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Bioinformatics Analysis Reveals Potential Microorganisms and Enzymes for Microplastic Remediation in Antarctic Waters

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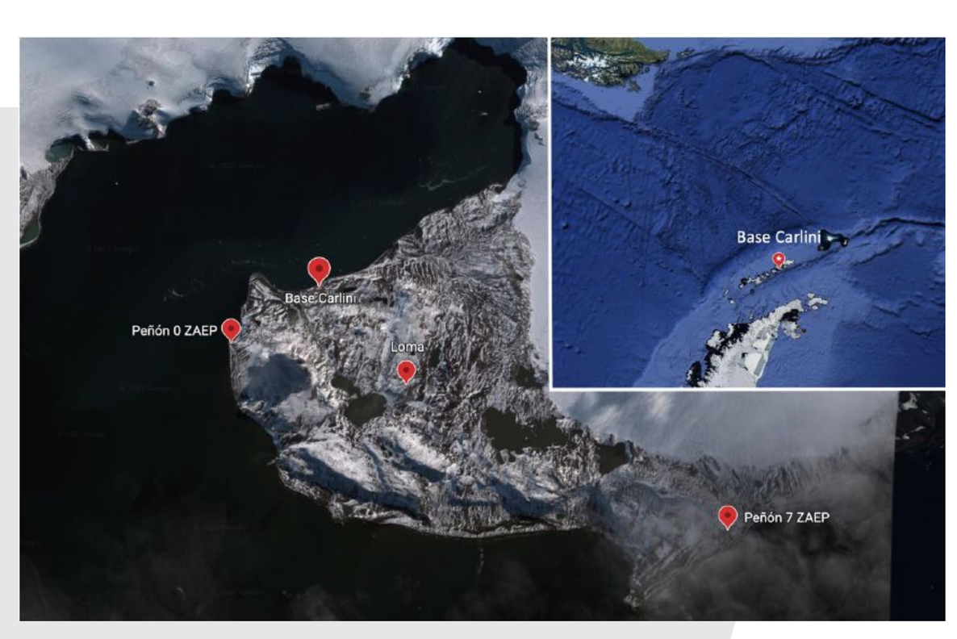
INTRODUCTION

- **The global issue of plastics and microplastics pollution demands urgent remediation measures. This study focused on the bioremediation of microplastic-contaminated[1] waters, specifically targeting the Antarctic region. Plastic (PET) sheets were exposed exposed to environmental conditions in seawater were collected, and metagenomic analysis was performed on cells recovered from these samples, DNA was extracted and sequenced using Illumina technology. The objective of this study is the bioprospection of different plastic degrading related enzymes[2] in these samples through a genome-mining approach.**

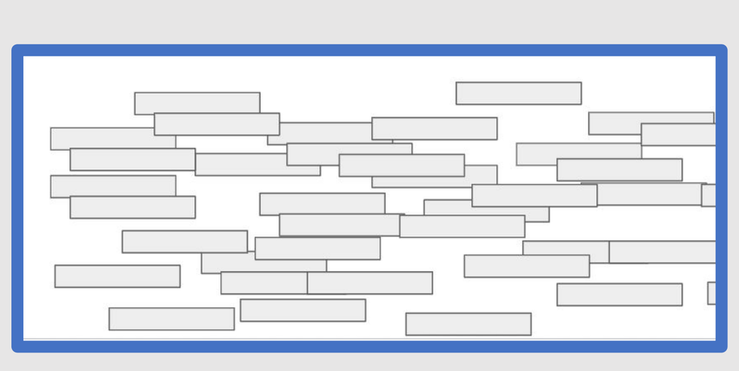
MATERIALS AND METHODS

Samples of PET were submerged in the Antarctic sea. They were collected one week and one month after time zero



The samples were taken to mainland Argentina and processed, where DNA extraction was performed

Shot-gun Metagenomic DNA sequencing using Illumina technology, resulting in paired-end reads of 151 bp



```
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QVASLNGTSSSPIYGKVDTARMGVMGWSMGG
SVTVPTLIFACENDSIAPVNSSALPIYDSMS
KGVAWMKRFMDNDTRYSTFACENPNSTRVSD
```
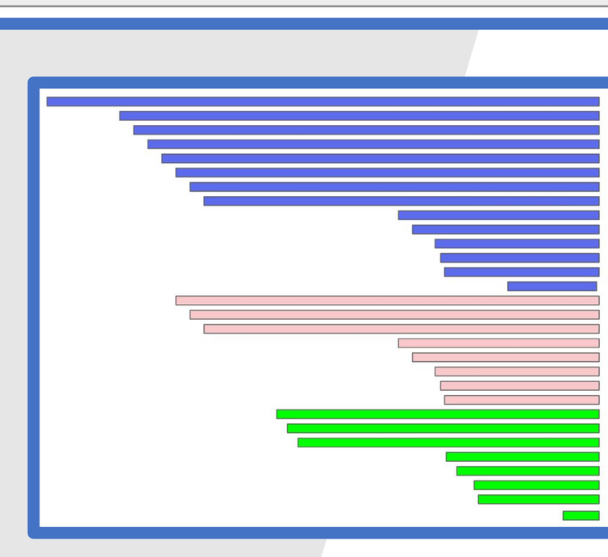

Analysis of metagenomes, key steps include quality control, demultiplexing to separate samples, and filtering to remove contamination

Assembly in contigs: This process involves piecing together overlapping reads to reconstruct the original genetic material, providing a more complete representation of the genome



Binning: the contigs were clustered into bins, resulting in nine Metagenome-assembles genomes (MAGs)

Annotation: Gene calling and Taxonomic profiling with prokka and MIGA, Kraken

Genome-Mining process, Protein BLAST was employed to search for homologous protein sequences in order to identify potential functional genes within the genome.

PAZy - The Plastics-Active Enzymes Database

```
>PETsem_C109_k77.4_00208 hypothetical protein
Length=350

 Score = 39.7 bits (91),  Expect = 2e-05, Method: Compositional matrix adjust.
 Identities = 22/53 (42%), Positives = 35/53 (66%), Gaps = 2/53 (4%)

Query  104  ALNHMINRASSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDL-KAAIPLTP  155
            A+N ++   +S +++ +D SR+ ++GHSMGGG TL  A   PDL +AA+   P
Sbjct  180  AVNAVLALKNSNIKN-LDFSRVGMLGHSMGGGVTLNAALAAPDLIQAAVIWGP  231
```

Protein analysis: Analysis of potential putative enzymes for PET and other polymer degradation through the study of their sequences using InterProScan and SignalP, to find importat domains.

Structural modeling: AlphaFold2 was usaed to predict the three-dimensional structures of these proteins. This is crucial for understanding protein function, interactions, and potentially for drug design.

RESULTS

Bin Id	Taxonomic assignment	Completeness	Contamination	Strain heterogeneity	Annotated proteins
PETsem_C109_k77.2	Flavobacteriaceae	72.54	02.08	66.67	1816
PETsem_C109_k77.4	Bacteria	58.92	2.25	0	848
PETmes_k_auto.2	Bacteria	43.35	6.94	0	1006
PETmes_k_auto.1	root	41.67	83.48	16.46	319
PETsem_C109_k113.3	Bacteria	39.20	0.56	0	641
PETsem_C109_k113.2	Bacteria	29.03	1.75	0	215
PETsem_C109_k77.1	Rhodobacteraceae	25.66	0.15	100	673
PETsem_C109_k77.3	Hellicobacter	15.62	0	0	200
PETsem_C109_k113.1	Hellicobacter	15.62	0	0	200

Table 1: Nine bins were formed, with seven of these being Metagenome-Assembled Genomes (MAGs) identified in samples collected one week after the experiment's start (pet_sem), and two were discovered a month (pet_mes) into the experiment. The relatively low number of bins may be attributed to the harsh Antarctic climate conditions. a total of four bins had a successful taxonomic assignment. Another four bins were classified as bacteria, suggesting that they may represent rare bacterial species. In contrast, one Metagenome-Assembled Genome (MAG) did not fall under the bacterial classification. An analysis of the taxonomic assignment of reads of this MAG conducted by Kraken revealed that 86.5% of the reads appeared as 'unclassified

Bioprospecting of Enzymes for Plastic Degradation

Enzymes	PETmes_k_auto.1	PETmes_k_auto.2	PETsem_C109_k77.1	PETsem_C109_k77.2	PETsem_C109_k77.3	PETsem_C109_k77.4	PETsem_C109_k113.1	PETsem_C109_k113.2	PETsem_C109_k113.3	Total
PET	0	0	0	0	0	15	0	0	14	29
Polyurethane (PUR)	0	1	8	3	0	11	0	3	8	34
Polylactic acid (PLA)	0	7	8	18	0	4	0	1	4	42
Other	0	1	7	6	0	9	0	3	7	33
										138

Table2: The table shows the significant alingments obtained by the BLASTp algorithm with an Expected value (E-value) under 0.01. The PAZY database we used as the query contained 196 enzymes with plastic-degrading activity. The BLASTp results yielded 138 significant alignments.

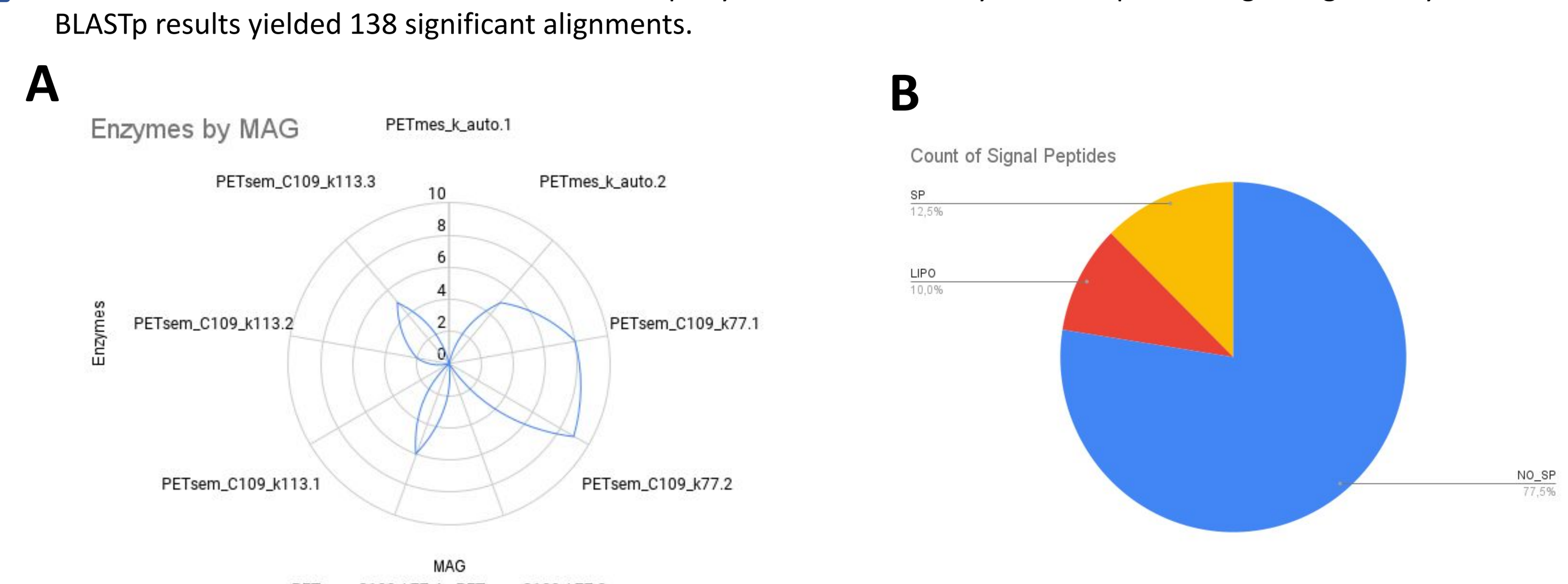


Figure 1: A. The plot shows the number of unique enzymes by MAG. This occurs because a single enzyme aligns well with more than one enzyme exhibiting degrading activity from the PAZY database. B. Count of signal peptides discovered by Signalp algorithm. Five enzymes has secretory peptidase SPase I (Sec/SPI), and four has SPase II (Sec/SPII), associated to prokaryotic lipoprotein. That implies that this enzymes are potentially exoenzymes capable of extracellular degradation since bacteria cannot incorporate microplastics into their cytoplasm

Figure 2: Enzymes featuring SPase I were modeled using AlphaFold2, resulting in these three-dimensional protein structures. A. PETsem_C109_k77.2_01003, B. PETsem_C109_k113.2_00089, C. PETsem_C109_k77.2_00999, D. PETmes_k_auto.2_00429 , E. PETsem_C109_k113.2_00089. The RMSD between D and E was 2.795

CONCLUSIONS

This study highlights the potential of Antarctic waters as a source of microorganisms and enzymes with the ability to degrade microplastics. The identification of PETases and other polymer-degrading enzymes opens up possibilities for the development of biotechnological solutions for microplastic pollution remediation. Further research is needed to explore the full potential of these microorganisms and enzymes in practical applications.

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