Evaluation of Methods to Study the Microbiome of Activated Sludge: Selection of 16S rRNA Fragment and Reference Database

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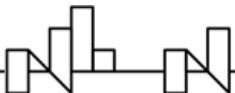
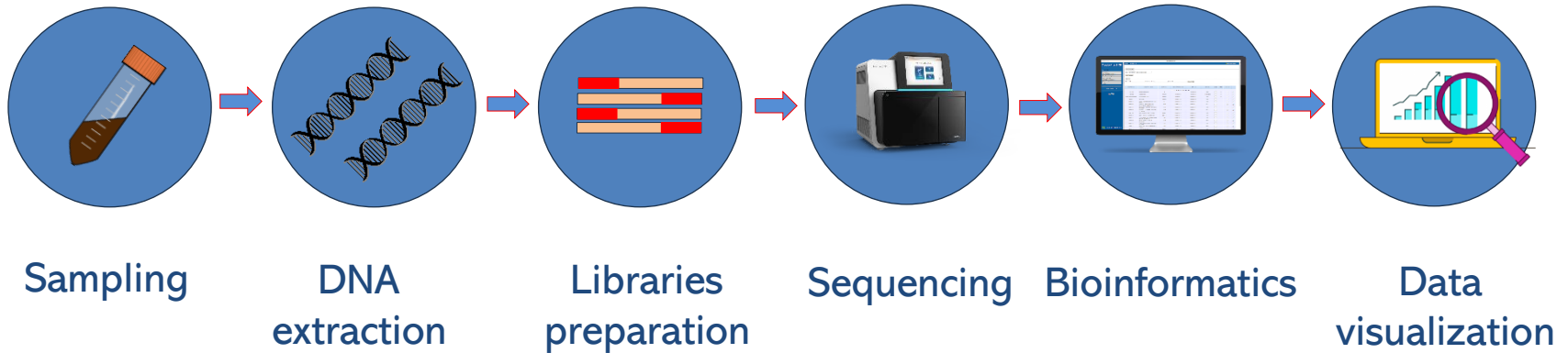## Presenter
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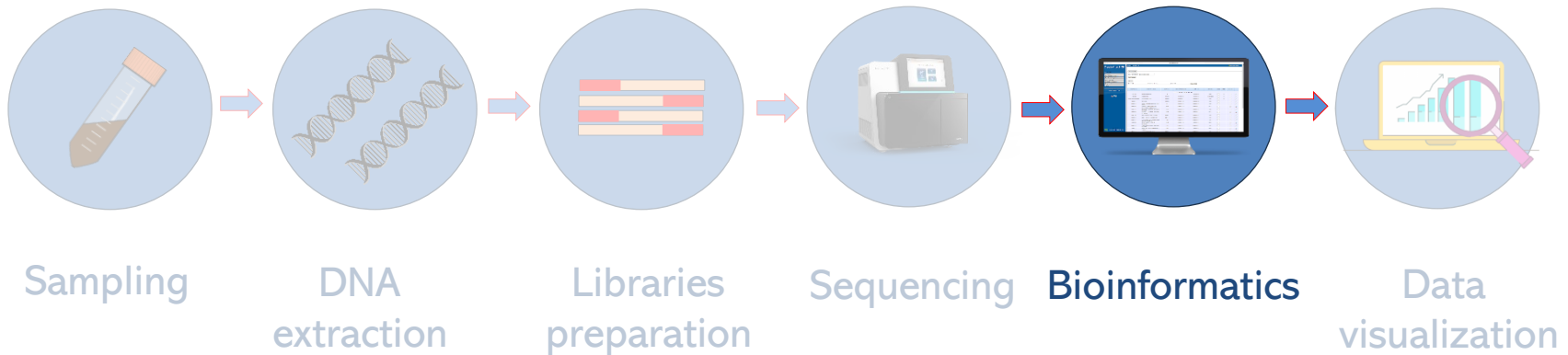
Scientific interests

- microbiomics of various environments

- microbiological synthesis of bioplastics, biosurfactants and pigments

- metagenomics and metatranscriptomics

# Workflow of metagenomic aproach used for amplicon analysis

Sampling → DNA extraction → Libraries preparation → Sequencing → Bioinformatics → Data visualization

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High-throughput sequencing methods are generally used for taxonomic barcoding and generate huge amounts of data that require bioinformatic analysis. The bioinformatics pipelines used can provide information on taxonomic composition, but results may vary depending on the parameters used.

Structure of gene coding for 16S rRNA
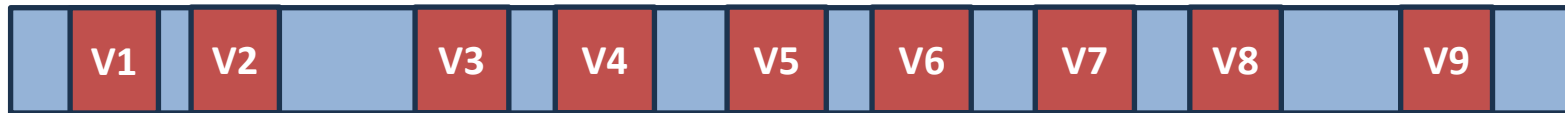
variable regions

The selection of the 16S rRNA region for sequencing can be crucial in the study of microbial communities. Each of the nine hypervariable regions of 16S rRNA can be used for identification purposes, but analyzing different regions, individually or in combination, does not always lead to identical results.
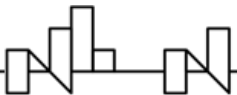
SILVA, Greengenes, and RefSeq are widely used 16S rRNA databases

Figure 1.
SILVA Logo
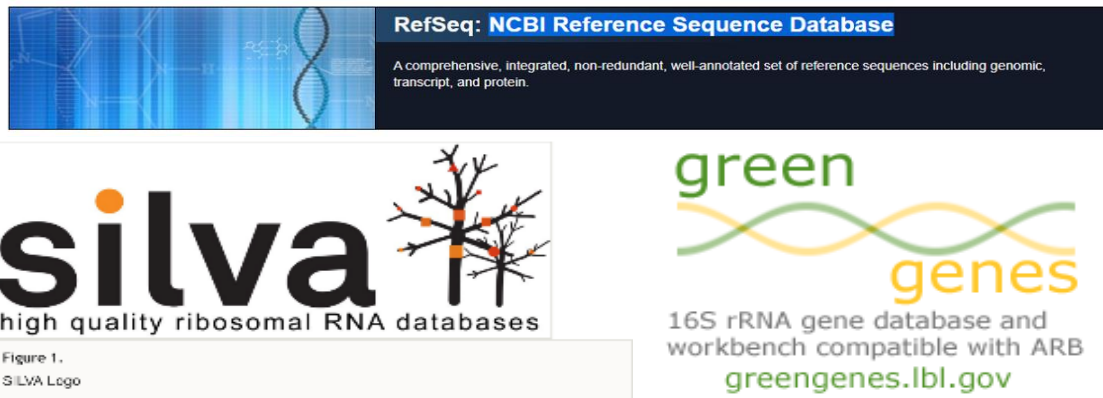
The selection of a suitable reference base may have an influence on the results obtained.

Aim of the work

Standarization of the barcoding methods by:

- the selection of the 16S rRNA region for sequencing

- the choice of a suitable reference database

In this work, the focus was on samples of activated sludge from a wastewater treatment plant.

The taxonomic characterization of activated sludge is extremely important as it could be used to improve biological wastewater treatment processes.
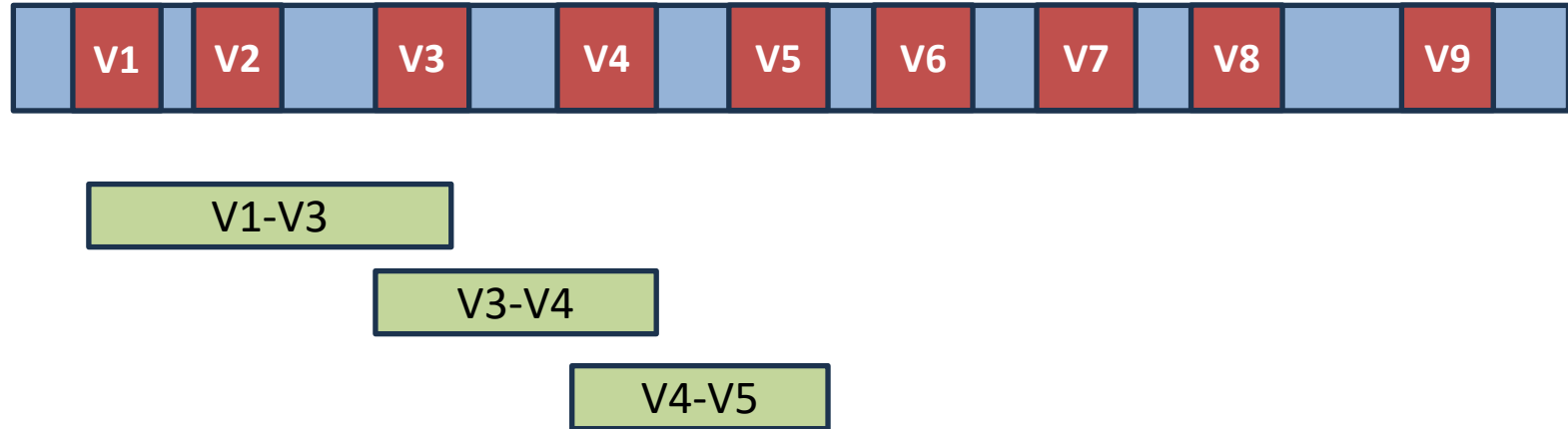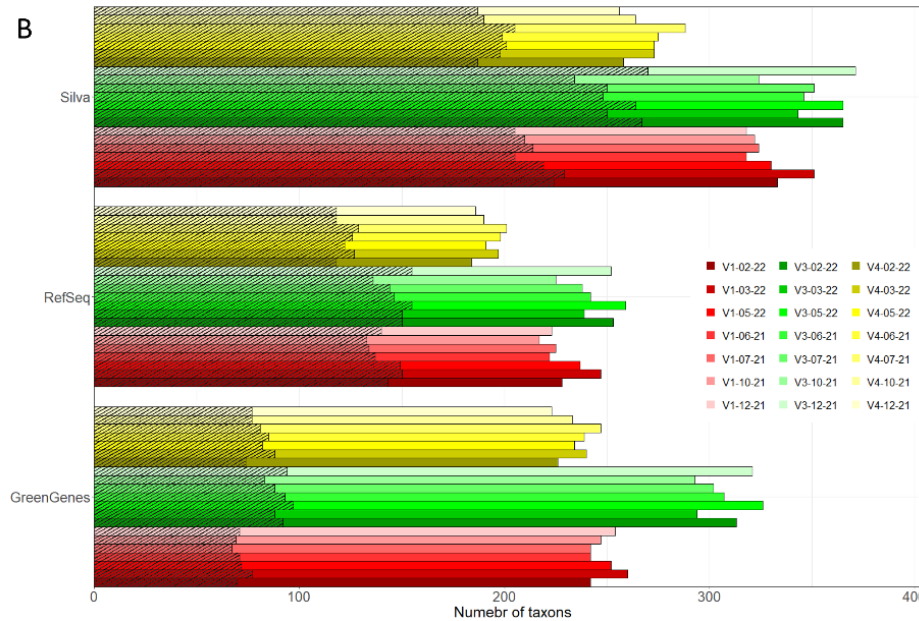
The average number of operational taxonomic units (OTUs) was highest for fragments V3-V4 with 6702 (SD ± 402), followed by V1-V3 with 2563 (SD ± 197) and V4-V5 with 1527 (SD ±58) OTUs.

Results

The taxonomic classification based on the SILVA database resulted in the largest number of genera. The same database had the highest number of unique taxons. GreenGenes had a larger number of genera than RefSeq, while RefSeq had a higher number of unique taxonomic groups.
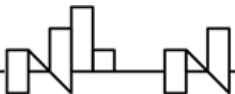
Summary and conclusions

The results confirmed that the V1-V3 region of the gene encoding 16S rRNA is the most suitable DNA fragment for the taxonomic characterization of activated sludge. The taxonomic classification based on the SILVA database yielded the largest number of genera and the highest number of unique taxons, which is why it is proposed as the database for 16S rRNA analysis.

The results obtained may be useful for the development of a research approach for microbiological characterization of activated sludge based on sequencing of 16S rRNA amplicons. The determination of the most appropriate reference database could be useful not only for the analysis of regions of the 16S rRNA gene but also for the whole gene using novel long-read sequencing techniques.
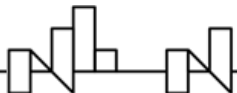
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Integrated system for SImultaneous Recovery of Energy, organics and Nutrients andgeneration of valuable products from municipal wastewater project (SIREN)