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Comparative metagenome of a stream impacted by the urbanization phenomenon

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ABSTRACT

Rivers and streams are important reservoirs of freshwater for human consumption. These ecosystems are threatened by increasing urbanization, because raw sewage discharged into them alters their nutrient content and may affect the composition of their microbial community. In the present study, we investigate the taxonomic and functional profile of the microbial community in an urban lotic environment. Samples of running water were collected at two points in the São Pedro stream: an upstream preserved and non-urbanized area, and a polluted urbanized area with discharged sewage. The metagenomic DNA was sequenced by pyrosequencing. Differences were observed in the community composition at the two sites. The non-urbanized area was overrepresented by genera of ubiquitous microbes that act in the maintenance of environments. In contrast, the urbanized metagenome was rich in genera pathogenic to humans. The functional profile indicated that the microbes act on the metabolism of methane, nitrogen and sulfur, especially in the urbanized area. It was also found that virulence/defense (antibiotic resistance and metal resistance) and stress response-related genes were disseminated in the urbanized environment. The structure of the microbial community was altered by uncontrolled anthropic interference, highlighting the selective pressure imposed by high loads of urban sewage discharged into freshwater environments.

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Introduction

Rivers and streams contain approximately 0.006% of the freshwater available on Earth,[1] but this resource is becoming limited. More than 30% of the renewable freshwater available for consumption is used for agricultural, industrial and domestic purposes. The main consequence of these activities, and of urbanization, is the injection of large quantities of waste into the water, contaminating it with xenobiotic compounds.[2] Pollution can modify the structure and composition of rivers and streams by altering their geomorphology, temperature, pH, nutrients, and biotic community.[3] The chemical pollution of natural waters can render environments dangerous for life. This problem occurs not only developing countries, which lack suitable waste management, but has already become a major public concern in most of the world.[2,4]

Numerous studies have sought to demonstrate the impacts of urbanization on freshwater ecosystems. Some of these studies, which focus on physicochemical parameters such as nutrients, show that urban environments contain increased levels of phosphorus, nitrogen, nitrate, ammonia, and potassium.[3,5–8] Metal and pesticide contaminants have also been identified in urban areas.[9] However, this type of analysis yields limited information when the objective is to understand the complexity of ecosystems, and, in this case biological components must be taken into account. Planktonic microorganisms (Bacteria, Archaea, members of Eukarya, and viruses) dominate these ecosystems in terms of abundance and biomass. They represent a large and diverse pool of species responsible for sustaining metabolic activities, including biogeochemical processes, and organic matter and nutrient recycling.[10–12] The microbial community of aquatic ecosystems is extremely important for the maintenance and sustainability of these environments since microbes are highly sensitive to anthropogenic stress.[13] However, only a few studies have analyzed the effect of urbanization on the microbial community.[12,14,15]

A previous study by our group focusing on an urban stream showed a higher concentration of dissolved nutrients in the urbanized waters. Using culture-independent methods such as Fluorescence *in situ* hybridization and PCR, we observed that urbanization alters the density of Nitrosomonadaceae, Nitrospiraceae, and *Nitrobacter*, microbes involved in the nitrogen cycle, and increases the occurrence of *Enterococcus*, *Streptococcus*, *Bacteroides/Prevotella/Porphyromonas*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and the diarrheagenic strains of *Escherichia coli*, which are considered potentially pathogenic to humans.[8] However, to date, no in-depth and comprehensive description is available about the taxonomy and functionality of microbes in urban freshwater ecosystems. Therefore, metagenomic comparisons of preserved and polluted areas of a stream may contribute significantly to a better understanding of the real anthropogenic impacts on aquatic environments.

Metagenomic is an important tool for understanding microbial ecosystems, given its ability to provide information about the diversity and distribution of the different members of a community and their metabolic potential.[16] This methodology has increased the knowledge about diverse microbiomes, such as oceans,[17] the human body,[18–20] and soil,[21–23] especially due to high-throughput sequencing technologies. In this context, the aim of this study was to make a comprehensive description of the taxonomic and functional profile of the microbial community in an urban stream, comparing a polluted and a preserved area. This was achieved by means of a metagenomic approach using 454-pyrosequencing.

Material and methods

Sample collection and DNA extraction

Approximately 6 and 12 L of samples were collected from the subsurface water of the urbanized and non-urbanized sites, respectively, of the São Pedro Stream located in Juiz de Fora, Brazil, in December 2010. The water samples were stored separately in 15 L bottles that had been previously rinsed three times with sample from each site. The sites were characterized in a previous study,[8] as follows: the urbanized site (661799E/7591070N), which is surrounded by houses, is polluted with sewage release. The system at this point is considered eutrophic, since it has extremely high contents of ammonia, nitrite, nitrate, total organic nitrogen, and total phosphorus. The non-urbanized site (668307E/7591772N), located in a farming region, is upstream from the urban area and has a low concentration of dissolved nutrients, characterizing it as a preserved system.

The water samples were sonicated on ice three times for 60 s, at an amplitude of 90%, using a Vibra-Cell VCX 130 PB ultrasonic processor (Sonics & Materials, USA). The samples were filtered twice, first through a paper filter (3M, USA) and then through a GF/F filter (Whatman Ltd, UK).[8] The filtered water was centrifuged at 8000 rpm for 15 min in 500 mL bottles. The microbial DNA was extracted using a PowerMax Soil DNA Isolation Kit (MoBio, USA). DNA integrity was checked by agarose gel electrophoresis and quantified spectrophotometrically in a NanoDrop ND 1000 instrument (Thermo Scientific, USA).

Sequencing and analysis

Five micrograms of DNA were used for sequencing in the 454 Sequencing GS FLX Titanium platform at the National Laboratory for Scientific Computation (LNCC) (Petrópolis, Rio de Janeiro, Brazil). The DNA from each of the two areas constituted one-quarter of the plate, without replicates. The obtained reads were quality-trimmed to remove short sequences (fewer than 180 bp) or sequences with Phred quality ≤20, using LUCY software.[24] To eliminate artificially replicated sequences, 454 Replicates[25] were used. The resulting sequences were uploaded to the Metagenomics RAST server (MG-RAST)[26] and made publicly accessible under code numbers 4464295.3 and 4464296.3 for non-urbanized and urbanized metagenomes, respectively. The NCBI access number for the sequences is SRA051287.