


Coursework Declaration and Feedback Form

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Abstract

Plastics have a high degree of stability, which enables them to be utilized in a broad variety of applications while also accumulating in huge numbers. Inadequate disposal of plastic waste in a non-biological environment results in the breaking of huge plastic pieces, resulting in a significant quantity of microplastic and nanoplastic contamination. Microplastics and nanoplastics have long been a source of worry for the health of creatures. Microplastics and nanoplastics may ultimately make their way into our intestines through the food chain and accumulate in the body over time. As a result, biodegradable polymers are presently undergoing significant research. Because microorganisms have enzymes that can degrade plastic and utilize it as a source of carbon and energy, the breakdown products of plastic will be absorbed by cells and used as a source of carbon and energy, before being reduced to end products such as carbon dioxide, methane, and water. Return to the biosphere in the form of photosynthesis and carbon fixation. The whole degrading process will be completely non-polluting to the environment. Among the many bacteria, *Pseudomonas putida* is one of the most frequently utilized biotech hosts in synthetic plastic degradation studies due to its broad metabolic capabilities. As a result, the emphasis of this study is on *Pseudomonas putida*'s capacity and pathway for degrading certain plastic polymers. The polyethylene terephthalate (PET) and polyurethane are the plastic high-molecular polymers that are being studied (PUR). The whole genome analysis of *Pseudomonas putida* will be the primary technique used in this study, and the tools used will be Prokka, Roary, Coinfinder, METABOLIC, and Rstudio.

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1 Introduction

1.1 Plastic application and background introduction

Plastics are high molecular weight synthetic polymers derived from long-chain hydrocarbons derived from petrochemical products (Ahmed et al., 2018). Various forms of plastics are the ubiquitous cornerstone of modern civilization. The production and application of plastics have made great contributions to a more efficient society, such as reducing packaging weight, extending food shelf life, and insulating homes and refrigerators (Li et al., 2020).

The unique durability and low-cost characteristics of plastics and the rapid development of cities have led to a sharp increase in the demand for plastic products in the global market (Jaiswal, S., Sharma, B., & Shukla, P., 2020). In 2014 alone, approximately 311 million tons of plastics were produced globally and used in different fields (Wilkes, R. A., & Aristilde, L., 2017). By 2018, this output has risen to 359 million tons, and it is expected that this value will reach 500 million tons in 2050 (Ackermann et al., 2021).

Plastic has the advantages of good stability, durability and low cost, which has made plastics gradually occupy the market share of original packaging materials for paper and other cellulose products. At present, plastics have been widely used in packaging in different fields. During manufacturing (Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S., 2008).

According to statistics, about 30% of the synthetic plastics produced annually in the world are widely used in different packaging purposes, and this ratio is increasing at a rapid rate of 12% per year (Wilkes, R. A., & Aristilde, L., 2017). Among the most widely used synthetic plastic materials are polyethylene (PE), polyurethane (PUR), polyamide (PA), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC) and polypropylene (PP) (Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S., 2008).

1.2 The status quo of plastic problems

Although humans undoubtedly benefit from the versatility and durability of plastics, the burden they create on the environment has gradually emerged. In the environmental matrix, the degradation rate of these synthetic plastics is very slow and the degradation difficulty is very high (Devi et al. 2016).

The current wide application of plastics worldwide and their good stability characteristics have led to their accumulation in all major terrestrial and aquatic ecosystems on the earth (Ackermann et al., 2021). Because synthetic plastics exist in nature for a short time, nature has only a short time to evolve highly active enzymes, but natural evolution cannot design new enzyme structures that can degrade synthetic polymers in a short time, so this makes plastic products have the ability to exist in nature for a long time. If plastic waste is not treated properly, the amount of plastic waste accumulated in the ecosystem will increase year by year (Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S., 2008).

According to statistics, 275 million tons of plastic waste was generated globally in 2010 alone (Wierckx et al., 2015). For the disposal of plastic waste, under ideal circumstances, the complete recycling of plastics is realized, that is, all plastics can be recycled and will not leak into the environment. However, this is quite unrealistic at the moment, and the reality is also very different from it (Ackermann et al., 2021). Even though some plastic waste has been incinerated or landfilled in controlled factories, the high processing costs and poor management still cause a large amount of plastic waste to be discharged into nature. At present, the improper handling and unregulated discharge of a large amount of plastic waste has become an important source of environmental pollution, and plastic pollution has also seriously affected the marine ecological environment (Katsnelson, 2015).

Investigations show that the level of plastic pollution in the ocean is increasing year by year,

and about 4 to 12 million tons of plastic waste enters marine habitats every year (Matar, R., 2020). As far as the Great Pacific Garbage Belt is concerned, more than 1.8 trillion pieces of plastic waste have been stored so far, and it is estimated that the total weight of these plastic waste will be as high as 80,000 tons (Danso, D., Chow, J., & Streit, W. R., 2019). According to the statistics of a research report in 2012, about 165 million tons of plastic waste have been stored in the global oceans (Jaiswal, S., Sharma, B., & Shukla, P., 2020).

However, improper handling of plastic waste is increasing its pollution impact on the natural environment. For example, the degradation of plastic waste in a non-biological environment will greatly promote the fragmentation and fracture of large plastic fragments, resulting in microplastic and nanoplastic pollution (Wei, R., & Wierckx, N., 2021). The treatment of plastic waste in the non-biological environment mainly refers to the exposure of plastic to ultraviolet rays in the ocean and the mechanical destruction of plastic caused by waves and wind, or the grinding and destruction of plastic by marine rocks and sediments. The treatment of plastics in non-biological environment has accelerated the fragmentation of large plastic fragments, resulting in a large number of microplastics and nanoplastics (MP, with size <5 mm, NP, size <0.1 μm) (Danso, D., Chow, J., & Streit, W. R., 2019). The impact of microplastics and nanoplastics on the health of organisms has always been a concern. Studies have shown that microplastics and nanoplastics will eventually enter the intestines of organisms through the food chain, and microplastics and nanoplastics will gradually accumulate and transfer in different organisms and environments (Danso, D., Chow, J., & Streit, W. R., 2019). This will have adverse effects on animals, humans and habitats, and will also cause certain environmental pollution (Matar, R., 2020). Relevant studies have confirmed that microplastic pollutants have adversely affected the reproductive behavior of invertebrates in the soil (Santacruz-Juárez et al., 2021).

Therefore, the reasonable disposal of plastic waste has become a global problem. Making plastics can be removed from the environment has become the focus of research for

environmental protection workers. To reduce this impact, in addition to incineration, landfill and inefficient recycling, new plastic waste treatment strategies are currently the most concerned. Among the new plastic waste treatment methods, microbial degradation of plastics is currently considered to be a promising and highly feasible method, and studies have shown that some microorganisms can use plastic waste as a source of carbon (Li et al., 2020).

1.3 Biodegradable plastics

Biodegradable plastic is an environmentally friendly method. Biodegradation is a process in which microorganisms induce polymer degradation through assimilation or enzyme release. Microbes mainly include bacteria and fungi (Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S., 2008). Since microorganisms have enzymes that can use plastics as substrates, (such as oxidoreductase, laccase, and peroxidase), the secretion of microbial enzymes and adhesion to the surface of the polymer will catalyze the cleavage of ester bonds. The catalyzed redox reaction will destroy the chemical bonds in the plastic polymer (Jaiswal, S., Sharma, B., & Shukla, P., 2020). This allows the plastic polymer to be decomposed into monomers, and the resulting monomers can quickly pass through the cell membrane, which can be further used by microorganisms as carbon and energy sources, making the plastic completely degraded (Jaiswal, S., Sharma, B., & Shukla, P., 2020). Therefore, this environmentally friendly degradation method makes microorganisms very suitable for the task of reducing plastic pollutants. However, this does not completely mean that all synthetic plastics have the conditions to be degraded by microorganisms. Due to this mechanism of microbial enzymes, the plastic polymer that is required to be degraded needs to contain ester bonds itself, or it needs to be oxidized by other methods in advance before being catalyzed by microorganisms (Vague et al., 2019).

Microbial degradation of plastics is caused by many factors, including the surface area of

the polymer, the type, and the nature of the pretreatment (Jaiswal, S., Sharma, B., & Shukla, P., 2020). The spontaneous hydrolysis, photooxidation and mechanical separation of plastics have been proven to promote biodegradation by introducing cleavable bonds or simply increasing the surface area of plastics (Vague et al., 2019). At the same time, the different characteristics of different polymers, such as fluidity, molecular weight, the types of functional groups and substituents present in the structure, and the plasticizers or additives added to the polymer, are all in the degradation of plastics by microorganisms. It plays an important role in the process (Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S., 2008).

The complete biodegradation process in which plastic is degraded by microorganisms first requires the decomposition of plastic polymers into smaller oligomers to form monomers that can pass through the cell membrane. The larger polymers are initially degraded into smaller subunits by secreted enzymes. Monomer (multimer, dimer) (Danso, D., Chow, J., & Streit, W. R., 2019). Then these subunit monomers can be incorporated into microbial cells for assimilation and subsequent intracellular metabolism. After entering the cell, these monomers or their degradation products will generate energy through classical degradation pathways or serve as catabolism or metabolic construction (Wilkes, R. A., & Aristilde, L., 2017). Once decomposed into monomers, oligomers, aldehydes, ketones, and other small molecules, plastic polymers may be ingested by cells and utilized as a source of carbon and energy. Finally, the breakdown products are reduced to their ultimate forms of carbon dioxide, methane, and water and mineralized (Wilkes, R. A., & Aristilde, L., 2017). The CO₂ is subsequently taken by plants or photosynthetic microorganisms, and the photosynthesis and carbon fixation processes return the carbon from the plastic to the biosphere (Vague et al., 2019).

Effective microbial digestion of the monomers included in biodegradable polymers is required. *Pseudomonas* is a particularly promising microbial catalyst in this area due to its metabolic diversity and ability to thrive on a variety of plastic monomers (Ackermann et al., 2021). Studies have shown that different non-pathogenic strains of this genus have good

inherent characteristics, such as high tolerance to chemical stress and rapid and efficient growth (Ackermann et al., 2021). Therefore, among the many microorganisms, bacteria and fungi, members of the genus *Pseudomonas* have received special attention due to their ability to degrade and metabolize plastics.

Pseudomonas species are ubiquitous in aquatic and terrestrial environments, and studies have shown that species of this genus can degrade polyethylene, polypropylene, polyvinyl chloride, polystyrene, polyurethane, and polyparaben with varying degrees of efficiency. Polymer materials of ethylene glycol phthalate, polyethylene succinate, polyethylene glycol and polyvinyl alcohol (Wilkes, R. A., & Aristilde, L., 2017). Therefore, *Pseudomonas* species has become the most frequently cited degradation agent for various plastic polymers.

1.4 *Pseudomonas putida*

Pseudomonas putida is a widely used microorganism among members of the genus *Pseudomonas*. Because *Pseudomonas putida* has high tolerance and metabolic ability to a variety of plastic polymers, *Pseudomonas putida* has become one of the most widely used biological hosts in the research of biodegradation of plastics. (Nikel and de Lorenzo, 2018). *Pseudomonas putida* can use plastic polymers as its main carbon source.

Modern biotechnology has shown that the genome of *Pseudomonas putida* contains a wide range of oxygenases, oxidoreductases, hydrolases, transferases, and dehydrogenases (Belda et al., 2016). The enzymes contained in its genome can make *Pseudomonas putida* have the ability to modify a large number of alcohols and aldehydes (Li et al., 2020). At the same time, the study pointed out that when *Pseudomonas putida* oxidizes alcohol and aldehydes, *Pseudomonas putida* will carry out a large amount of metabolic input.

Many studies have verified the metabolic ability of *Pseudomonas putida* to different plastic polymers. Polyethylene (PE) plastic has been discovered by researchers as the main carbon

source in the redox reaction of *Pseudomonas putida* (Matar, R., 2020). Additionally, *Pseudomonas putida* cells have been demonstrated to lyse and breakdown LDPE (powdered low-density polyethylene plastic) into polyhydroxyalkanoate polymers. (Montazer et al., 2019).

Pseudomonas putida also acts on the oligomer monomers produced during the decomposition of different plastics. Among them, polyethylene terephthalate (PET) monomer ethylene glycol, and polyurethane (PUR) monomer 1,4-butanediol have been studied to show that they can provide *Pseudomonas putida* Carbon source and energy (Ackermann et al., 2021).

Under certain conditions, *Pseudomonas putida* strains can metabolize ethylene glycol and use it as the only source of carbon and energy (Franden et al., 2018). The application of ethylene glycol in daily life is also very extensive. Ethylene glycol is an important starting material for the production of polyester plastics, such as polyethylene terephthalate (PET) (Franden et al., 2018).

Pseudomonas putida can also grow in 1,4-butanediol (Li et al., 2020). 1,4-Butanediol is one of the main chain extenders used in the production of polyurethane (PUR), and it is also a common comonomer in many polyesters, such as polybutylene terephthalate and polyadipate. According to surveys, a large amount of 1,4-butanediol will be used to make plastics and polyester products every year.

1.5 Method overview

This research mainly used the study of microbial genome to infer the ability of microbial ecology. In this process, *Pseudomonas putida* will be sequenced through the use of different genome software, as well as gene annotation and other analysis. And through the obtained

genomic information to detect the relationship between *Pseudomonas putida* and polymers with different characteristics, as well as the relationship between the monomers that make up the polymer.

The specific software tools and methods used are as follows. The first is to download all available genomes of *Pseudomonas putida* from the gene database. Then use Prokka to perform genome annotation on the genome of *Pseudomonas putida*. Genome annotation is the process of identifying and marking all relevant features on the genome sequence (Seemann, T., 2014). Prokka can quickly annotate *Pseudomonas putida* genes, and can identify coding sequences in the prokaryotic genome and identify the characteristic coordinates of the *Pseudomonas putida* genome in the contig (Riccardi et al., 2021). Running Roary (pan-genome pipeline) can analyze and construct *Pseudomonas putida* pan-genome within a reasonable calculation time (Sitto, F., & Battistuzzi, F. U., 2020). Roary analysis contains thousands of *Pseudomonas putida* pan-genomic data sets, as well as rapid construction of *Pseudomonas putida* pan-genomic data from a large number of prokaryotic samples (Riccardi et al., 2021). Coinfinder will detect important associations and separations in the pangenome of *Pseudomonas putida*, and predict genes that are associated and separated from other genes in the pangenome of *Pseudomonas putida* at a higher frequency than expected. Coinfinder can be used to study the pan-genome structure of strains or species, and is not limited to the input of prokaryotic or eukaryotic genomes (Whelan, F. J., Rusilowicz, M., & McInerney, J. O., 2020). Use METABOLIC to perform genome annotation, protein annotation, identification of metabolic markers, and metabolic pathway analysis of *Pseudomonas putida*. And METABOLIC will also predict the metabolic and biogeochemical functional profile of the *Pseudomonas putida* genome dataset (Zhou et al., 2020).

At the same time, the research also runs Vegan and CNA on the RStudio software to conduct in-depth analysis of the gene sequences and protein species related to plastic degradation

in the data generated by METABOLIC. Vegan describes the tools of species and community ecology, mainly used to analyze diversity and difference. This research will mainly use its "Non-Metric Multidimensional Scaling" (NMDS/MDS) function. Coincidence Analysis (CNA) is a method used to analyze joint causality (component causality) and equivalence (alternative causality). At the same time, CNA is currently the only method for INUS-discovery that allows for multiple effects.

1.6 Gaps in existing knowledge

This study can analyze the degradation effect of *Pseudomonas putida* on plastics to a certain extent, but it cannot fully understand all the information of *Pseudomonas putida* in the biodegradation process. At the same time, the results of this genetic analysis are only about *Pseudomonas putida* and plastic or its monomers directly undergo natural degradation. As current studies have shown that *Pseudomonas putida* can promote or enhance the degradation of some plastics by *Pseudomonas putida* in some customized biocatalysts. At present, experiments have confirmed that the use of antibiotics or *sacB* selection and counter-selection systems for *Pseudomonas putida* can change the metabolism of *Pseudomonas putida* to polymer materials after gene replacement (Franden et al.,2018). However, studies on unnatural degradation methods such as bioengineering to improve *Pseudomonas putida* or adding multiple substrates to improve the degradation ability of *Pseudomonas putida* cannot be fully or accurately explained and proved in this study. On the other hand, in this study on the genome of *Pseudomonas putida*, only part of the gene analysis software was used, and the true and natural degradation of plastics by *Pseudomonas putida* could not be fully studied. Therefore, this study has a certain degree of knowledge limitations. The research content is only part of the research and discussion on the degradation ability of *Pseudomonas putida* under natural conditions.

1.7 Research purpose

The focus of this research is on the exploration of *Pseudomonas putida* degrading ability of some plastic polymers and the path of microbial degradation. Through genome sequencing and gene annotation analysis of the whole genome of *Pseudomonas putida* to study the relationship between *Pseudomonas putida* and plastics and monomers produced by plastic degradation. At the same time, the analysis of the path of plastic biodegradation by *Pseudomonas putida* and the pan-genome report generated by the software will be used to further explore the relationship between *Pseudomonas putida* genes and different protein enzymes. Polyethylene terephthalate (PET) and its monomer ethylene glycol, polyurethane (PUR) and its monomer 1,4-butanediol are the focus of this research on polymer materials. This study aims to discuss under natural conditions, *Pseudomonas putida* on the metabolism of polyethylene terephthalate (PET) and polyurethane (PUR), as well as the monomers ethylene glycol and 1,4-butanediol produced by them. And explore the path of these polymer materials in the process of degradation by *Pseudomonas putida*. Furthermore, through the study of its biodegradation pathway, how *Pseudomonas putida* plays a role in the degradation process. And will explore whether it is feasible to use *Pseudomonas putida* to biodegrade plastics and whether there is a more suitable biodegradation pathway.

2. Literature review

2.1 Polyethylene terephthalate

PET is mostly utilized in everyday life to manufacture products such as PET bottles, PET foils, and fibers in the textile sector. PET is a polar linear polymer composed of aromatic terephthalic acid and ethylene glycol repeating units (Danso, D., Chow, J., & Streit, W. R., 2019). Research on the direct biodegradation of PET by *Pseudomonas putida* has not been accurately confirmed. However, the biodegradation analysis of monomers produced by PET pyrolysis has been widely discussed. Studies have shown that some PET pyrolysis product monomers can be used as the only carbon source for *Pseudomonas putida* (Vague et al., 2019). Studies have also suggested that the polymer components in polyethylene terephthalate waste can provide a new matrix for the conversion of industrial biotechnology into value-added products, such as ethylene glycol and terephthalic acid (Franden et al., 2018).

Heat treatment (pyrolysis) of PET in the absence of air. In general, one of the principal products of PET degradation is the monomer mono(2-hydroxyethyl) terephthalic acid (MHET), which eventually degrades to bis(2-hydroxyethyl) terephthalic acid (BHET). PETase is secreted and converts PET to MHET, which is then converted to terephthalic acid (TPA) and ethylene glycol by secreted MHETase (Vague et al., 2019). TPA generated during PET pyrolysis could be used as a starting material for microbial production of a high-value-added biodegradable polymer called polyhydroxyalkanoate (PHA) (Kenny et al., 2008).

Among them, the ethylene glycol produced by the pyrolysis of PET is currently widely used in the production of polyethylene terephthalate (PET) polyester plastics, and it is also a common pollutant in the environment. Improper handling of ethylene glycol or its intermediate glycolaldehyde can cause serious environmental problems (Franden et

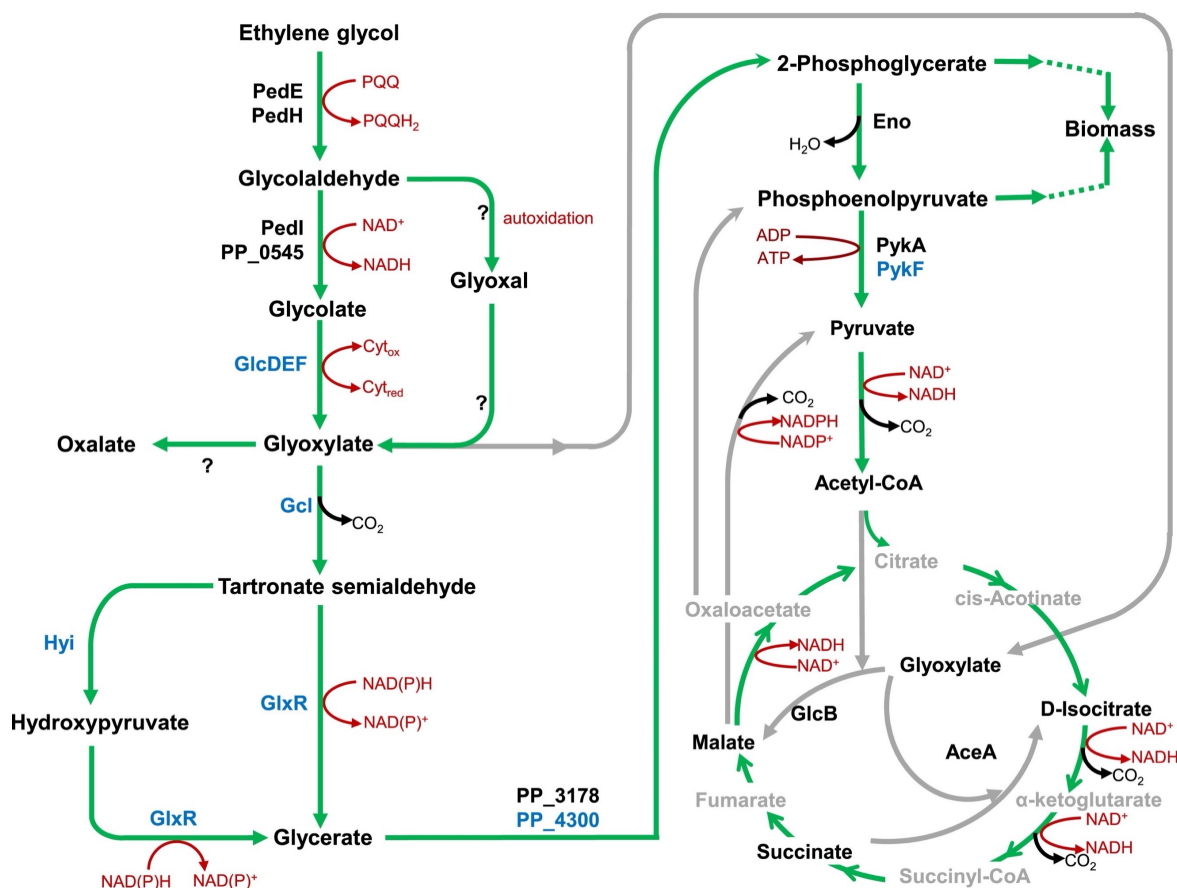
al.,2018). Therefore, the focus of the degradation of PET by *Pseudomonas putida* is to study the mechanism of biodegradation of ethylene glycol by *Pseudomonas putida*. At present, studies have shown that *Pseudomonas putida* can biodegrade ethylene glycol. *Pseudomonas putida* has the genes necessary to convert ethylene glycol into cell biomass, and the obligate aerobic organism *Pseudomonas putida* can use only ethylene glycol as a source of reducing equivalents and energy (Mückschel et al., 2012). Studies have also found that the process of ethylene glycol metabolism by *Pseudomonas putida* is achieved by the transcription regulator GclR (glyoxylic ligase repressor) inhibiting the glyoxylic ligase pathway (Li et al., 2019).

In addition, experiments have compared the proteome of *Pseudomonas putida* strains, and proposed that the catabolism pathway of *Pseudomonas putida* to ethylene glycol is mainly through two redundant periplasmic quinone proteins PedE and PedH, and the following two. The activities of two cytoplasmic aldehyde dehydrogenases PP_0545 and PedI, together with the membrane-anchored oxidase GlcDEF, finally produce glyoxylic acid (Wehrmann et al., 2017). Then, glyoxylic acid is further metabolized by the dicarboxylic acid route, which is begun by the acetyl-CoA linkage catalyzed by malate synthase, or via the partial utilization of TCA-cycle processes, which are initiated by the AceA-dependent ligation of glyoxylate and succinate to generate isocitrate (glyoxylate cycle) (Frandsen et al.,2018).

Pseudomonas putida uses ethylene glycol as a genomic list of carbon sources, and initially connects two glyoxylic acid molecules to tartrate semialdehyde by glyoxylate carbonase. According to the prediction of the *Pseudomonas* genome database, glyoxylate sugar ligase can work with glycerate kinase to allow the conversion of glyoxylate into biomass (Winsor et al., 2016). At the same time, studies have shown that *Pseudomonas putida* can effectively convert ethylene glycol into polyhydroxyalkanoate (mcl-PHA), a high-value chemical building block. Therefore, it has been pointed out that *Pseudomonas putida* strains can be used as the basis for the conversion of ethylene glycol in plastic waste and glycolaldehyde

in biomass-derived wastewater streams (Franden et al.,2018). However, the research on the ability of *Pseudomonas putida* to biodegrade ethylene glycol requires further sequencing and protein analysis of its genome. The degradation path of ethylene glycol in *Pseudomonas putida* and the reaction of enzymes involved in biodegradation are shown in the figure.

Figure1: Ethylene glycol degradation process



2.2 Polyurethane

Different polyether or polyester polyols can be used to manufacture polyurethane (PUR). PUR is a polymer composed of organic units connected by carbamate; the inclusion of an aromatic ring structure alters the polymer's physical and chemical characteristics (Danso, D., Chow, J., & Streit, W. R., 2019). Polyurethane is a synthetic polymer that is widely utilized in the manufacturing of foams, insulating materials, textile coatings, and anti-corrosion

coatings (Danso, D., Chow, J., & Streit, W. R., 2019). However, due to the diversity of polyurethane molecules and most of them are thermoset materials, as well as the characteristics of inability to melt and difficult to reshape, it is difficult to systematically recycle polyurethane materials (Li et al., 2020).

Polyurethane is synthesized by reacting aliphatic or aromatic diisocyanates with chain extenders such as polyols and α,ω -diols. The features of the polymer vary depending on the monomer composition and chain length, which contributes to the polymer's adaptability. Polyurethane may be found in a variety of end-user goods, including paints and coatings, building insulation materials, and sealants, as well as soft foams and absorbents such as pillows and mattresses (Li et al., 2020).

Biodegradation studies have shown that *Pseudomonas putida* degrades PUR at a relatively high rate (Danso, D., Chow, J., & Streit, W. R., 2019). Polyurethane polymers are degraded into polyurethane monomers under the action of a series of polyurethane degrading esters and polyurethane hydrolase. The typical polyurethane monomer is 1,4-butanediol (Li et al., 2020). The focus on the biodegradation of polyurethane by *Pseudomonas putida* will focus on the polyurethane monomer it produces, 1,4-butanediol.

1,4-Butanediol is a commonly utilized chain extender in the manufacture of polyurethane. (Yim et al., 2011). Butanol is a substrate with a structure comparable to that of 1,4-butanediol. *Pseudomonas putida* has been found to grow on 1,4-butanediol. In this process, butanol can be rapidly oxidized to butyric acid through the action of various alcohol and aldehyde dehydrogenases (Cuenca et al., 2016). Prominent among them are PedE, PedH and PedI alcohol and aldehyde dehydrogenase. They have a very broad substrate specificity, which enables them to oxidize butanol, and the resultant butyrate is activated by acyl-CoA synthase to form CoA, which is subsequently β -oxidized. (Wehrmann et al., 2017).

1,4-Butanediol may be metabolized through three distinct metabolic routes, all of which terminate in 4-hydroxybutyrate. This 4-hydroxybutyric acid is rapidly formed in the culture of *Pseudomonas putida* and 1,4-butanediol. Subsequently, 4-hydroxybutyric acid oxidizes 1,4-butanediol to 4-hydroxybutyrate in a high-speed reaction process through the action of alcohol and aldehyde oxidase in *Pseudomonas putida* (Li et al., 2020). PedE, PedH and PedI alcohol and aldehyde dehydrogenase are the main players in these oxidation steps (Franden et al., 2018). The 4-hydroxybutyric acid formed during the oxidation reaction is further oxidized by the same enzyme, and the resulting succinate semialdehyde can be oxidized by the annotated succinate semialdehyde dehydrogenase, or by methylmalonate-semialdehyde dehydrogenase (MmsA-II), the succinate semialdehyde that results may be further metabolized through the TCA cycle (Li et al., 2020).

At the same time, in *Pseudomonas putida*, 4-hydroxybutyrate, the oxidation product of 1,4-butanediol under the action of alcohol and aldehyde dehydrogenase, can also be activated by CoA through CoA ligase or transferase. After CoA is activated, 4-hydroxybutyryl-CoA can undergo β -oxidation. The reflection process is carried out by the enzyme encoded by the transcription regulator of the alcohol dehydrogenase operon, which will lead to the production of glycolyl-CoA and acetyl-CoA. It is then metabolized through the natural pathway of *Pseudomonas putida* (Li et al., 2019). When it is active on 4-hydroxyl substrates, 4-Hydroxybutyryl-CoA can also be further oxidized by acyl-CoA dehydrogenase to produce succinyl-CoA (Li et al., 2020). There are also studies and analyses that support the direct oxidation of 1,4-butanediol to succinate through 4-hydroxybutyrate, or simultaneous operation with β -oxidation. But this requires further genome resequencing and proteomic analysis to confirm it (Li et al., 2020).

3. Method

3.1 Method background

This research is based on metagenomics and single-cell genomics. The research method used is to mark and analyze the whole genome of *Pseudomonas putida*. The research method is based on the establishment and analysis of the pan-genome of prokaryotes. Microbial pan-genome is used to describe the combination of genes shared by the genome of interest (Sitto, F., & Battistuzzi, F. U., 2020). The latest applications of pan-genomes can better define the concept of species in prokaryotes (Moldovan and Gelfand 2018). The application of the pan-genome method makes genetic research and analysis not only consider the gene structure of identity (-i parameter in Roary), but also consider orthology/paralogy and gene flow, thus increasing the perspective of studying the species genome (Sitto, F., & Battistuzzi, F. U., 2020).

The purpose of this study is to explore the biodegradability of *Pseudomonas putida* to plastics, so a comprehensive analysis of the entire genome of *Pseudomonas putida* is needed. However, by using different genetic analysis software to analyze the pan-genome of *Pseudomonas putida*, we can have a certain understanding of the biodegradation mechanism of *Pseudomonas putida* to a certain extent. The different genetic analysis software used in the whole research process will conduct in-depth research around the pan-genome of *Pseudomonas putida*. The genome sequence of *Pseudomonas putida* in FASTA format will provide input files for Prokka and METABOLIC.

Prokka converts a single genome of *Pseudomonas putida* into Roary's input file. Then the *Pseudomonas putida* gene of a single genome was constructed by Roary's operation and the pan-genome of *Pseudomonas putida* was estimated. At the same time, the pan-genome is also an input file in the genetic analysis software. By running Coinfinder, important

associations and separations in the pan-genome of *Pseudomonas putida* can be detected. Therefore, this research method will be based on the analysis of the entire gene of *Pseudomonas putida* and the analysis of the pangenome of *Pseudomonas putida*.

3.2 Genetic analysis

Prokka

Prokka is a command-line software tool for annotating whole genomes. Genome annotation is the process of identifying and marking all relevant features on the genome sequence (Seemann, T., 2014). Due to the accurate and fast design of Prokka, this method is very suitable for sequence analysis and iterative models integrated into the genome pipeline.

Therefore, this study will use Prokka to quickly identify and label the entire genome of *Pseudomonas putida*. First enter the genome sequence of *Pseudomonas putida* in FASTA format in Prokka. Then Prokka uses external feature prediction tools to identify the coordinates of the genome-wide features of *Pseudomonas putida*, and identify the coding sequence in the genome of *Pseudomonas putida*, and search for the characteristics of the genome of *Pseudomonas putida*, all these tools provide coordinates and appropriate labels to describe features (Seemann, T., 2014). The tools that Prokka will use to annotate genome sequences are shown in the table. Finally, a standard-compliant output file is generated, and the output file format is shown in the table.

Table1: Feature prediction tools used by Prokka

Tool (reference)	Predicted characteristics
Prodigal	Sequence of coding (CDS)
RNAmmer	Genes encoding ribosomal RNA (rRNA)
Aragorn	RNA transfer genes

SignalP	Signal leader peptides
Infernal	Non-coding RNA

Table2: Description of Prokka output files

Suffix	Description of file contents
.gff	This is the GFF3-format master annotation, which contains both sequences and annotations. It is accessible immediately via Artemis or the IGV.
.gbk	This is a typical Genbank file that was generated from the master.gff file. If prokka was fed a multi-FASTA, this will result in a multi-Genbank, with one record for each sequence.
.fna	FASTA file containing the input contig sequences' nucleotide sequences.
.faa	Protein FASTA file of the translated CDS sequences.
.ffn	FASTA file containing the nucleotide sequences of all predicted transcripts (CDS, rRNA, tRNA, tmRNA, and misc RNA).
.sqn	A file in the ASN1 format called "Sequin" for submission to Genbank. It should be updated to include the proper taxonomy, authors, and linked publications, among other things.
.fsa	The input contig sequences' nucleotide FASTA file, which is utilized by "tbl2asn" to generate the.sqn file. It is mostly identical to the.fna file, except for the addition of Sequin tags to the sequence description lines.
.tbl	File containing the Feature Table, which is utilized by "tbl2asn" to generate the.sqn file.
.err	Unacceptable annotations - a report from the NCBI on discrepancies.
.log	Contains every output generated by Prokka throughout its execution. This is a log of the settings you used, regardless of whether the <code>--quiet</code> option was used.
.txt	Statistics on the annotated features discovered.
.tsv	All characteristics are tab-separated in this file: locus tag,ftype,len bp,gene,EC number,COG,product.

During this process, Prokka will also create Roary's input file. This process is to run Prokka to convert the .fna file to the GFF3 file format.

Roary

Roary is a tool for constructing and applying large-scale pan-genomes. Through multi-species pan-genome construction, species genomes can be described in more detail.

Roary can quickly construct a large-scale pan-genome and analyze and generate results of tens or hundreds of pedigrees within a reasonable calculation time (Sitto, F., & Battistuzzi, F. U., 2020).

Roary uses GFF3 format files as input files. This format file contains a series of information in a specific order, and each sample has an annotated assembly (Page et al., 2015). GFF3 format files can be obtained by various methods. In this study, Prokka software will be used to obtain GFF3 format files of *Pseudomonas putida* genes. After Roary runs, a series of files will be output, this contains statistical information on all shared genes、most core and soft core gene lineages、and some accessory genomes (shell gene and cloud gene) (Sitto, F., & Battistuzzi, F. U., 2020).

In this study, Roary software will be used to construct the pan-genome of *Pseudomonas putida*. First, input the GFF3 file of *Pseudomonas putida*. Then enter parameters and commands in Roary, run Roary and complete three types of operations: file access, analysis settings, and visualization. Finally, output the generated series of files and get the visualization results.

Roary will generate 17 output files after the completion of the operation and a series of operations; among the output files, the most significant are summary statistics.txt and gene presence absence.csv (Page et al., 2015). The summary statistics text file contains

information on the number of genes classified into four groups (core, soft, shell, and cloud) as well as the overall number of genes in the pan-genome. These numbers accurately reflect the pan-genome of the species under analysis. The gene presence absence file contains extra information, including the specific gene IDs of sequences included in the summary statistics for each category (Sitto, F., & Battistuzzi, F. U., 2020). Additional output files (beginning with "number of_") include data unique to each category (ie core or attachment). These data are supplied in the R format for further genetic study (Sitto, F., & Battistuzzi, F. U., 2020). Simultaneously, in order to view the results graphically, two outputs (ending in _graph.dot) enable users to gather information on the relative placements of genes belonging to subsidiary or core categories.

In the simulation data, Roary is the only application that can correctly identify all clusters. Roary has good scalability on large real data sets, even if there are various open pan-genomes, it can identify a large number of core genes. Therefore, in this study, running the *Pseudomonas putida* genome in Roary is an important part of genetic analysis.

METABOLIC

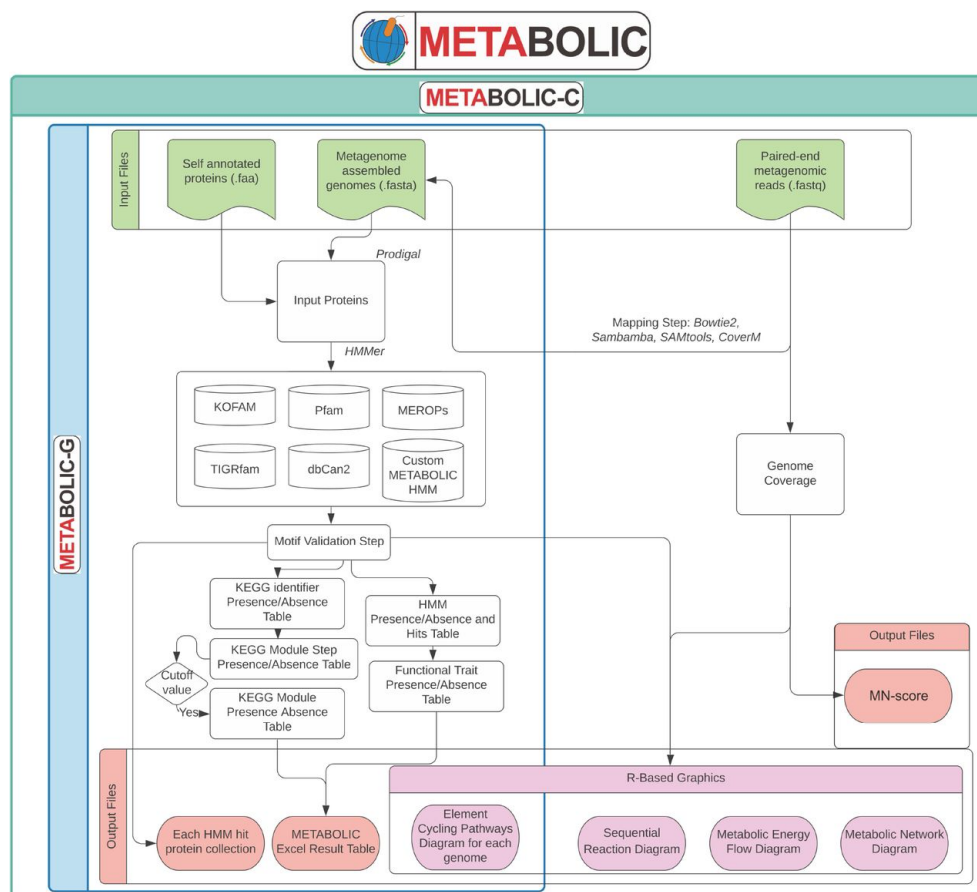
METABOLIC: METabolic And Biogeochemistry anaLyses In miCrobies.

METABOLIC is a toolkit based on the analysis of metabolic and biogeochemical features of microbial genomes (Zhou et al., 2020). METABOLIC can realize microbial metabolism switching and interaction and community-scale visualization of the contribution of microorganisms to the biogeochemical cycle. METABOLIC will make it possible for metagenomics and genomes to more easily explain microbial metabolism and biogeochemistry, and make microbiome research possible in different fields (Zhou et al., 2020). METABOLIC can extract input genomes from isolates, metagenome assembled genomes, or single-cell genomes. The results generated after running METABOLIC are presented in the form of metabolism tables and various visualizations.

Regarding the entire process of METABOLIC operation, the input file format is still required to use FASTA format file. The input folder requires the microbial genome sequence in FASTA format and an optional set of genome/metagenomic reads to reconstruct these genomes. The genome sequence was annotated by Prodigal (Zhou et al., 2020). Then use hmmsearch implemented in HMMER to query the HMM database for proteins. This search application method is as sensitive and effective as possible to detect remote homologs (Ghatge, S., Yang, Y., Ahn, J. H., & Hur, H. G., 2020). After the hmmsearch step, METABOLIC then verifies the primary output through the subject inspection step of the protein family subset.

METABOLIC makes use of database comparisons to infer the presence of particular metabolic pathways in the microbial genome. To improve the explanation of the metabolic pathway, a single KEGG annotation is inferred within the context of the KEGG module. The KEGG module is divided into several stages, each representing a distinct metabolic activity. (Zhou et al., 2020). The workflow of METABOLIC is shown in the figure.

Figure2: An outline of the workflow of METABOLIC



The prediction of microbial metabolism depends on the established database. METABOLIC uses KEGG, TIGRfam, Pfam and custom hidden Markov model (HMM) in the process of predicting microbial metabolism for protein annotation. Determine the presence or absence of metabolic pathways based on the KEGG module, and produce user-friendly output in the form of tables and numbers, this includes a functional overview、 biogeochemical related pathways、 a single genome and community-scale metabolic network abstract (Zhou et al., 2020). The output file is shown in the table.

Table3: Overview of METABOLIC output files

Output File	File Description
All_gene_collections_mapped.depth.txt	The gene depth of all input genes
Each_HMM_Amino_Acid_Sequence/ intermediate_files/	The faa collection for each hmm file Intermediate files are generated by hmmsearch, peptides (MEROPS), CAZymes (dbCAN2), and GTDB-Tk (only for METABOLIC-C).
KEGG_identifier_result/	Each genome's hit and result from the Kofam database
METABOLIC_Figures/	All numbers generated during the execution of METABOLIC
METABOLIC_Figures_Input/	All files used to create diagrams in R
METABOLIC_result_each_spreadsheet/	Each sheet in the generated METABOLIC result.xlsx file is represented by a TSV file.
MN-score_result/	The table that results for MN-score
METABOLIC_result.xlsx	METABOLIC's resulting excel file

This research will focus on the analysis of the 'METABOLIC_result.xlsx' table of *Pseudomonas putida* output by METABOLIC. The output Excel spreadsheet contains detailed information about protein hits and consists of six different results. The specific information is shown in the table. These contain detailed information about protein hits, these include the presence/absence of protein names, the presence/absence of functional features, the presence/absence of KEGG modules, the presence/absence of KEGG module steps, CAZyme hits, and peptidase/inhibitor hits. For each HMM profile, protein hits from all input genomes can be used to construct a phylogenetic tree, or further combine with other data sets or reference protein collections for detailed evolutionary analysis.

Table4: METABOLIC result table (METABOLIC_result.xlsx)

Results	Description
HMMHitNum	The presence or absence of bespoke HMM profiles inside each genome, the number of times the HMM profile was detected within a genome, and the ORF(s) encoding the identified protein are all reported.
FunctionHit	The presence or absence of sets of proteins that have been discovered and shown separately in the sheet labeled "HMMHitNum". The functions are classified as "Present" or "Absent" for each genome.
KEGGModuleHit	Each genome is annotated with modules from the KEGG database, which are grouped by metabolic type. The functions are classified as "Present" or "Absent" for each genome.
KEGGModuleStepHit	Resence or absence of modules from the KEGG database within each genome separated into the steps that make up the module. The functions are classified as "Present" or "Absent" in each genome, depending on their presence or absence.
dbCAN2Hit	The dbCAN2 annotation results against all genomes (CAZy

	numbers and hits). For each genome, there are two separate columns that display the number of times a CAZy was found as well as the ORF(s) that encode the protein that was detected.
MEROPSHit	The outcome of the MEROPS peptidase searching (MEROPS peptidase numbers and hits). The number of times a peptidase was discovered and the ORF(s) that encoded the protein are each listed in two separate columns for each genome in the table.

Coinfinder

Coinfinder is a command-line software program used to detect important associations and separations in pan-genomes. Since Coinfinder can focus on overlapping gene pairs. Therefore, Coinfinder will be used to identify a set of overlapping (associated or dissociated) genes in the input genome, assess whether homologous genes in the pan-genome are associated or separated more frequently than expected by chance, as well as an accurate and effective tool to identify overlapping gene relationships in pan-genome (Whelan, F. J., Rusilowicz, M., & McInerney, J. O., 2020). At the same time, Coinfinder is used to study the pan-genome structure of strains or species and is not limited to the input of prokaryotic or eukaryotic genomes (Whelan, F. J., Rusilowicz, M., & McInerney, J. O., 2020). Therefore, this study will use Coinfinder to detect significant association and dissociation in the pangenome of *Pseudomonas putida*.

Coinfinder is designed to accept as input data sets including pan-genomes and the genes included within them. Ideally, genes will be grouped into homologous gene clusters by utilizing pan-genomic techniques to identify homologous gene pairs (such as Panaroo, Roary, PIRATE, or Pandora). Coinfinder is then used to a particular pan-genomic data collection in order to discover the gene sets that are coincident (Whelan, F. J., Rusilowicz, M., & McInerney, J. O., 2020).

In the process of running Coinfinder, the input of pan-genomic data is first required. Coinfinder can accept genomic content data in two formats, this study will use the Roary --- `gen_presence_absence.csv` genome content data of *Pseudomonas putida* as the pan-genome data input (Tiso et al., 2020). Then run Coinfinder to identify the significant associated and dissociated genes in the pangenome of *Pseudomonas putida*.

Coinfinder will examine the presence/absence patterns of gene pairs in each set of genes in the input genome in order to evaluate whether or not they reflect a coincidence connection between them. In other words, whether gene *i* and gene *j* are seen together or separately in the input genome at a greater frequency than would be predicted by chance is determined.

Coinfinder offers two ways for identifying coincidence relationships: affiliation and dissociation (Whelan, F. J., Rusilowicz, M., & McInerney, J. O., 2020). For the purpose of determining gene connections, Coinfinder examines the input genome for instances in which gene *i* and gene *j* of a particular gene pair are seen together more often than they were discovered by chance. When looking for gene dissociation, Coinfinder examines if gene *i* and gene *j* of a particular gene pair are seen separately in the input genome more often than would be anticipated by chance, according to the manufacturer. In each mode, the default behavior of Coinfinder is to use Bonferroni corrected binomial accurate test statistics to assess whether each gene pair is clearly consistent. Finally, the output of Coinfinder running results is performed.

Coinfinder presents the findings of its research in two ways (Whelan, F. J., Rusilowicz, M., & McInerney, J. O., 2020). To begin, Coinfinder creates a network in which each node represents a gene family and each edge is a declaration of substantial genetic linkage (adjusted for pedigree effects) or dissociation. The node's size is proportional to the gene's *D* value. Second, Coinfinder produces a presence-absence heat map, showing the existence of a co-occurring gene in the input system's occurrence. The heat map is ordered

by D value and colored according to the pattern of coincidence. Finally, Coinfinder created a large number of output files with the default suffix `coincidnet_`. The specific output data format is shown in the table.

Table5: Description of Coinfinder output files

Suffix	File description
<code>_pairs.tsv</code>	Tab-delimited list of significant coincident gene pairs
<code>_nodes.tsv</code>	Node list of all unique coincident genes and their D value
<code>_edges.tsv</code>	Edge list of notable gene–gene pairings along with their related P-values.
<code>_network.gexf</code>	Network file structured in GEXF (Graph Exchange XML Format) v1.2. Nodes are colored according to their linked component (i.e. coincident gene set) and scaled according to their D value; edge thickness is equal to the P-value of any two connected genes' coincident connection.
<code>_components.tsv</code>	A tab-separated list of all the components that are linked inside the gene–gene coincident network.
<code>_heatmap[0-X].pdf</code>	Heatmap visualizations of the presence–absence patterns of coincident components across input genomes. When necessary, the heatmap is divided into several files for increased visibility.

3.3 RStudio

Vegan: A primer on ordination

The paper details common, straightforward labor procedures for vegetation ordination. Unconstrained ordination demonstrates how to understand the findings of detrended correspondence analysis and non-metric multidimensional scaling by fitting environmental

vectors and factors or smooth environmental surfaces to the graph. The papers detail how to conduct the most often used ordination techniques in a vegan manner.

CNA: A R Package for Causal Inference and Modeling in Configurational Spaces

Coincidence Analysis (CNA) is a configurational comparison technique for causal data analysis that was developed for binary data in and significantly expanded, revised, and modified for multi-value and fuzzy-set data in. In contrast to more conventional data analysis techniques, which focus only on effect sizes, CNA is a family of methods for grouping causal impact variables conjunctively (i.e. in complicated bundles) and disjunctively (i.e. on alternative pathways). It is based on the so-called regularity theory of causation and is the only technique of its type capable of recovering causal structures with numerous outcomes (effects).

4. Results

4.1 Prokka

After Prokka hierarchically applied external feature prediction tools to *Pseudomonas putida*, the final output file obtained is shown in the following table. In this process, Prokka accurately and quickly completed the gene annotation of 194 genes of *Pseudomonas putida*. While getting the Prokka output file, Prokka also completed the creation of the Roary input file. The output file obtained from the operation of Prokka also became the basis of this genetic analysis of *Pseudomonas putida*.

Table6: Prokka Output Files

File name		
PROKKA_07052021.err	PROKKA_07052021.fsa	PROKKA_07052021.sqn
PROKKA_07052021.faa	PROKKA_07052021.gbk	PROKKA_07052021.tbl
PROKKA_07052021.ffn	PROKKA_07052021.gff	PROKKA_07052021.tsv
PROKKA_07052021.fna	PROKKA_07052021.log	PROKKA_07052021.txt
PROKKA_07062021.err	PROKKA_07062021.fsa	PROKKA_07062021.sqn
PROKKA_07062021.faa	PROKKA_07062021.gbk	PROKKA_07062021.tbl
PROKKA_07062021.ffn	PROKKA_07062021.gff	PROKKA_07062021.tsv
PROKKA_07062021.fna	PROKKA_07062021.log	PROKKA_07062021.txt

4.2 Roary

By running the pan-genome of *Pseudomonas putida* constructed by Roary, genetic analysis to correctly identify all clusters was realized. After Roary's operation, 17 output files were

generated. Among these, the summary statistics text file summarizes the overall number of genes in the pan-genome and the total number of genes in each of the four categories.

These numbers accurately reflect the pan-genome character of *Pseudomonas putida*. The output file obtained by Roary analysis is provided in R format, which provides convenience for the application of data in the following research. The output file after running Roary is shown in the table.

Table7: Roary output file

File name	
accessory_binary_genes.fa	gene_presence_absence.csv
accessory_binary_genes.fa.newick	gene_presence_absence.Rtab
accessory_graph.dot	number_of_conserved_genes.Rtab
accessory.header.embl	number_of_genes_in_pan_genome.Rtab
accessory.tab	number_of_new_genes.Rtab
blast_identity_frequency.Rtab	number_of_unique_genes.Rtab
clustered_proteins	pangenome_frequency.png
conserved_vs_total_genes.png	pangenome_matrix.png
core_accessory_graph.dot	pangenome_pie.png
core_accessory.header.embl	pan_genome_reference.fa
core_accessory.tab	picture
core_alignment_header.embl	Rplots.pdf
core_gene_alignment.aln	summary_statistics.txt

At the same time, through the successful operation of Roary, basic visualization results were also generated, pangenome_matrix.png, pangenome_pie.png, and pangenome_frequency.png. The three basic visualization results are presented by Matrix, Pie and Frequency respectively. Matrix is a comparison chart showing the presence and

absence of a tree and a matrix with core and auxiliary genes, Pie is a pie chart of gene breakdown and the number of isolates in which they are located, and Frequency is a graph of the relationship between gene frequency and the number of genomes. The specific visualization chart is shown below.

Figure3: Matrix

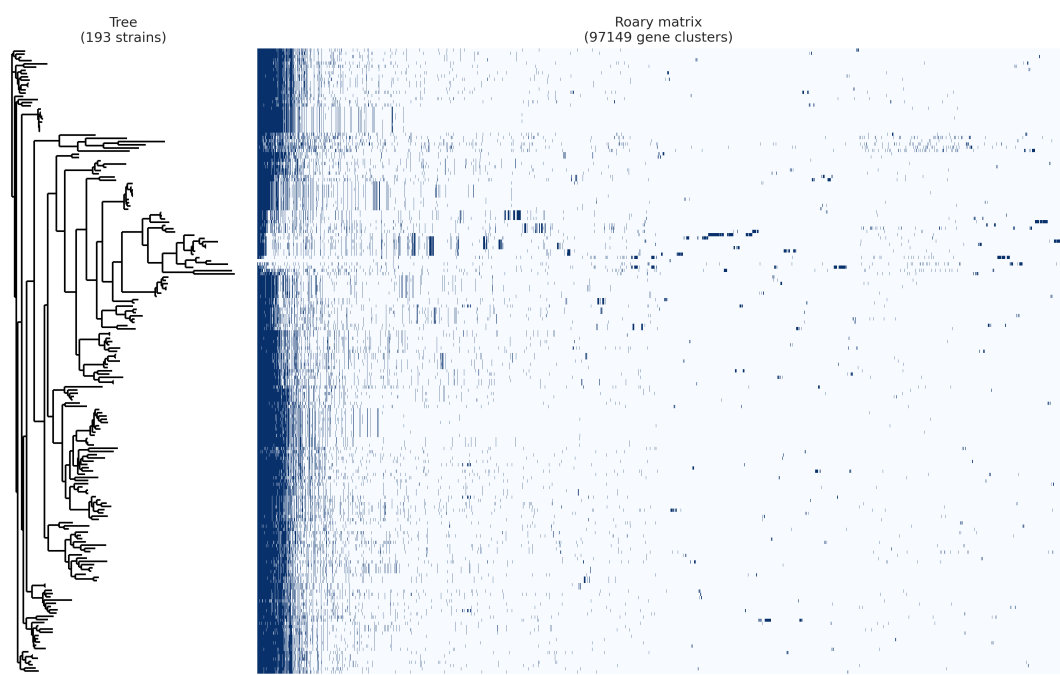


Figure4: Pie

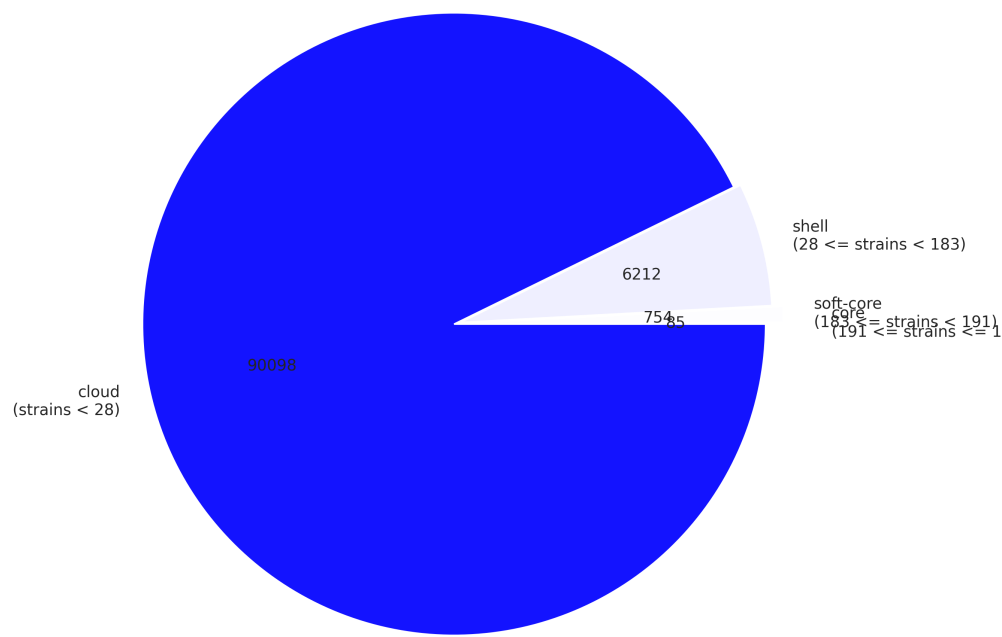
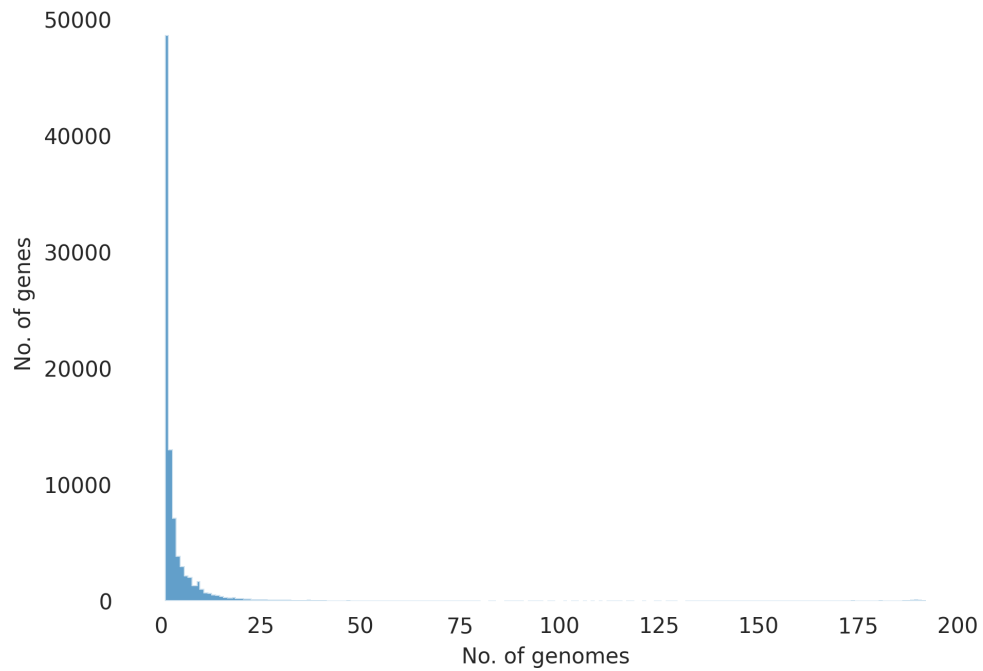
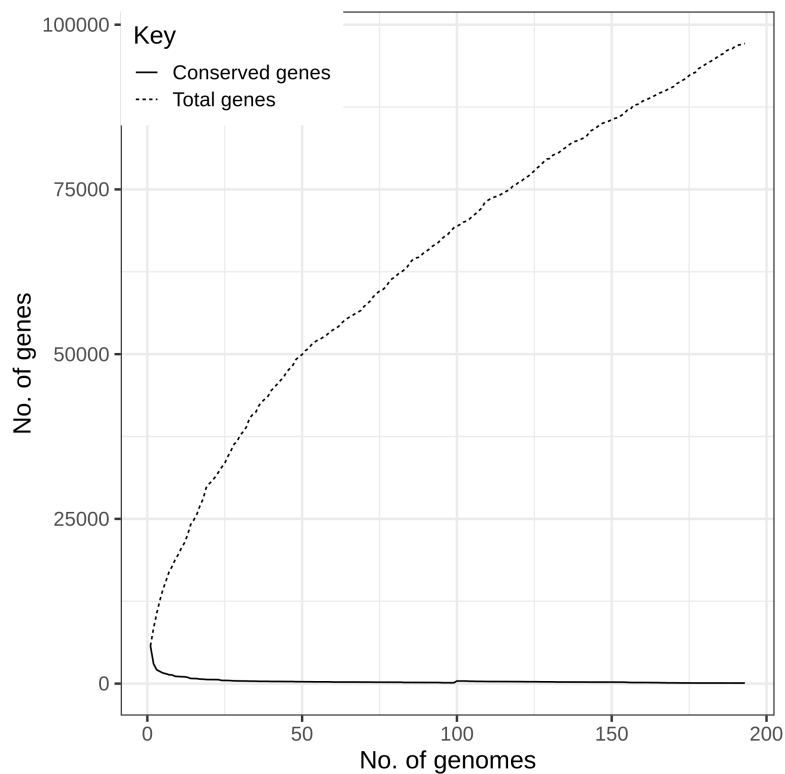


Figure5: Frequency



At the same time, a graph of how the pan-genome changes with the addition of genomes was also generated in this study, conserved_vs_total_genes.png. The specific visualization chart is shown below.

Figure6: Pan-genome change map



4.3 METABOLIC

By running METABOLIC, the expanded analysis of *Pseudomonas putida* metabolic pathways and the visualization of microbial metabolic networks have been realized. Metabolic relies on microbial genome and metagenomic readings to infer the existence of specific metabolic pathways in the *Pseudomonas putida* genome, and outputs important metabolic marker protein data related to the metabolic pathways of *Pseudomonas putida*. The resulting data list is as follows.

Table8: METABOLIC output file list

File name	
Each_HMM_Amino_Acid_Sequence	METABOLIC_Figures_Input
intermediate_files	METABOLIC_result_each_spreadsheet
KEGG_identifier_result	METABOLIC_result.xlsx
METABOLIC_Figures	METABOLIC_run.log

The purpose of this research is to investigate the biodegradation of plastic polymer materials by *Pseudomonas putida*. The polymer materials that are focused on are polyethene terephthalate (PET) and polyurethane (PUR), and their monomer products, ethylene glycol and 1,4-butanediol.

For this research, in the output file of METABOLIC, the degradation mechanism and pathways of polyethene terephthalate (PET) and polyurethane (PUR) and their monomer products in *Pseudomonas putida* will be analyzed emphatically. The key analysis of the METABOLIC output file information exists in the METABOLIC result table.

The METABOLIC result table contains important protein gene analysis data such as protein names, functional characteristics, KEGG modules, and KEGG module steps. And these data

show whether there is information about these genetic data for different kinds of proteins. In this study, the protein enzymes that play a role in the degradation pathway were compared and analyzed with six different protein information results in the METABOLIC result table. At the same time, the statistical calculation of the "presence/absence" of the protein result is performed. The final statistical results are as follows.

Polyethylene terephthalate-Ethylene glycol

Table9: HMMHitNum

Gene.name	Percentage
propionyl-CoA synthetase	0.835051546
malonyl-CoA/succinyl-CoA reductase (NADPH)	0

Table10: KEGGModuleHit

Module	Percentage
<u>Glyoxylate</u> cycle	0.96907216
C4- <u>dicarboxylic acid</u> cycle, <u>phosphoenolpyruvate</u> carboxykinase type	0
C4- <u>dicarboxylic acid</u> cycle, NAD - malic enzyme type	0
C4- <u>dicarboxylic acid</u> cycle, NADP - malic enzyme type	0
<u>Pyruvate</u> oxidation, <u>pyruvate</u> => <u>acetyl-CoA</u>	0.99484536
MalK-MalR (<u>malate</u> transport) two-component regulatory system	0
PgtB-PgtA (<u>phosphoglycerate</u> transport) two-component regulatory system	0

Table11: KEGGModuleStepHit

Module	Percentage
C4-dicarboxylic acid cycle, phosphoenolpyruvate carboxykinase type-K01595	0.98969072
C4-dicarboxylic acid cycle, phosphoenolpyruvate carboxykinase type-K01610	0.99484536
C4-dicarboxylic acid cycle, NAD - malic enzyme type-K01595	0.98969072
C4-dicarboxylic acid cycle, NADP - malic enzyme type-K01595	0.98969072
C4-dicarboxylic acid cycle, NADP - malic enzyme type-K00029	0.95360825

Polyurethane-1,4-butanediol

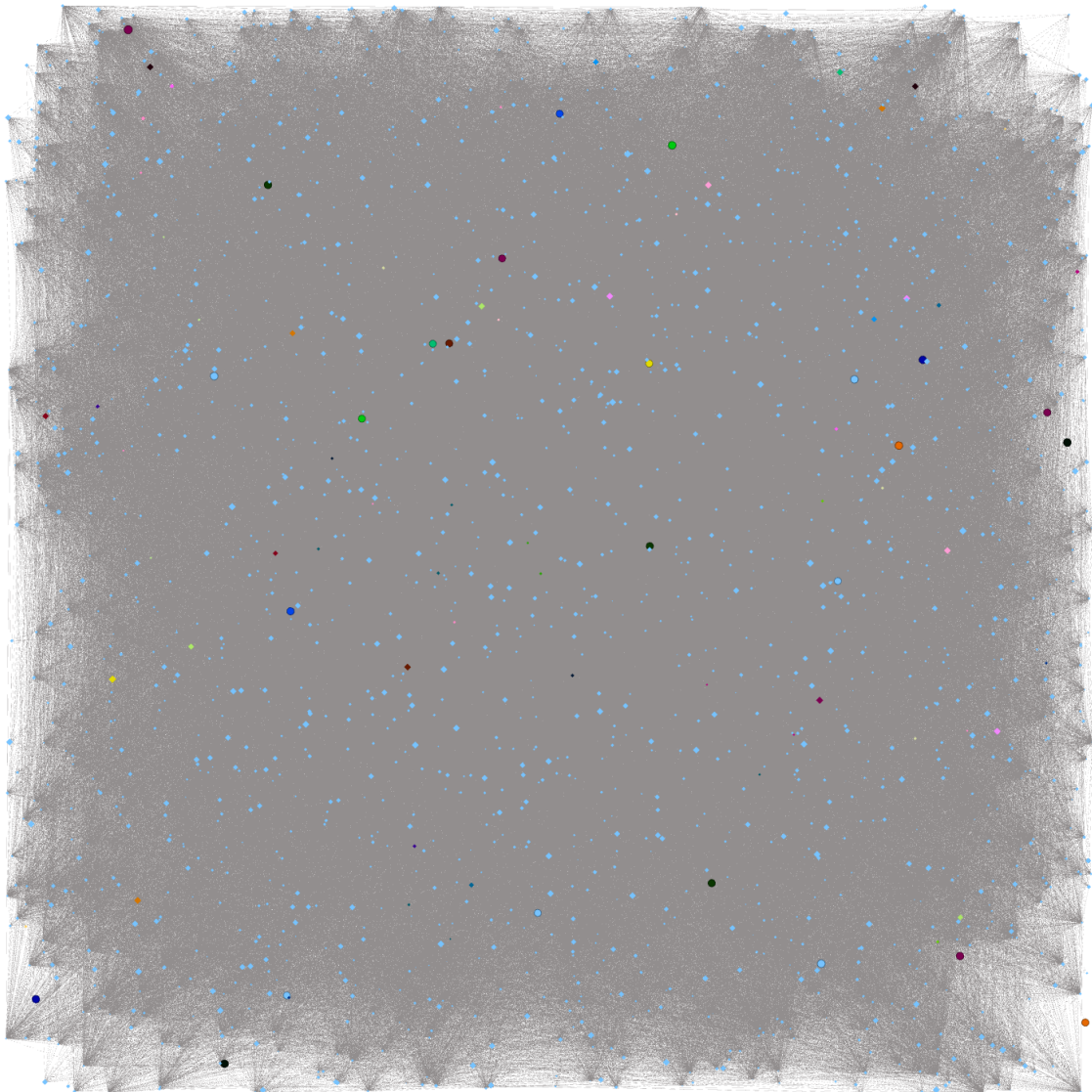
Table12: HMMHitNum

Gene.name	Percentage
<u>4-aminobutyrate</u> aminotransferase and related aminotransferases	0.963917526
<u>ornithine</u> /acetylornithine aminotransferase	0.974226804
<u>acyl-CoA dehydrogenase</u>	0.989690722
<u>alcohol dehydrogenase</u>	0
S-(hydroxymethyl)glutathione dehydrogenase / <u>alcohol dehydrogenase</u>	0.984536082
malonyl-CoA/succinyl-CoA reductase (NADPH)	0
<u>4-hydroxybutyrate</u> --- <u>CoA ligase</u> (ADP-forming)	0
<u>4-hydroxybutyryl-CoA</u> dehydratase / vinylacetyl-CoA-Delta-isomerase	0
ATP-citrate lyase alpha-subunit	0
ATP-citrate lyase beta-subunit	0
DMS <u>oxygenase beta</u> subunit	0.118556701

4.4 Coinfinder

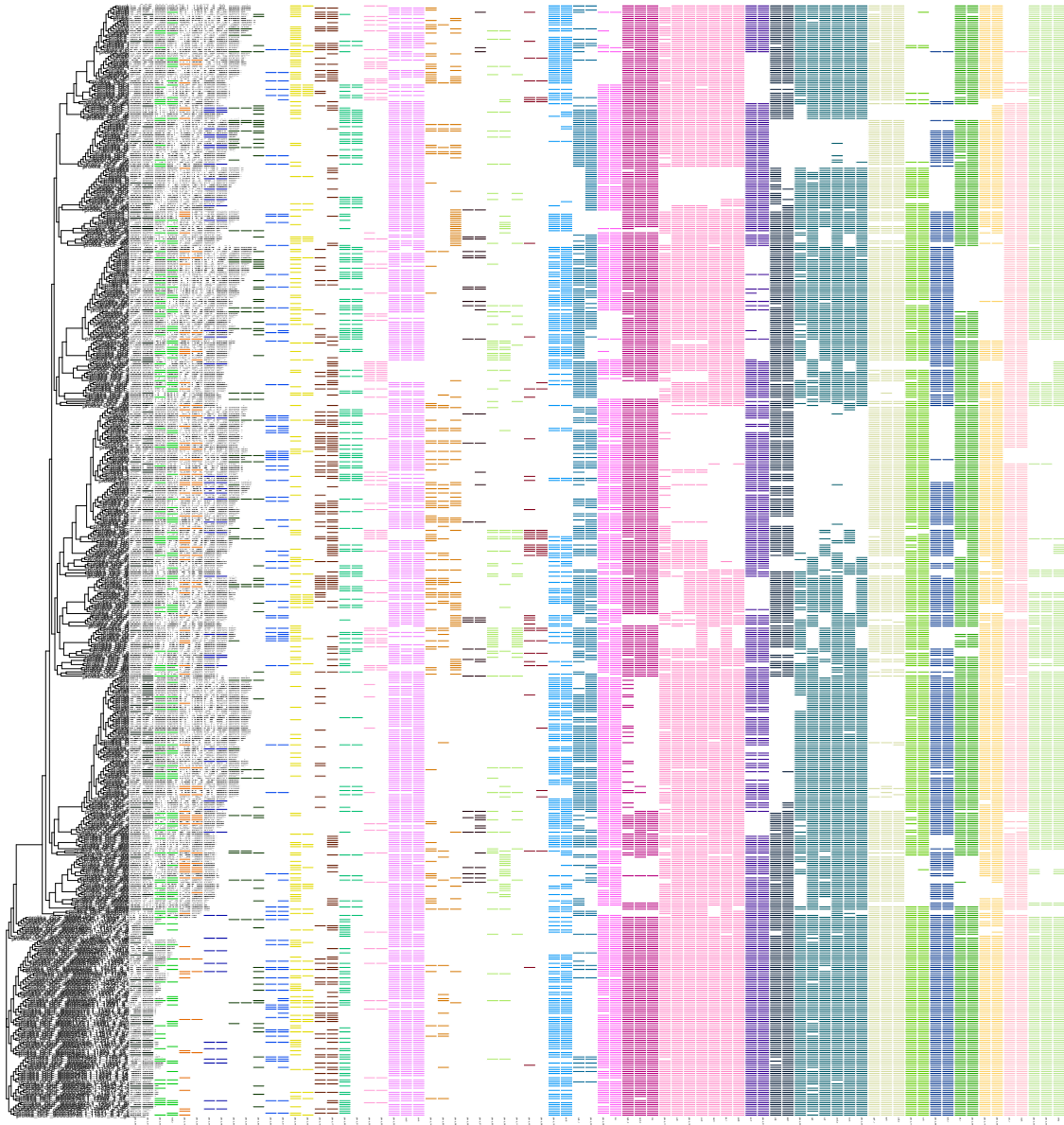
Run Coinfinder to identify genes that are associated or isolated in the accessory genome of the pan-genome of *Pseudomonas putida*. Genes that accidentally associate or segregate more frequently than expected will indicate that these genes have an attractive or repulsive link. The identification of the pan-genome of *Pseudomonas putida* through Coinfinder will further deepen the understanding of the importance of auxiliary genes in this field. The output files of Coinfinder are presented in two visual charts, network and heat map. The visualization chart is shown below.

Figure7: Gene association and dissociation network



The *Pseudomonas putida* gene association and dissociation network has nodes representing gene families, and edges representing statements of substantial gene connection or significant gene separation between those genes. The size of the node is proportional to the D value of the gene.

Figure8: Presence-absence heat map



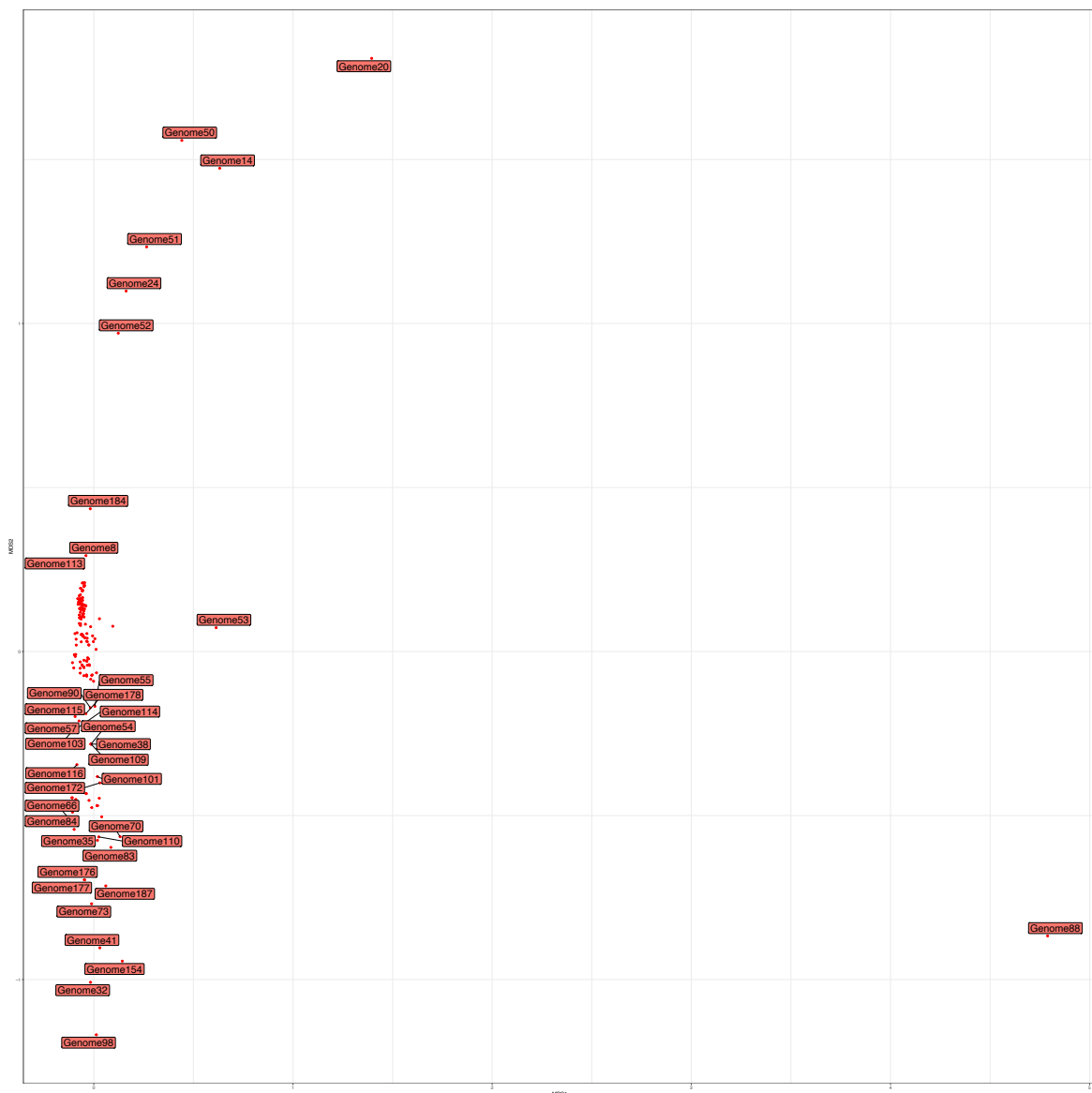
The presence-absence heat map generated by Coinfinder indicates the presence of overlapping genes in the context of input phylogeny. The genes in the heat map are sorted by D value and colored according to the coincidence pattern.

4.5 RStudio

Vegan

Through the operation of Vegan on RStudio, the MDS chart of genes related to *Pseudomonas putida* genome and plastic degradation will be drawn. The chart analyzes the abundance relationship between different genes in the genome of *Pseudomonas putida* through a certain sorting method.

Figure9: Non-metric multidimensional scaling-Ordination plot.



CNA

Through the operation of CNA on RStudio, Boolean operations are performed on the genome of *Pseudomonas putida*, and the concepts of sufficiency and necessity are defined. To this end, a list of causal correlations between different genes of *Pseudomonas putida* will be obtained. Finally, RStudio outputs the ASF and MSC list. See the attachment for the table.

5. Discussion

Pseudomonas putida may biodegrade ethylene glycol and 1,4-butanediol to a considerable degree, according to the output files acquired by various genetic analysis tools and the examination of the output files. Through in-depth research on the biodegradation path of ethylene glycol, the experiment found that in a series of degradation products of *Pseudomonas putida* degrading ethylene glycol, the expression ratio of propionyl-CoA synthetase gene in *Pseudomonas putida* HMMHitNum protein name table is about 83.5 percent, at the same time in the KEGGModuleHit module table, an important reaction module in the biodegradation path of ethylene glycol, Glyoxylate cycle and Pyruvate oxidation, pyruvate => acetyl-CoA are also shown in the table, and the gene expression rate is as high as 96.9 percent and 99.4 percent respectively. On the other hand, about *Pseudomonas putida* 1,4-butanediol. In the HMMHitNum protein name table, 4-aminobutyrate aminotransferase and related aminotransferases (96.4 percent), ornithine/acetylornithine aminotransferase (97. percent), acyl-CoA dehydrogenase (98.9%), alcohol dehydrogenase (98.4 percent) and DMS oxygenase beta subunit (11.9 percent). these five protein genes that exist in the 1,4-butanediol degradation pathway are all shown in the protein name table. and the expression ratio between these protein genes and *Pseudomonas putida* genes is very high. Therefore, this shows that *Pseudomonas putida* can degrade plastic polymers (polyethylene terephthalate and polyurethane) or participate in the biodegradation reaction of plastic polymers to a certain extent. At the same time, the biodegradation of *Pseudomonas putida* has also been confirmed in Vegan. It has been discovered that when the genome of *Pseudomonas putida* has a degrading impact on a polymer, the protein genomes that have a degrading effect on the polymer are summarized in a table produced by Vegan. Current research, on the other hand, cannot conclusively demonstrate that *Pseudomonas putida* is a bacterium that may be utilized to enhance the natural environment or to biodegrade plastics. Studies have also shown that the oxidation of 4-hydroxybutyric acid, which occurs during the natural breakdown of 1,4-butanediol by

the bacteria *Pseudomonas putida*, may be restricted, and that it may only occur at a slow pace at its most extreme (Li et al., 2020). As a result, our research did not establish that *Pseudomonas putida* is capable of biodegrading plastic polymers with high efficiency under naturally occurring circumstances. At the same time, the research data has not confirmed that polymer materials such as ethylene glycol can be used as the sole carbon source of *Pseudomonas putida*.

However, some studies have shown that the upstream metabolite allantoin can be added to the medium of *Pseudomonas putida* and ethylene glycol to activate the glyoxylic acid metabolism pathway, thereby improving or changing the biodegradation mechanism of *Pseudomonas putida*, and improve the biodegradation efficiency (Ackermann et al., 2021). By pretreating plastics, inert polymers can be more easily degraded by bacteria. For example, the plastic will be pretreated by ultraviolet light, or use other methods to introduce ester bonds into the inert polymer backbone, this makes plastics easier to recognize and cut by bacterial lipases (Wilkes, R. A., & Aristilde, L., 2017). Furthermore, the research demonstrates that if no pretreatment is performed to enhance the hydrophilicity of the polymer surface, plastic polymers may take decades to deteriorate until they are no longer useful. As a result, while investigating the biodegradability of plastics using *Pseudomonas putida*, it is necessary to take into account a wide range of variables.

6. Conclusion

Pseudomonas putida can naturally biodegrade plastics to a certain extent. With the use of *Pseudomonas putida* whole genome analysis, it has been shown that the biodegradation of polyethylene terephthalate and polyurethane, as well as its breakdown monomer, by this bacteria is possible at the whole genome analysis level. Given that this research is based on an investigation of how *Pseudomonas putida* affects the biodegradation of plastics in the natural environment, it has certain limitations and cannot conclusively demonstrate that *Pseudomonas putida* may be utilized as a biodegradable microbe for the treatment of plastic waste in its entirety. Following the findings of the present study, it can be shown that the enzyme included in *Pseudomonas putida* has the ability to biodegrade and oxidize certain plastic substrates to a limited degree. The use of genetic engineering to modify *Pseudomonas putida*, on the other hand, has shown that it is possible to improve the degradation efficiency while also altering the presence of specific enzymes that impede biodegradation in the process of biodegradation. In addition, the conversion of polyethylene terephthalate into biodegradable plastic polyhydroxyalkanoate is an issue that has drawn the attention of many people who are worried about the disposal of plastic trash (Kenny et al., 2008). Therefore, this can also become a new direction in the study of plastic biodegradation by *Pseudomonas putida*. The research on the biodegradation of *Pseudomonas putida* needs to further explore the impact of changing other factors on biodegradable plastics in the future.

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Appendix

Appendix I. CNA – MSC Result

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Appendix II. CNA – ASF Result

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