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The Potential of Gram-Positive Bacteria in Eco-Enzymes from Peatland Plants: An Innovative Biotechnology Learning Resource for Developing Scientific Explanation Skills

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Abstract: This study aims to observe Gram-positive bacteria in eco-enzymes produced from the fermentation of peatland plants in Central Kalimantan and to analyze their potential as biotechnology learning resources for developing students' scientific explanation skills. The research employed a descriptive qualitative method through eco-enzyme preparation, bacterial identification using Gram staining, and analysis of the role of observational results in the learning context. The findings revealed four Gram-positive bacteria, namely *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis*, and *Lactobacillus bulgaricus*. The presence of these bacteria demonstrates the active involvement of microorganisms in the fermentation process, which can serve as a concrete example for students to understand biotechnology concepts. These results emphasize that bacterial identification outcomes are not only scientifically valuable but also hold potential as contextual learning resources that support the development of scientific explanation. Through these observational results, students can practice constructing claims, presenting evidence, and connecting them with scientific reasoning. Therefore, this study concludes that exploring Gram-positive bacteria in eco-enzymes derived from peatland plants has the potential to enrich biotechnology learning while simultaneously developing students' scientific explanation skills.

Keywords: Biotechnology learning; Eco-enzyme; Gram-positive bacteria; Peatland; Scientific explanation

Introduction

Eco-enzymes are liquid products generated from the fermentation of organic residues with sugar and water, which contain diverse bioactive compounds with antibacterial, antifungal, and insecticidal properties (Ningrum et al., 2024; Permatananda et al., 2023; Vidalia et al., 2023). Gram-positive bacteria, particularly *Bacillus spp.* and lactic acid bacteria, play a pivotal role in this fermentation process by producing lactic acid, lowering environmental pH, suppressing spoilage microorganisms, and enhancing the antimicrobial potential of eco-enzymes (Hirozawa et al., 2023; Shiraishi et al., 2016). These findings highlight eco-enzymes not only as products of environmental biotechnology but also as authentic resources for science education.

Central Kalimantan, known for its vast peatland ecosystems, holds rich yet underutilized biodiversity (Ramdhan, 2018). Peatland plants provide unique substrates for eco-enzyme production, but scientific documentation of Gram-positive bacterial diversity in peatland-based eco-enzymes remains scarce. This lack of exploration limits both scientific understanding and the opportunity to incorporate local microbial resources into contextual learning materials.

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Biotechnology is one of the subjects studied by students at the senior high school level (Gusti et al., 2023; Rangkuti et al., 2016; Suryanda et al., 2020). However, various studies have shown that this material is considered difficult for students to understand. The difficulty arises because biotechnological processes generally involve microorganisms in product development, making them difficult to observe directly in the classroom (Gusti et al., 2023).

At the same time, scientific reasoning encompasses both conceptual knowledge and the cognitive processes required for developing hypotheses (Mardhiyyah et al., 2022). This includes inductive reasoning for generating hypotheses, deductive reasoning for testing them, as well as strategies for conducting experiments and evaluating evidence (Dowd et al., 2018; McNeill & Krajcik, 2008). This situation reflects a broader challenge in equipping students with essential 21st century skills, including critical thinking, problem-solving, and scientific reasoning (Carlgren, 2013; Osborne, 2013). When biotechnology is taught in abstract and decontextualized ways, students struggle to transfer their knowledge into meaningful applications.

Thus, a clear gap emerges while eco-enzyme fermentation and Gram-positive bacteria represent authentic scientific phenomena with strong local relevance, their potential has not been systematically integrated into biotechnology learning in schools. This omission means that opportunities to strengthen students' biotechnology competence and foster 21st century scientific literacy are missed.

Therefore, this study aims to present the observation results of Gram-positive bacteria in eco-enzymes derived from peatland plants in Central Kalimantan and to integrate these findings into biotechnology learning resources designed to explicitly enhance students' scientific explanation skills. By embedding local microbial exploration into biotechnology education, this research not only contributes to documenting microbial diversity but also aligns with global demands for contextualized science education that cultivates higher-order thinking and 21st century competencies.

Method

Research Design

This study employed a descriptive qualitative design to explore the potential of Gram-positive bacteria in eco-enzymes derived from peatland plants and to integrate the findings into biotechnology learning resources. The research consisted of two main stages: (1) microbiological analysis to identify Gram-positive bacteria in eco-enzymes, and (2) adaptation of findings into a learning resource prototype framed through the Claim–Evidence–Reasoning (CER) model for developing students' scientific explanation skills.

Sample and Sampling

The study population comprised Gram-positive bacteria with the potential to thrive in eco-enzyme fermentations derived from peatland plant substrates. Samples consisted of bacterial colonies isolated from eco-enzyme liquids produced using selected peatland plants.

Instruments and Materials

A set of laboratory instruments was employed, including a light microscope (Olympus CX21), micropipettes, Petri dishes, inoculating loops, incubator, autoclave, and digital balances. Mannitol Salt Agar (MSA) was used as the selective medium for Gram-positive bacteria. Raw substrates for eco-enzyme production included lemongrass (*Cymbopogon citratus*), turkey berry (*Solanum torvum*), kelakai (*Stenochlaena palustris*), pineapple (*Ananas comosus*), dragon fruit (*Hylocereus spp*.), karamunting (*Rhodomyrtus tomentosa*), banana (*Musa spp*.), citrus (*Citrus spp*.), watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), and papaya (*Carica papaya*). Sucrose, palm sugar, and molasses served as carbon sources for fermentation.

Procedure

The procedure of bacterial identification from eco-enzyme can be seen in Figure 1.

Figure 1. Procedure of bacterial identification from eco-enzyme

Preparation of Eco-Enzyme

Eco-enzyme solutions were prepared using a 1:3:10 ratio of substrate (0.9 kg peatland plant material), sugar source (0.3 kg), and water (3 L), respectively, in 5-L fermentation containers. Three fermentation variants were produced using palm sugar, white sugar, and molasses. The mixtures were sealed and fermented at room temperature for 30 days.

Media Preparation

MSA was prepared by dissolving 11.1 g of dehydrated medium in 100 mL distilled water, sterilized at 121 °C for 20 minutes in an autoclave, and poured aseptically into Petri dishes (~10 mL/plate).

Bacterial Isolation

Eco-enzyme samples were inoculated onto MSA plates using the spread plate method. Plates were incubated at 37 °C for 72 hours. Colony morphology, including color, shape, and surface texture, was recorded.

Gram Staining

Single colonies were transferred to glass slides, heat-fixed, and subjected to Gram staining using crystal violet, iodine, ethanol, and safranin. Observations were made at 100× magnification under oil immersion.

Identification

Morphological and staining characteristics of bacterial isolates were compared with standard microbiological references.

Data Collection and Analysis

Data were collected from colony morphology, Gram-staining reactions, and photomicrograph documentation. The results were analyzed qualitatively by categorizing isolates based on morphological traits and Gram characteristics. Findings were then synthesized into descriptive profiles of Gram-positive bacteria in eco-enzymes.

The descriptive data were subsequently integrated into a prototype biotechnology learning resource. The integration explicitly employed the Claim–Evidence–Reasoning (CER) framework, enabling alignment between laboratory procedures and indicators of scientific explanation skills. In this way, students are expected not only to learn microbiological techniques but also to practice constructing scientific explanations through structured connections between claims, supporting evidence, and logical reasoning.

Result and Discussion

Results of Gram-Positive Bacterial Identification

Based on the results of isolation and identification, four types of Gram-positive bacteria were found in eco-enzymes derived from peatland plants. The study was conducted in June at the Biology Education Laboratory, Universitas Palangka Raya, with weekly observations carried out during the fermentation process.

In the first week, no Gram-positive bacteria were detected in the three media with different sugar variations; only Gram-negative bacilli were observed. In the second week, three Gram-positive isolates were obtained, exhibiting coccus and bacillus morphologies. In the third week, two Gram-positive isolates were identified with similar coccus and bacillus forms. In the fourth week, three Gram-positive isolates were again detected, also showing both coccus and bacillus morphologies.

Overall, the findings indicate that four types of Gram-positive bacteria could be isolated from the second to the fourth week of fermentation. There are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Lactobacillus bulgaricus* as shown in Figure 2.

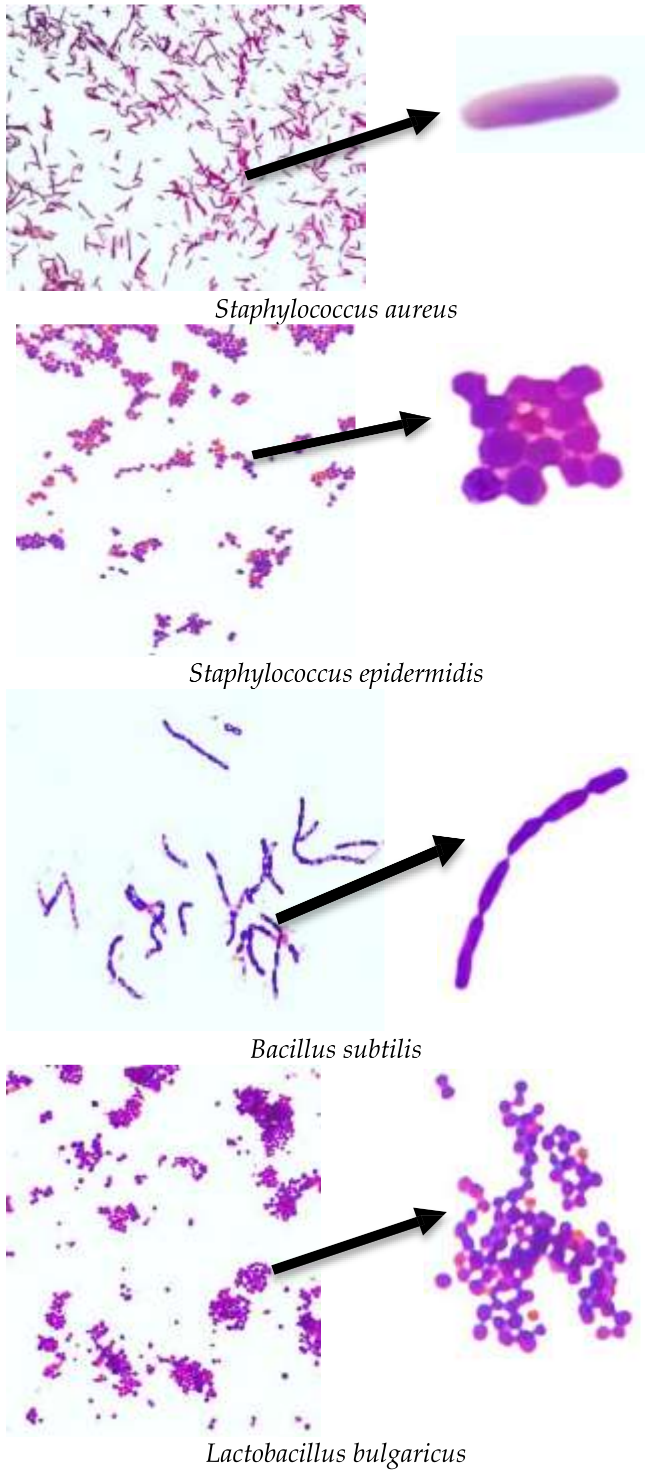

Figure 2. Results of gram-positive bacterial identification

Based on the results of Gram staining and colony morphology observations, several types of Gram-positive bacteria were successfully identified from the eco-enzyme samples. The morphological characteristics of each isolate are consistent with previous descriptions of their respective genera.

Staphylococcus aureus was identified as a Gram-positive coccus bacterium with a distinctive grape-like cluster arrangement (Abbas et al., 2025; Fayisa & Tuli, 2023; Pal et al., 2023). The cells appeared round and retained a purple color after Gram staining, indicating a thick peptidoglycan layer in the cell wall. Colonies of *S. aureus* were observed to be round, smooth, and golden yellow in color, which aligns with typical characteristics reported in literature for this species.

Similarly, *Staphylococcus epidermidis* exhibited a coccoid shape and clustered arrangement, also retaining the violet coloration of Gram-positive bacteria. The colonies were small, convex, circular, and opaque with a pinkish appearance (Namvar et al., 2014). These features correspond to the general morphology of *S. epidermidis*, which is known as a commensal species commonly found on human skin but capable of adapting to different environments.

Bacillus subtilis was identified as a Gram-positive rod-shaped (bacillus) bacterium (Arnaouteli et al., 2021; Hirozawa et al., 2023; Lu et al., 2018). The cells maintained the purple coloration after Gram staining, confirming the presence of a thick cell wall typical of Bacillus species. Colonies of *B. subtilis* were large, round, cream-colored, opaque, and had undulated margins with a convex surface. This species is known for producing extracellular enzymes that accelerate the decomposition of organic matter, thereby contributing to nutrient cycling during eco-enzyme fermentation.

Finally, *Lactobacillus bulgaricus* was identified as a Gram-positive, facultatively anaerobic bacterium belonging to the lactic acid bacteria group (Hakim et al., 2023; Tahyat et al., 2025; Tumbarski et al., 2021; Wahyuni et al., 2023). The cells were rod-shaped, and the colonies appeared small, round, convex, with smooth edges and a cream color. This bacterium plays a crucial role in lactic acid fermentation, converting lactose and other sugars into lactic acid. The production of lactic acid lowers the pH of the medium, creating an unfavorable environment for pathogenic microorganisms and enhancing the antimicrobial properties of the eco-enzyme.

Integrating the Potential of Gram-Positive Bacterial Observation Procedures in Eco-Enzymes for Developing Scientific Explanation Skills

The potential of Gram-Positive bacterial observation procedures in Eco-Enzymes for developing scientific explanation skills is presented in Figure 3.

The integration of each observation stage in the eco-enzyme experiment demonstrates its potential for developing students' scientific explanation skills through the Claim–Evidence–Reasoning (CER) framework (Mardhiyyah et al., 2022). By connecting practical microbiological procedures with explanatory reasoning, students are guided not only to follow the procedure but also to construct and justify scientific claims.

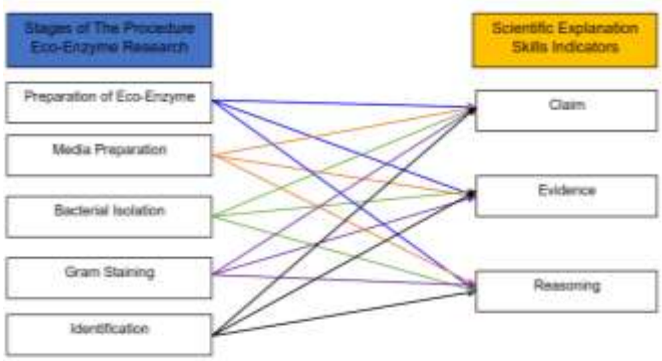

Figure 3. The potential of gram-positive bacterial observation procedures in eco-enzymes for developing scientific explanation skills

Preparation of Eco-Enzyme

In this stage, students recognize that raw materials such as peatland plants combined with sugar can be fermented into eco-enzyme. The claim is that the mixture can undergo fermentation. The evidence is represented by the standardized ratio of 1:3:10 (substrate:sugar:water), which ensures that the fermentation process can occur effectively. The reasoning is grounded in microbiology: sugar provides the primary energy source for microbial metabolism, while the plant substrate contributes additional nutrients, thereby supporting bacterial growth. This stage trains students to connect experimental design with the biochemical rationale underlying microbial activity (Samosa, 2021; Wangmo et al., 2025).

Media Preparation (MSA)

The preparation of Mannitol Salt Agar (MSA) provides a direct link to the selectivity of culture media. The claim here is that MSA can be used to grow bacteria from eco-enzyme samples. The evidence lies in the composition of MSA, which contains high concentrations of NaCl suitable for Gram-positive bacteria, especially Staphylococcus species. The reasoning explains that the high salt concentration inhibits the growth of Gram-negative bacteria, ensuring that the colonies observed are predominantly Gram-positive. This stage encourages students to see how selective media embody both empirical utility and theoretical justification (Diola et al., 2025; Hardcastle et al., 2021)

Bacterial Isolation

Observation of colony growth on MSA plates provides an opportunity to develop claims about microbial presence. The claim is that bacterial colonies successfully grow on the medium. The evidence consists of visible colonies with distinct morphology (color, shape, and surface texture) after 72 hours of incubation. The reasoning emphasizes that colony morphology provides preliminary insights into bacterial identity, although further tests are required for confirmation. This trains students to base claims on observable data while recognizing the limits of evidence (Masters & Docktor, 2022).

Gram Staining

This classical microbiological technique strengthens the link between observation and reasoning. The claim is that the bacteria are Gram-positive. The evidence is the purple coloration of cells after staining with crystal violet, iodine, ethanol, and safranin. The reasoning highlights that Gram-positive bacteria retain the primary stain due to their thick peptidoglycan layer, a principle explained in Gram's theory of bacterial differentiation. Here, students practice articulating causal reasoning that connects microscopic evidence with structural and chemical explanations (Diola et al., 2025; Hardcastle et al., 2021; Kartika et al., 2023; Masters & Docktor, 2022; Samosa, 2021).

Identification

The final stage synthesizes the findings into a coherent conclusion. The claim is that the isolates belong to Gram-positive bacteria such as Staphylococcus or Bacillus. The evidence combines colony morphology, Gram-staining results, and reference comparisons with authoritative sources such as Bergey's Manual of Systematic Bacteriology. The reasoning demonstrates how empirical data are validated through established scientific literature, showing students the importance of aligning their claims with accepted scientific frameworks (Samosa, 2021).

The development of scientific explanation skills through laboratory activities can be effectively structured into five stages, each contributing to a comprehensive understanding and application of scientific explanation. These stages are designed to enhance students' ability to articulate claims, provide evidence, and justify their reasoning, thereby fostering a deeper engagement with scientific concepts. The integration of these stages into laboratory activities not only improves scientific explanation skills but also enhances overall scientific literacy (Achor et al., 2018).

Conclusion

This study highlights the potential of Gram-positive bacteria in eco-enzymes derived from peatland plants as an authentic and locally relevant context for strengthening biotechnology learning in senior secondary schools. The identification of bacterial isolates such as Lactobacillus bulgaricus, Bacillus subtilis, Staphylococcus aureus, and Staphylococcus epidermidis not only provides scientific insight into the microbial dynamics of eco-enzyme fermentation but also offers meaningful opportunities to integrate these findings into biotechnology instruction. By adapting the results into a prototype instructional resource, this study demonstrates how laboratory-based phenomena can be systematically aligned with the Claim–Evidence–Reasoning (CER) framework to foster students' scientific explanation skills. Ultimately, this approach supports the development of 21st-century competencies, particularly critical thinking, problem solving, and evidence-based reasoning, while also addressing the persistent learning gap in biotechnology education.

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Author Contributions

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Conflicts of Interest

The authors declare no conflict of interest.

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