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Metagenomic analysis of the bacterial communities and their functional profiles in water and sediments of the Apies River, South Africa, as a function of land use

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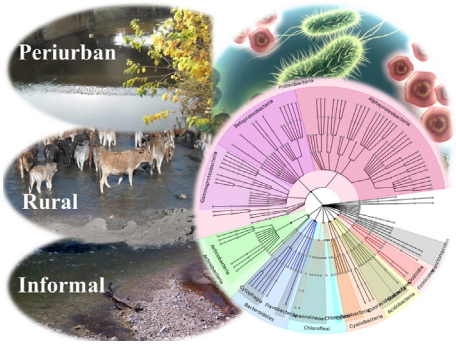
HIGHLIGHTS

- We studied bacterial communities in river water and sediments using metagenomics.
- Samples were collected from a rural, peri-urban and an informal settlement.
- Sediment samples had the highest bacterial diversity and abundance.
- The informal settlement contributed most to the bacterial diversity and abundance.
- Functional profiling revealed involvement of the bacteria in human diseases pathways.

GRAPHICAL ABSTRACT



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ABSTRACT

Water quality is an important public health issue given that the presence of pathogenic organisms in such waters can adversely affect human and animal health. Despite the numerous studies conducted to assess the quality of environmental waters in many countries, limited efforts have been put on investigating the microbial quality of the sediments in developing countries and how this relates to different land uses. The present study evaluated the bacterial diversity in water and sediments in a highly used South African river to find out how the different land uses influenced the bacterial diversity, and to verify the human diseases functional classes of the bacterial populations. Samples were collected on river stretches influenced by an informal, a peri-urban and a rural settlement. Genomic DNA was extracted from water and sediment samples and sequenced on an Illumina® MiSeq platform targeting the *16S rRNA* gene variable region V3-V4 from the genomic DNA. Metagenomic data analysis revealed that there was a great diversity in the microbial populations associated with the different land uses, with the informal settlement having the most considerable influence on the bacterial diversity in the water and sediments of the Apies River. The *Proteobacteria* (69.8%), *Cyanobacteria* (4.3%), *Bacteroidetes* (2.7%), and *Actinobacteria* (2.7%) were the most abundant phyla; the *Alphaproteobacteria, Betaproteobacteria* and *Anaerolineae* were the most recorded classes. Also, the sediments had a greater diversity and abundance in

Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes; OTUs, Operational Taxonomic Units; PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; SA, South Africa; SRA, Sequence Read Archive.

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bacterial population than the water column. The functional profiles of the bacterial populations revealed an association with many human diseases including cancer pathways. Further studies that would isolate these potentially pathogenic organisms in the aquatic environment are therefore needed as this would help in protecting the lives of communities using such rivers, especially against emerging bacterial pathogens.

1. Introduction

The fast growth of the world's population demands more land to establish settlements to accommodate new and emerging communities. In most cases, there is migration from rural areas to urban cities which usually results in overcrowding (UNICEF, 2011). Due to the lack of space, many of these communities create informal settlements, most of which are built along rivers (Govender et al., 2011). In the urban and peri-urban areas, the growing populations put tremendous pressure on the basic services such as water supply and wastewater treatment (Teklehaimanot et al., 2015). As a result, many wastewater treatment plants, unable to meet the challenge of treating the large volumes of waste received, discharge these wastes into receiving water bodies without proper treatment or at times without any treatment (Teklehaimanot et al., 2014). In the rural and informal settlements, the lack of these basic facilities usually results in the use of nearby rivers and streams as areas for dumping refuse (Vollmer and Grêt-Regamey, 2013). The ultimate result is the heavy pollution of these water bodies, which most of the time serve as alternative water sources and, at times as the only water sources for these rural and informal settlements (Durand, 2012; Lim et al., 2017).

Within these aquatic ecosystems, especially within the bed sediments, bacteria are among the most abundant group of microorganisms (Wang et al., 2013). These bacteria are involved in the cycling of nutrients and play a significant role in the breakdown of some of the pollutants that enter the aquatic environment (Haley, 2006; Torsvik and Øvreås, 2002; Zhang et al., 2013; Zhi et al., 2015). The pollutants that enter water bodies because of different land use could be chemical or biological. The chemical pollutants may exert pressure on the microbial communities in these systems, thus resulting in community change, with possible negative impact on environmental health (Scown et al., 2010; Matranga and Corsi, 2012). Biological pollutants may include pathogenic microorganisms with the potential of causing infections in humans and animals if they get exposed to such untreated water for drinking or during recreation (Prieto et al., 2001; Griffin et al., 2003; Abraham, 2011; Leonard et al., 2015). Another important aspect of aquatic ecosystem pollution is that the pollutants that enter these systems may settle into the bottom sediments. In the sediments, they usually go unnoticed as most countries do not consider sediments during routine monitoring programmes. These pollutants could be resuspended when the sediments are disturbed, thereby affecting the quality of the water column and increase the risk of infections to humans (Abia et al., 2017).

Studies have been conducted in many countries around the globe to determine the quality of environmental water bodies. In most developing countries, limited efforts have been put on investigating the microbial quality of the sediments. In South Africa, for example, although such studies have been conducted, most of them have used culture-based methods and have focused only on a selected number of organisms. For example, Abia et al. (2016) demonstrated that the water and sediments of the Apies River, a highly used river by populations in the Gauteng Province of South Africa, harboured considerable amounts of indicator and pathogenic organisms. Culture-based methods are insufficient in that they can only determine certain organisms (only those that can grow on culture media) and are labour- and time-demanding (Theron and Cloete, 2002; Yashiro et al., 2011; Stefani et al., 2015). Also, studies on the impact of different land use on the overall microbial population in these aquatic environments are limited. Thus, the current study was conducted to investigate the impact of peri-urban, rural and informal settlements on the microbial diversity in the Apies River using metagenomics. The study also sought to compare the microbial diversity and abundance in water and sediments and to examine the bacterial functional profiles in this river.

2. Material and methods

2.1. Study site and sample collection

The study was conducted in the Apies River, in the Gauteng Province of South Africa. The River and the various land uses around it have been fully described previously (Abia et al., 2015). Water and sediment samples were collected in 1-L sterile plastic bottles and 100-mL plastic cups, respectively, as previously described. Grab samples were collected in 2016 from three sites in the Apies River: downstream of an informal settlement (AP1), a peri-urban settlement (AP7) and a rural settlement (AP9) (Abia et al., 2015) as illustrated in the graphical abstract. Each sample type was collected in triplicates from each sampling sites and the samples were immediately transported in a cooler box to the laboratory for further analysis.

2.2. DNA extraction and high throughput amplicon sequencing

In the case of sediment samples, genomic DNA was directly extracted from 250 mg of sediment sample using the ZR Soil Microbe DNA MicroPrep™ (Zymo Research Corp., Irvine, California, USA), according to the manufacturer's instructions. Given the turbid nature of the water samples, 100 mL of water from each site was centrifuged at 7500 revolutions per minutes (rpm) for 10 min (Tekere, 2011). Genomic DNA was extracted from the resulting pellets using the kit as previously described for the sediment samples. The extracted DNA from the triplicate samples of each sample type were pooled together for each sample site. The concentration and quality of the extracted DNA were measured using a NanoDropsND-2000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA). The extracted DNA was sent to Inqaba Biotechnology, South Africa, for sequencing. The sequencing was performed on an Illumina® MiSeq platform using the MiSeq Reagent Kit v3 (600-cycle) (Illumina, Inc. San Diego, CA, USA) targeting the *16S rRNA* gene variable region V3–V4 from the genomic DNA.

2.3. Data analysis

To identify the microbial composition of the various sampling sites, data were analysed using Mothur (Schloss et al., 2009) and CLC Genomics Workbench (CLC Bio Qiagen). In brief, the quality of the data was checked, and quality trimming ($Q > 30$) and length trimming were conducted to obtain clean data. Operational Taxonomic Units (OTUs) were obtained based on Greengene database (DeSantis et al., 2006) with a percent similarity threshold of 97%. Venn diagrams were plotted based on the number and similarity of OTUs using Venn plotter at http://bioinformatics.psb.ugent.be/webtools/Venn/. The OTUs were further analysed and visualised using different software and packages as outlined below.

The stacked bar plots and rarefaction curves were plotted using R package Phyloseq (McMurdie and Holmes, 2013) and Microsoft Excel (2013). A rarefaction curve typically plots the number of OTUs as a function of the number of sequenced reads. In addition, GraPhlAn (Asnicar