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SHORT COMMUNICATION: TAXONOMIC PROFILING OF BACTERIA IN THE BIO-ENZYME THAT PRODUCED FROM ORANGE FRUIT WASTE

KOMUNIKASI SINGKAT: PROFIL TAKSONOMI BAKTERI PADA BIO-ENZIM YANG DIPRODUKSI DARI LIMBAH BUAH JERUK

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Abstract

Fruit wastes are reported as among the main contributors to food waste production. They have an impact on the environment so they need to be treated. To reduce the negative effects, fruit wastes must be treated before they are released into the environment. Meanwhile, the fruit wastes have been reported about their functional properties and nutritional as bio-enzyme. This preliminary study aimed to provide information about metagenome bacteria of bio-enzymes from orange fruit wastes treated with different concentrations of probiotics in anaerobic fermentation. Bio-enzyme was a fermented solution made from a mixture of 15 g brown sugar: 5 kg orange fruit waste: and 12 L water. The fermentation process was carried out for 1 month and then the bioenzyme sample was taken 1 L for metagenome analysis. The metagenomic analysis was carried out using the next-generation sequencing (NGS) method for the 16S rRNA gene of bacteria by amplicon full-length sequencing with nanopore sequencing. In this research, phylum *Pseudomonadota* (*Proteobacteria*), class *Betaproteobacteria*, order *Burkholderiales*, and family *Comamonadaceae* were the predominant bacterial group of the bio-enzyme that produced from orange fruit waste. Identification of bacterial species in the next research becomes important to understand the role of bacterial species in the biochemical metabolism of bio-enzyme products from orange fruit waste.

Keywords: Bacteria; Bio-enzyme; Metagenome; NGS; Orange waste

Abstrak

Limbah buah-buahan dilaporkan sebagai salah satu penyumbang utama produksi limbah makanan. Limbah buah mempunyai dampak terhadap lingkungan sehingga perlu ditangani. Limbah buah harus diolah terlebih dahulu untuk mengurangi dampak negatifnya sebelum dibuang ke lingkungan. Sementara itu, limbah buah telah dilaporkan mengenai sifat fungsional dan nutrisinya sebagai bio-enzim. Penelitian awal ini bertujuan untuk memberikan informasi mengenai diversitas metagenom bakteri yang terdapat di produk bioenzim yang diproduksi dari limbah buah jeruk yang diberi perlakuan konsentrasi probiotik berbeda dalam fermentasi anaerobik. Bio-enzim merupakan larutan fermentasi yang terbuat dari campuran 15 g gula merah : 5 kg limbah buah jeruk : 12 L air. Proses fermentasi dilakukan selama 1 bulan dan kemudian sampel bioenzim diambil sebanyak 1 L untuk dianalisis metagenom. Analisis metagenomik bakteri dilakukan dengan menggunakan metode next-generation sequencing (NGS) untuk analisis 16S rRNA gene melalui sekuensing amplikon dengan nanopore sequencing. Pada penelitian ini, keberadaan bakteri pada bio-enzim yang diproduksi dari limbah buah jeruk. Filum Pseudomonadota (Proteobacteria), kelas Betaproteobacteria, ordo Burkholderiales, dan famili Comamonadaceae merupakan kelompok bakteri yang dominan di bio-enzim yang diproduksi dari limbah buah jeruk. Identifikasi spesies bakteri pada penelitian selanjutnya menjadi penting untuk mengetahui peranan spesies-spesies bakteri di metabolisme biokimiawi dari produk bio-enzim dari limbah buah jeruk.

Kata Kunci*: Bakteri; Bio-enzim; Limbah Jeruk; Metagenom; NGS*

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INTRODUCTION

The fruit industry including production, and post-harvest until retail and consumption can produce food waste or food by-products (Plazzotta et al., 2017) and contribute to environmental quality. This organic waste contributes to environmental pollution due to its odor, contamination, and beauty reducing of environment when the wastes are disposed of in open landfills without further processing (Fadlilla et al., 2023). Whereas, The fruit wastes have been reported that the wastes still contain nutrients, functional properties, and bioactive compounds with functional ingredients (Lucarini et al., 2021; Zhu et al., 2023) such as dietary fiber, flavonoids, phenolic, antioxidants, polysaccharides, and several other phytochemicals (Nirmal et al., 2023).

These compounds can be extracted and implemented for many utilizations, including products in industrial applications. Lakra et al. (2022) explain that bio-enzymes are also called eco-enzymes or garbage enzymes as one of the extracted products from fruit wastes. According to Patel et al. (2023), bio-enzyme is a natural substance that is produced from fresh or wasted fruit or vegetables by the fermentation process. It has many functionalities, including bio-fertilizer, environment-contaminated reduction, care products, and other functions. Benny et al. (2023) explain one of the organic waste sources that can be processed into bio-enzyme is orange fruit due to it has unique properties, such as phytochemicals, polyphenols, vitamins (a good source of vitamin C), minerals, dietary fibers, essential oils, amino acids, pectin, carotenoids, flavonoids, a high acidity value, to smell and a strong flavor.

One of the bio-enzyme applications is to treat domestic wastewater and to clean water bodies, mostly ponds (Penmatsa et al., 2019; Kerkar & Salvi, 2020). We want to implement bio-enzymes in ex-tin mining pond waters that have acidic water and heavy metal contaminations. However, the quality of bio-enzymes must be analyzed to ensure its effectiveness. One of the analyses is the presence and diversity of microorganisms in the bio-enzyme.

Microorganisms, including bacteria, are the most abundant, oldest, and most biodiverse biological agents in the environment (Vitorino & Bessa, 2018; Rosenberg & Zilber-Rosenberg, 2022). The bacteria play important roles in the biogeochemical and nutrient cycles in the environment and function of the ecosystem (Meng et al., 2022; Zhou et al., 2023).

There are two methods to identify bacterial communities, the culture and non-culture methods (Akram et al., 2023). The culture method is a well-established, low-cost, and simple-to-use approach to bacterial isolation, but, this method can only be used to identify a small number with the estimated less than 20% of species bacterial in the habitat (Eckburg et al., 2005; Costa & Weese, 2019). The identification of modern methods such as next-generation sequencing (NGS) can answer the problems due to this method can investigate the composition and diversity of bacteria by the 16 sRNA gene sequences of bacteria (An et al., 2022; Chen et al., 2022). The NGS method is more comprehensive and has a higher accuracy for metagenomic analysis and to identify the uncultured bacteria (Bahram et al., 2018; Ashraf et al., 2022; Navgire et al., 2022).

In this preliminary research, we focus on bacterial metagenome identity that is found in bio-enzyme from orange. This study supported the further analysis of biochemical characteristics and quality of the bio-enzyme and its implementation for treatment in e in ex-tin mining pond waters that have acidic and heavy metal-contaminated water. The information on bacterial metagenome identity and their role in biochemical metabolism were novelties of this research. Furthermore, the bio-enzyme implementation for recovering the poor quality of the environment, especially acidic, and heavy metal-contaminated water can become a novelty for the next research.

MATERIALS AND METHODS

This research took place from March to June 2023. The study was conducted to analyze the metagenome diversity of bacteria as biological properties of bio-enzymes from orange fruit waste. Bio-enzyme sample was collected 1 L for metagenome analysis. The sample was collected by a plastic bucket and put into a sample bottle.

The metagenomic analysis through 16S rRNA gene sequencing was investigated by amplicon full-length sequencing with nanopore sequencing. Genomic DNA was extracted using the

ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). DNA concentration was determined using both NanoDrop spectrophotometers and Qubit fluorometers. Library preparations were conducted using kits from Oxford Nanopore Technology. Nanopore sequencing was operated by MinKNOW software version 22.05.7.

RESULTS

The presence of bacteria community from this habitat was observed to identify their diversity and composition. The taxonomic profiling and the relative abundance of bacteria composition in the bio-enzyme were presented in Figures 1 to Figure 4. The relative abundance showed phylum *Pseudomonadota* (*Proteobacteria*), class *Betaproteobacteria*, order *Burkholderiales*, and family *Comamonadaceae* to be the predominant bacteria of the bio-enzyme from orange fruit waste.

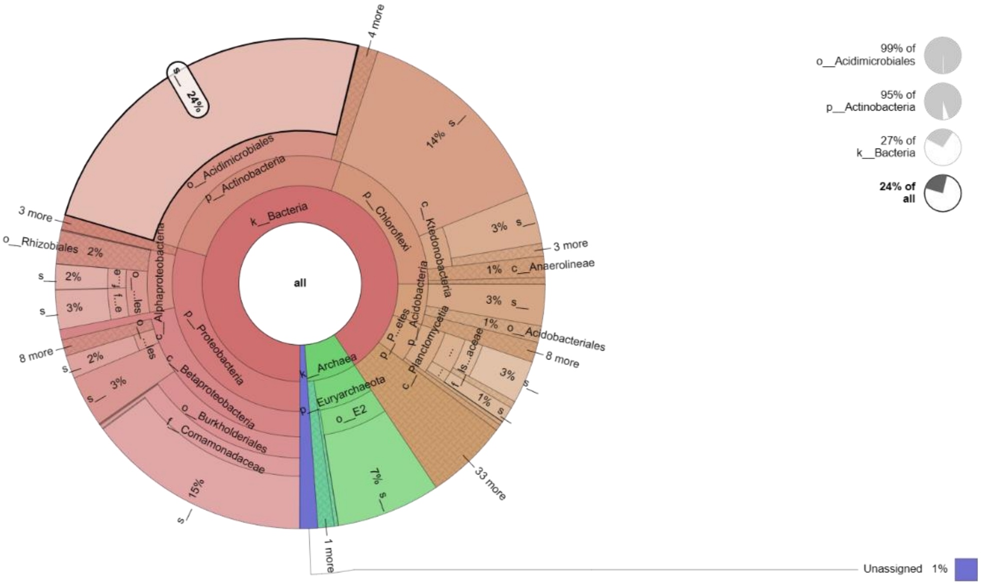

Figure 1. Relative abundance of the phyla of bacteria in bio-enzyme from orange fruit waste

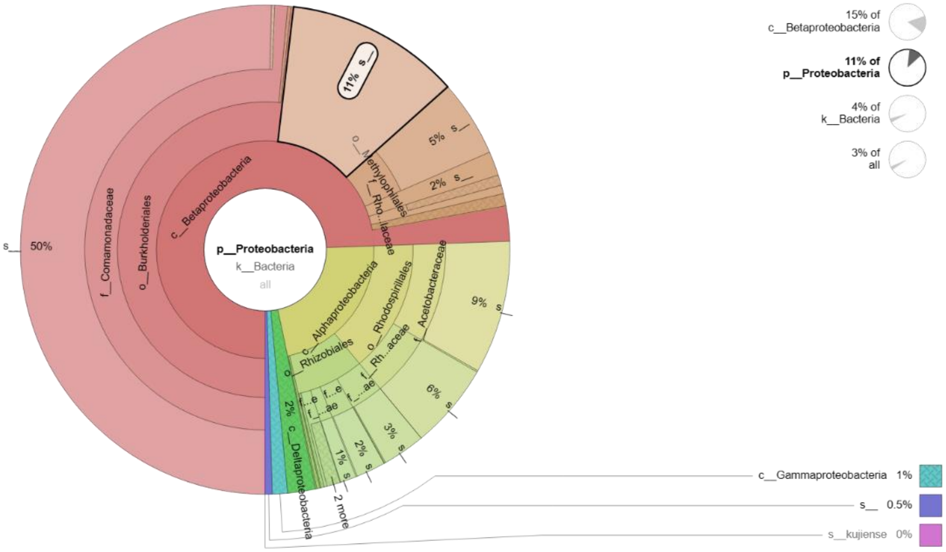

Figure 2. Relative abundance of the class of bacteria in bio-enzyme from orange fruit waste

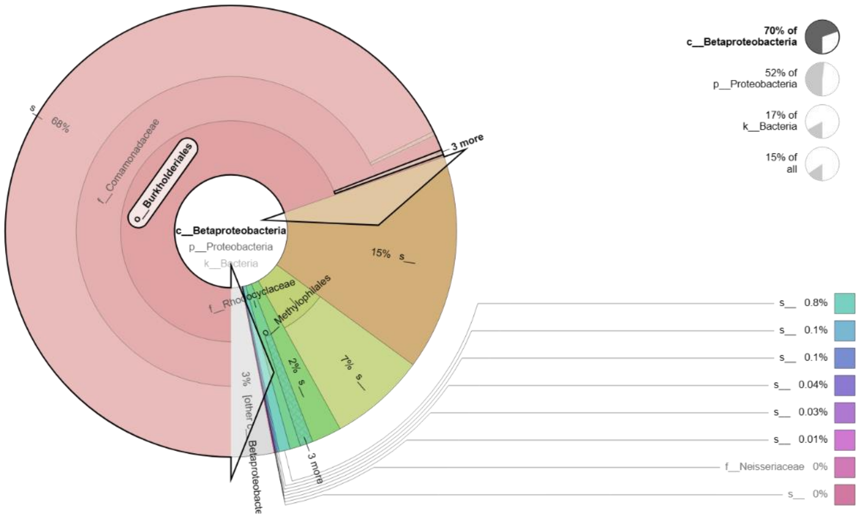

Figure 3. Relative abundance the order of bacteria in bio-enzyme from orange fruit waste

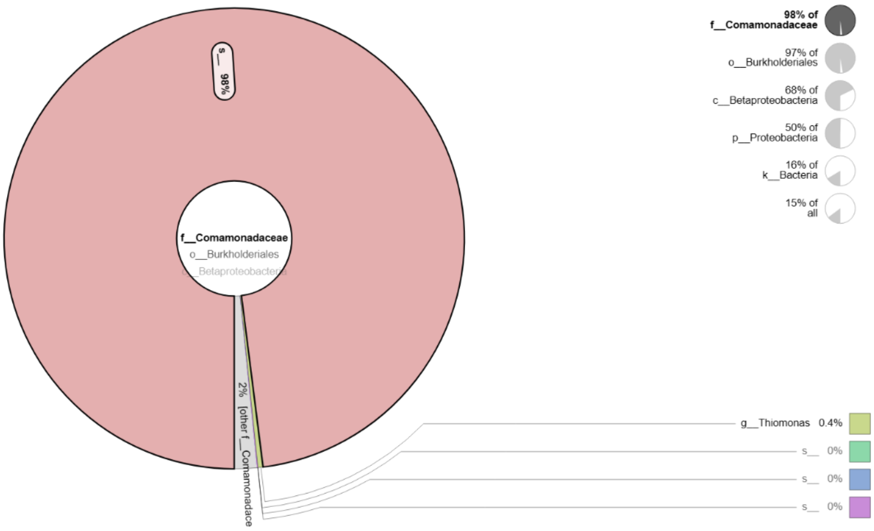

Figure 4. Relative abundance of the family of bacteria in bio-enzyme from orange fruit waste

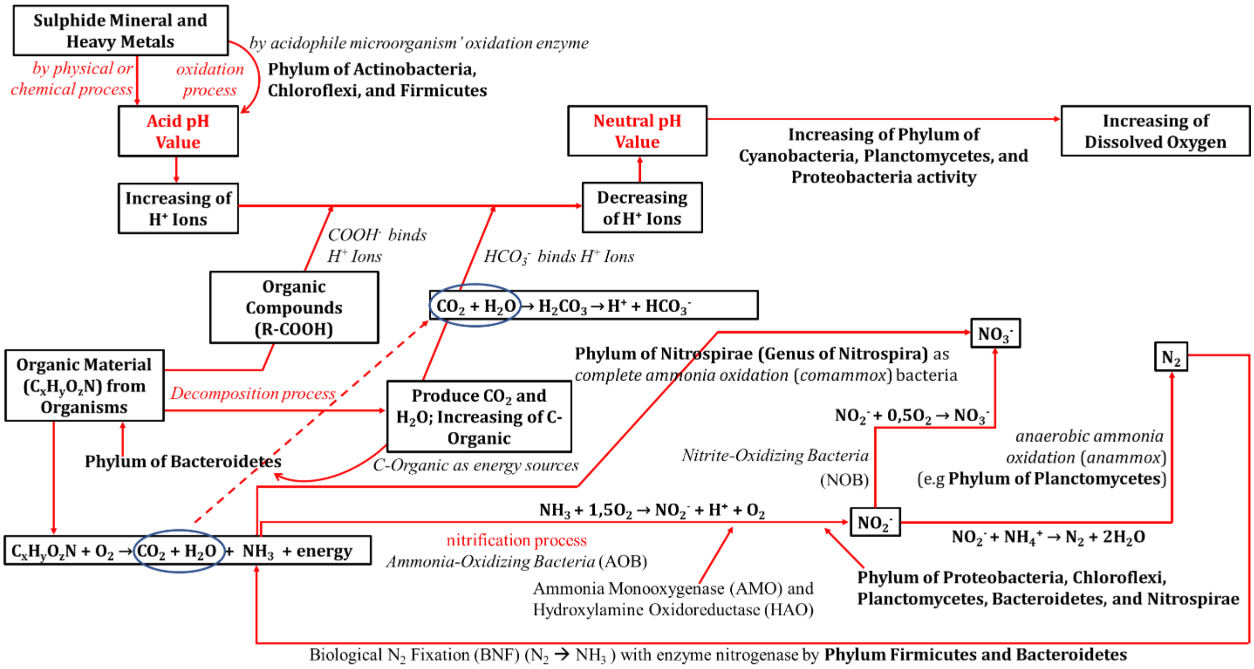

Figure 5. Biogeochemical flow involved some bacterial found in abandoned tin mining pits, which red line indicated a biogeochemical process (Kurniawan et al., 2023)

Figure 5 explains the pathway of the biogeochemical process that indicated the potential for improving of water quality in abandoned tin mining pits. The red line showed the involvement of some species of bacteria and the presence of organic compounds for increasing water quality with increasing pH value and dissolved oxygen, and also decreasing H^+ ions, including heavy metal toxicity. Our hypothesis showed the utilization of bio-enzymes from fruit waste can contribute to the presence of organic matter. The presence of organic compounds can become a nutrient for bacterial growth. Furthermore, the organic compound can increase the role of the carboxyl group to bind ion H^+ impact to decrease of ion H^+ and make the increase of pH value and decrease of ion cation form the other elements such as heavy metal. This role of organic compounds and bacterial can make an improvement of this water quality to produce pH value increasing and heavy metal toxicity decreasing.

DISCUSSION

Pseudomonadota is the synonym of *Proteobacteria* (Kochetkova et al., 2023). Many gram-negative bacteria are found within the phylum *Proteobacteria*. This phylum is a diverse group of bacteria that are generally Gram-negative (Vaz-Moreira et al., 2017; Zheng et al., 2022), opportunistic bacteria (Leão et al., 2023), the richest phylum in water and sediments (Qin et al., 2016; Shen et al., 2022), and the *Pseudomonadota* plays an important role in the biogeochemical cycle, including nutrient cycling and mineralization of organic compounds (Madsen, 2011; Newton et al., 2011; Liu et al., 2023). Pękala-Safińska (2018) and Breijyeh et al. (2020) explain that Gram-negative bacteria are commonly known to be more pathogenic and more resistant to antibiotics than Gram-positive, while Mekasha and Linke (2021) explain that phylum *Pseudomonadota* is the most prominent Gram-negative bacterial pathogen. Gram-negative bacteria, mainly members of the phylum *Proteobacteria*, represent the most frequent bacteria in drinking water, and their ubiquity and physiological versatility implicate in human health (Vaz-Moreira et al., 2017).

Nevertheless, the phylum *Pseudomonadota* or *Proteobacteria* is the most abundant in the environment and plays a role in the cycle of nitrogen, phosphorus, sulfur, and in the degradation of organic matter (Mellado & Vera, 2021). In the environment, the Gram-negative is not a single player which makes them dominate in causing pathogenic infection. At the same time, when Gram-negative work together with Gram-positive and other microorganisms, they can contribute to environmental recovery. The consortium of the dominant bacteria such as *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, and *Firmicutes*, precisely, play a role in the nitrogen, carbon, and sulfur cycles that are needed by land and plants. *Actinobacteria* in the C-cycle, *Acidobacteria* in the N-cycle, *Bacteroidetes* decompose the organic matter, and *Firmicutes* are required for biocontrol and plant growth (Purbalisa et al., 2022).

Bio-enzyme is a nutritive liquid that is fermented from fruit and vegetable waste. Bio-enzyme is the result of fermentation for 3 months from a mixture of sugar, fruit and vegetable wastes, and water with a ratio of 1 : 3 : 10 (Rasit et al., 2019; Galintin et al., 2021). However, Kurniawan et al. (2024) did a preliminary study about the physico-chemical properties of bio-enzymes produced from orange fruit waste treated with different concentrations of probiotics in 35 days with a ratio of 15 g brown sugar: 5 kg orange fruit waste: 12 L water.

Weisheng et al. (2015) explain using enzymes is one of the remediation methods for environment-contaminated toxic (e.g. heavy metals). In the bio-enzyme, organic matter from fruit or vegetable waste is a nutrient source for microorganisms. The Microbial communities in this bio-enzyme can produce high amounts of enzyme substances that have potential applications in a broad range of industrial, agricultural medical, and environmental. Bollag (2008), microorganisms are ultimately responsible for degrading most organic matter to carbon dioxide, minerals, and water. Microorganisms cause transformation, mineralization, or complexation by directing those capabilities toward environmental pollutants

This bio-enzyme can be a medium for the microorganism's growth and then implement the bio-enzyme and microorganism to remediate the environment. In bioremediation methods, enzymes play the most crucial role (Mousavi et al., 2021) and these enzymes act as bio-catalysts to perform reactions in bio-processes of the environmentally friendly (Nigam, 2013). Kurniawan et al. (2023) explain the interaction and role of microorganisms in the environment and can exchange the environment, including water quality (Figure 5). Figure 5 showed the presence of organic matter, organic compounds, and microorganisms can contribute to the environment recovery.

Group of *Proteobacteria*, class *Betaproteobacteria* contribute to ammonia oxidation and decomposition of organic matter (Teira et al., 2011). *Betaproteobacteria*, order *Burkholderiales* were isolated mostly at pH 4 and play an important role in the cycling of carbon in the aquatic habitat (Klann et al., 2016), and the family *Comamonadaceae* contributed to the phosphorus removal in this system (Lu et al., 2023) and an electron donor in an environment (Khan et al., 2002). Electron donor microbes, together with organic matter role in reduction-oxidation (redox) reactions and have the effectiveness of specific functional groups for metal reduction (Chen et al., 2003). These properties showed that microorganisms, especially bacterial in bio-enzymes may contribute to organic matter decomposition, improve acidic pH value, and reduce the acidic elements like Fe, Zn, Sn, Cu, Pb, etc that present in ex-tin mining water (Kurniawan et al., 2019).

CONCLUSION

We have identified the presence of bacteria from the bio-enzyme of orange fruit waste. The phylum *Pseudomonadota* (*Proteobacteria*), class *Betaproteobacteria*, order *Burkholderiales*, and family *Comamonadaceae* were the predominant bacterial groups of the bio-enzyme produced from orange fruit waste. For the next research, we will apply this bio-enzyme to recover the poor water quality of the abandoned ex-tin mining water and investigate the risk of the bio-enzyme implementation at this ecosystem.

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