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

Karolina Furtak 1,*, Jarosław Grządziel 1, Anna Gałązka 1, Jacek Niedźwiecki 2

1 Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation—State Research Institute, Czartoryskich 8, 24-100 Pulawy, Poland; jaroslaw.grzadziel@gmail.com (J.G.);
agalazka@iung.pulawy.pl (A.G.)
2 Department of Soil Science Erosion and Land Protection, Institute of Soil Science and Plant Cultivation—State Research Institute, Czartoryskich 8, 24-100 Pulawy, Poland; jacekn@iung.pulawy.pl
* Correspondence: kfurtak@iung.pulawy.pl; Tel.: +814786961

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 (Illumina) 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.

<table>
<thead>
<tr>
<th>pH</th>
<th>EC</th>
<th>Metals content (mg L⁻¹)</th>
</tr>
</thead>
</table>

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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 λ = 590 nm [7] at 24-intervals using a MicroStation ID (Biolog Inc., Hayward, CA, USA) plate reader.

Substrates from EcoPlate™ were classified 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>
<thead>
<tr>
<th>Number of sequences</th>
<th>Dominance (D)</th>
<th>Simpson (1/D)</th>
<th>Shannon (H')</th>
<th>Evenness (E)</th>
<th>Chao-1</th>
<th>Fisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.014</td>
<td>0.986</td>
<td>4.939</td>
<td>0.348</td>
<td>401</td>
<td>62.570</td>
</tr>
<tr>
<td>Classified to genus</td>
<td>36</td>
<td>0.014</td>
<td>0.986</td>
<td>4.939</td>
<td>0.348</td>
<td>401</td>
</tr>
<tr>
<td>Unclassified</td>
<td>377</td>
<td>0.014</td>
<td>0.986</td>
<td>4.939</td>
<td>0.348</td>
<td>401</td>
</tr>
</tbody>
</table>

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 sequences present above 1% in the water, 23 were unclassified, including the dominant Unclassified_CL0182 (5.05%), belonging to the family Planomycetaceae (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.](image1)

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.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Carbohydrates</th>
<th>Amines &amp; amides</th>
<th>Amino acids</th>
<th>Carboxylic acids</th>
<th>Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>AWCD</td>
<td>Shannon (H')</td>
<td>Evenness (E)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.642</td>
<td>3.301</td>
<td>0.841</td>
<td>30.93</td>
<td>6.44</td>
<td>28.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15.46</td>
<td></td>
<td>18.65</td>
</tr>
</tbody>
</table>

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.

Acknowledgments: The research was conducted within the grant for young scientists in IUNG-PIB (MNiSW): Influence of summer floods on changes in structural and functional biodiversity of microbiomes of selected river muds (2018).

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

References


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