. The percentage of the physiological group of GB among the population of HB was calculated. The accuracy of the methods of CFU and percentage of the physiological group of GB determination was estimated by 30 repeating measurements of the same sample.

A parallel control estimation of HB according to the standard methods (APHA 1995) was done. A 0.1-mL of sample from the serial dilution was assayed by a spread-plate method in duplicate onto standard TGYA and incubated in the dark for 3 d at 22 ± 0.1 °C.