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Bacterial Structure and Community-Level Physiological Profiles in Water From Vistula River, Lubelskie, Poland

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Abstract: The Vistula River is Poland's longest river, and its section called the Lesser Poland Gorge of the Vistula (Polish: Małopolski Przełom Wisły) belongs to two Natura 2000 Areas. The waters of the Vistula River periodically flood neighbouring areas during winter or summer floods. In this study we decided to determine the composition of the bacterial community and its metabolic potential in Vistula River water. In order to analyse the structure of bacterial communities, the next generation V3/V4 16S rDNA region sequencing (NGS) using Miseq (Ilumina) and the community-level physiological profiles (CLPP) method using the Biolog® EcoPlate™ system were applied. A total of 413 operational taxonomic units (OTU) were obtained, of which 377 could not be classified at genera level. Among those identified, *Arenimonas* (2.13%), *Brevundimonas* (1.44%) and *Flavobacterium* (1.05%) predominated. The most intensively degraded group of substrates were carbohydrates (30.93%) and the least amines and amides (6.44%). The NGS analysis did not indicate a sanitary risk to humans. A large number of unclassified bacteria indicate a still low level of knowledge and limitations concerning the world of bacteria.

Keywords: bacteria; CLPP; EcoPlate; NGS; Vistula River; water

1. Introduction

The Vistula River is Poland's longest river, and its section called the Lesser Poland Gorge of the Vistula (Polish: Małopolski Przełom Wisły) belongs to two Natura 2000 Areas [1]. The waters of the Vistula River periodically flood neighbouring areas during winter or summer floods [2]. Among studies on the microbiome of river waters, sanitary analyses to control drinking water dominate [3]. The most frequently determined bacteria are *Escherichia coli* and *Enterobacter*, *Citrobacter* and *Klebsiella*.

The aim of this study was to determine the composition of the bacterial community and its metabolic potential in Vistula River water.

2. Materials and Methods

2.1. Water from the Vistula River

Water was taken from the Vistula River from Janowiec, Lubelskie Voivodeship (51°19′06.8″N 21° and 54′53.5″E) in August 2018. The water was taken from the river at a distance of about 27 m from the riverbank into three sterile 50-litre containers (total ~ 150 L). Selected water parameters are presented in Table 1.

Table 1. Selected chemical parameters and trace elements contents (mg/L) in water.

pH	EC	Metals content (mg L^{-1}) [1]

	(μS cm^{-1})	K	Ca	Mg	Na	Fe	Mn	Cu	Cd	Al	Pb
8.54	370	4.567 ±0.257	49.395 ±2.423	10.760 ±0.547	60.97 ±0.85	0.035 ±0.001	0.035 ±0.001	0.002 ±0.000	<0.001 ±0.000	<0.001 ±0.000	<0.001 ±0.000

[1] Values are the average of three replicates (n = 3); means ± standard deviation (SD).

The water pH was slightly alkaline and was 8.54 and the electrical conductivity (EC) was 370 μS cm^{-1} (Table 1).

2.2. The colony PCR and next generation sequencing (NGS)

The direct PCR, also known as colony PCR [4] was performed on water from Vistula. All details of the procedure are described in Furtak et al. (2020) [5]. The resulting product (about 1500 nt) was then used as a template for V3–V4 region sequencing.

The V3–V4 region of the 16S rRNA gene was sequenced using 341F and 785R primers [6] at Genomed S.A. (Warsaw, Poland), in 2 bp × 250 bp paired-end technology using the Illumina MiSeq system.

The sequences obtained are available at the NCBI database under the bioproject accession number: PRJNA552453 (*https://www.ncbi.nlm.nih.gov/bioproject/PRJNA552453/*).

2.3. The community-level physiological profiles (CLPP)

The diversity of the metabolic potential of the bacterial community from water was determined using the Biolog EcoPlate™ (Biolog Inc., Hayward, CA, USA) system with 31 different carbon sources in three replicates.

The water was applied (without dilution) to the EcoPlate™ plate in a volume of 120 μl to each well. EcoPlates™ were incubated at 25 °C for 120 h in the OmniLog® ID System multiplate reader (Biolog Inc., Hayward, CA, USA). The intensity of the wells' colour development was determined spectrophotometrically at $\lambda = 590$ nm [7] at 24-intervals using a MicroStation ID (Biolog Inc., Hayward, CA, USA) plate reader.

Substrates from EcoPlate™ weere classificated into five biochemical groups: ; according to Weber and Legge (2009) [8].

2.4. Data analysing

Details of bioinformatic data processing are described in detail in Furtak et al. (2020) [5]. The analyses were performed using the DADA2 (1.12) package [9] in R software (3.6.0) [10].

On the basis of the data from Biolog EcoPlate™ (after 120 h of incubation), the average well colour development (AWCD), richness (*R*), Shannon diversity (*H'*) and evenness (*E*) indices were calculated following Garland and Mills (1991) [11]. Heatmaps were generated on OmniLog units in R software (3.6.0) using the *phyloseq* package [12].

3. Results

3.1. Structural diversity of the bacterial community

A total of 413 operational taxonomic units (OTU) were obtained, of which 377 could not be classified at genera level (Table 2). Based on this data the calculated Shannon's biodiversity index was 4.94.

Table 2. Selected diversity indices obtained for NGS results.

Number of sequences			Dominance (D)	Simpson (1/D)	Shannon (H')	Evenness (E)	Chao-1	Fisher
All	Classified to genus	Unclassified						
413	36	377	0.014	0.986	4.939	0.348	401	62.570

In the water taken from the Vistula River 53 different bacterial families were identified, the most numerous of which were Planctomycetaceae (18.5%) and Xanthomonadaceae (13.8%). Among the identified classes of bacteria the most abundant were Betaproteobacteria (21.5%).

Of the sequensec present above 1% in the water, 23 were unclassified, including the dominant Unclassified_CL0182 (5.05%), belonging to the family Plantomycetaceae (Figure 1). Among those identified, Arenimonas (2.13%), Brevundimonas (1.44%) and Flavobacterium (1.05%) predominated.

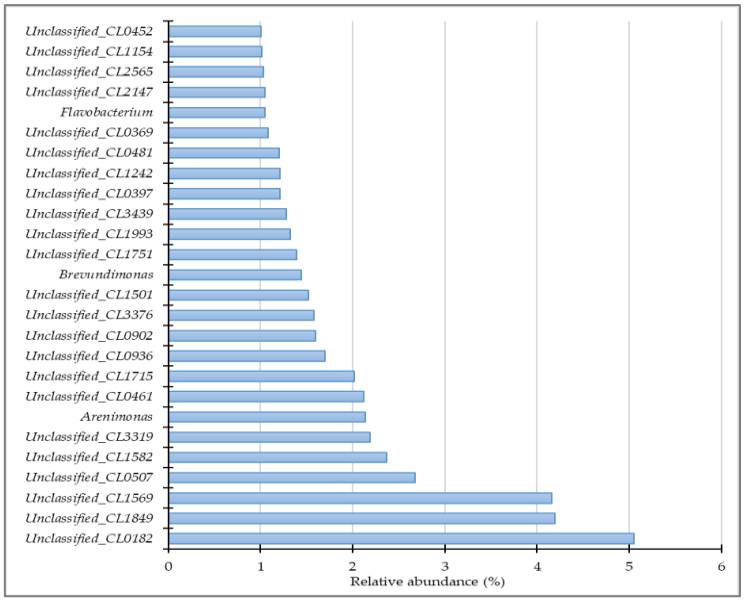

Figure 1. Sequences that occurred in more than 1% in water sample from Vistula River.

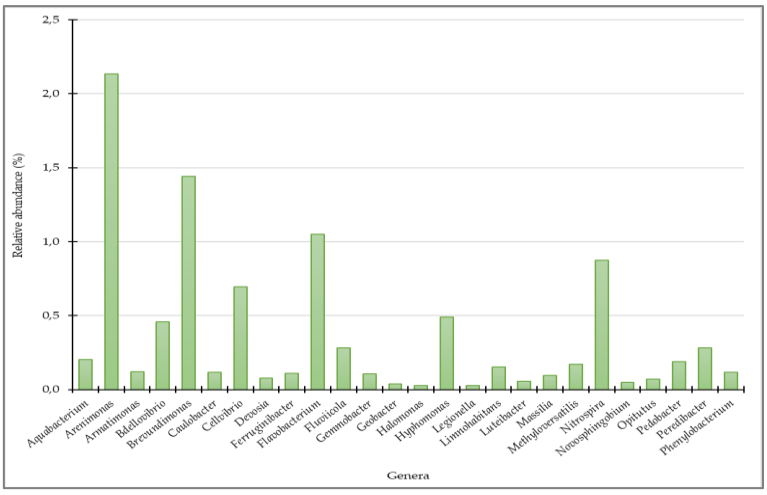

Figure 2. Relative abundance (%) of classified at genera level bacterial sequences.

While searching for bacteria pathogenic to humans, few representatives of Legionella sp. (0.03%) and Rickettsia sp. (0,03 %). Additionally, the presence of bacteria from the genus Brevundimonas (1.44%) and from the family Oxalobacteraceae (0.09%) was detected, which may cause opportunistic infections. The results obtained are surprising, as it is commonly believed that river waters are contaminated with pathogenic microorganisms.

3.2. Metabolic potential of the bacterial community

The highest AWCD and Shannon's diversity index (H') was recorded after 120 hours of incubation of EcoPlate™ (Figure 3).

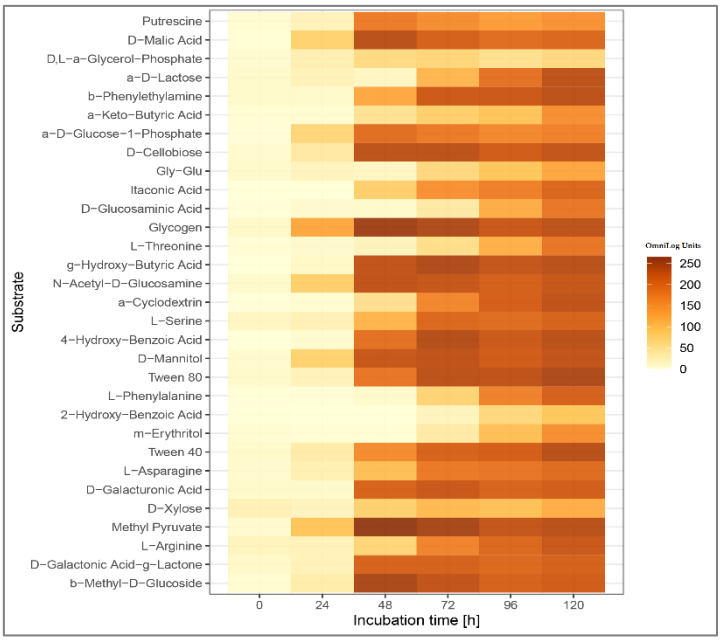

Figure 3. Heatmaps of EcoPlate™ substrate utilisation by bacterial community during incubation time.

Table 2. Indices obtained for EcoPlate™ results and utilization (%) of group of substrates by bacterial community after 120 h incubation.

Indice			Utilization (%) of 5 groups of substrates				
AWCD	**Shannon (H′)**	**Evenness (E)**	**Carbohydrates**	**Amines & amides**	**Amino acids**	**Carboxylic acids**	**Polymers**
1.642	3.301	0.841	30.93	6.44	15.46	28.52	18.65

The microorganisms present in the water most intensively decomposed carbohydrate group substrates (30.93%) and the least intensively compounds classified as amines and amides (6.44%) (Table 2).

The NGS analysis did not indicate a sanitary risk to humans. A large number of unclassified bacteria indicate a still low level of knowledge and limitations concerning the world of bacteria.

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Conflicts of Interest: The authors declare no conflict of interest.

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