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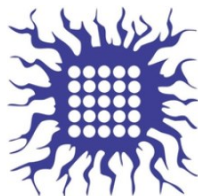
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2nd International Conference on Chemo and Bioinformatics

ICCBIKG_2023



BOOK OF PROCEEDINGS





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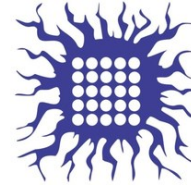
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Bacteria in drinking and bathing mineral waters of Serbia with polymer-degrading potential

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Abstract: Three mineral water occurrences, captured by wells with a depth of 6.5-442.5 m and used for drinking and bathing purposes, were sampled and cultivated under conditions favouring the growth of anaerobic, microaerophilic or CO₂ bacteria, in order to capture predominantly anaerobic portion of the bacteriome, which is dominant in water and soils. Cultivated bacteria were identified by next-generation 16S sequencing and their biotechnological potential in plastics and lignocellulose degradation was explored. Most abundant genera detected in examined samples mainly belong to facultative anaerobes that are common representatives of water and soil environments. In total, 17 genera were detected with a relative abundance over 1% in all three samples, including *Aeromonas*, *Exiguobacterium*, *Comamonas* and *Acinetobacter*. Half of the screened isolates demonstrated growth on at least one plastic or lignocellulosic polymer, with one isolate demonstrating growth on all tested substrates, one demonstrating carboxymethyl cellulose- and one arabinoxylan-degrading ability. Some of the representatives of genera identified with high relative abundance in mineral water samples, such as *Aeromonas*, *Klebsiella*, *Escherichia*, *Salmonella*, *Enterobacter*, *Pseudomonas* and *Staphylococcus*, have been previously documented to have pathogenic potential. Due to the use of investigated mineral waters for drinking and bathing, the health risk from such bacteria in these occurrences needs to be continuously monitored, while, on the other hand, mineral waters deserve special attention in the future from the aspect of screening for biotechnologically relevant enzymes.

Keywords: mineral water, bacteria, 16S rDNA sequencing, Serbia.

1. Introduction

Mineral water usually refers to groundwater with chemical characteristics that differ from water for public water supply. They are characterized by a content of dissolved mineral substances above 1000 mg/L or an increased content of certain specific components (CO₂, H₂S, Br, Rn, J, Rb, etc.), with a pharmacological effect on the human body, or by a higher temperature compared to ordinary low-mineralized waters [1]. There are approximately 230 recorded mineral water occurrences in Serbia, with reserves estimated at around 1000 L/s, positioning Serbia as one of the most promising countries in the region in terms of the richness of this natural resource. Mineral waters are used, to a certain extent, for drinking (as table bottled water) and bathing purposes (as spa water) [2]. Water,

while essential for all forms of life, can also act as a vector for pathogens and chemicals, and thus be associated with diseases. Exposure to pathogens, which are in mineral water most often of allochthonous origin [3], may have serious health consequences [4, 5]. Hydric infections caused by bacterial pathogens and opportunistic pathogens have been documented for drinking and bathing waters [5] and for the latter the risk of colonization by certain pathogens is significantly higher due to inhalation of aerosols or skin colonization than due to the digestive tract colonization [6]. Ecological data, especially the diversity of bacterial communities, are essential for the health risk assessment of mineral waters [7]. In addition, mineral waters can represent untapped reservoir of enzymes with possible biotechnological application [8]. Therefore, three mineral water occurrences in Serbia, captured by wells with depth of 6.5-442.5 m [9, 10] and used for bathing and drinking purposes, were selected for cultivation and identification of bacteria and evaluation of their biotechnological potential.

2. Methods

Mineral water occurrences of Obrenovačka Banja (OB), Velika Vrbnica (VV) and Lomnički Kiseljak (LK) were sampled and cultivated during September 2022. For each occurrence, both direct mineral water samples and pellets were cultivated on 3 different solid media: Luria Agar (LA), Yeast Extract-Malt Extract (ISP-2) and Mannitol-Soy Flour (MSF). Plates were incubated for 72 h in accordance with temperatures of the sampled occurrence using the GasPak™ EZ System to capture the predominantly anaerobic portion of the bacteriome. Full-length 16S rDNA next-generation sequencing was performed on DNA from cultivated OB, LK and VV samples using the MinION platform to assess the diversity of bacteria captured under applied cultivation conditions. Several isolates representative of distinct observed morphologies from OB occurrence, which is unique due to its depth (442.5 m) and temperature (22.8-31.3°C) [9], were screened for growth and/or enzymatic activity in the GasPak™ EZ System using plastic and lignocellulosic polymeric substrates as sole C source to assess their biotechnological potential. The Minimal Salt Medium agar was supplemented with different plastic polymers Impranal® DLN-SD and DL 2077, polycaprolactone diol and bis(2-hydroxyethyl) terephthalate and lignocellulosic polymers carboxymethyl cellulose (CMC), arabinoxylan (AXYL) and lignin for screening.

3. Results

For each cultivated mineral water sample, plate counts were in the range of $2 \times 10^2 - 1 \times 10^3$ CFU/mL and similar morphologies were observed across all three types of cultivation media used and between pellet and whole mineral water samples. In total, 17 genera were detected with a relative abundance over 1% in three cultivated samples. Of these, 13 and 11 genera were detected in VV and LK samples, while only 3 genera were detected in the OB sample (Table 1). Two genera (*Aeromonas* and *Acinetobacter*) are shared among all three samples, while 5 and 4 genera were unique for LK and VV cultivated samples, respectively.

In OB and LK samples, *Aeromonas* was the most abundant genus, while *Exiguobacterium* was detected at the highest relative abundance in the cultivated VV sample. In the OB sample, genera *Acinetobacter* and *Comamonas* were also detected with relative abundance >1%. *Aeromonas*, *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*, *Escherichia*, *Klebsiella* and *Salmonella* are shared

between LK and VV samples. In addition, in LK sample representatives of genera *Flavobacterium*, *Staphylococcus*, *Pantoea*, *Comamonas*, *Mammaliicoccus* and *Microbacterium* and in VV sample, representatives of genera *Exiguobacterium*, *Enterobacter*, *Proteus* and *Bacillus*, were also detected at relative abundance >1%. Four out of 8 screened isolates demonstrated growth on at least one plastic or lignocellulosic polymeric substrate. All 4 demonstrated growth on SD, 3 on DL and 2 on PCL, CMC and AXYL. One isolate had growth on all tested polymers; one demonstrated CMC- and one AXYL-degrading ability.

Table 1. Bacterial genera detected by 16S rDNA sequencing in cultivated mineral water samples.

Genus	OB	LK	VV
<i>Aeromonas</i>	88.2	52.3	1.7
<i>Pseudomonas</i>	0.0	10.1	3.0
<i>Acinetobacter</i>	6.2	7.6	24.1
<i>Stenotrophomonas</i>	0.0	4.2	3.7
<i>Escherichia</i>	0.4	4.2	13.3
<i>Flavobacterium</i>	0.0	3.7	0.0
<i>Klebsiella</i>	0.5	3.0	8.6
<i>Staphylococcus</i>	0.0	2.2	0.0
<i>Pantoea</i>	0.0	1.8	0.3
<i>Comamonas</i>	3.6	1.7	0.0
<i>Salmonella</i>	0.3	1.4	3.7
<i>Mammaliicoccus</i>	0.0	1.3	0.0
<i>Microbacterium</i>	0.0	1.1	0.1
<i>Bacillus</i>	0.0	0.7	1.3
<i>Exiguobacterium</i>	0.1	0.5	25.7
<i>Enterobacter</i>	0.0	0.4	8.2
<i>Proteus</i>	0.1	0.1	2.0

Genera detected with relative abundance >1% for all samples are included. Lowest Value – red, Percentile (50) – yellow, Highest Value – green. Mineral water samples: OB – Obrenovačka Banja; LK – Lomnički Kiseljak; VV – Velika Vrbnica.

Most abundant genera detected in examined samples mainly belong to facultative anaerobes that are common representatives of water and soil environments. Applied cultivation conditions seemed to favor the growth of facultative anaerobes over obligate anaerobic bacteria. Some representatives of genera identified at the highest relative abundance in three mineral water samples, such as *Aeromonas*, *Klebsiella*, *Escherichia*, *Salmonella*, *Enterobacter*, *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Stenotrophomonas*, *Proteus* and *Bacillus* have been previously documented to have pathogenic potential [5]. Some of the detected representatives, such as *Proteus* [11], *Stenotrophomonas* [12], *Pseudomonas* [13], *Aeromonas* [14] and *Microbacterium* [15], have a documented biotechnological potential in degradation of plastic or lignocellulose polymeric materials.

4. Conclusions

Since examined mineral waters are used for bathing and drinking purposes, the health risk from bacteria with pathogenic potential detected in these occurrences needs to be continuously monitored. On the other hand, two of the cultivable isolates demonstrated CMC- and AXYL-degrading ability and, in the future, mineral waters deserve special attention from the aspect of screening for biotechnologically relevant enzymes.

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