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Prebiotic And Enzymatic Indices of Split Gill Mushroom (*Schizophyllum Commune*)

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Abstrak: Penelitian ini bertujuan untuk mengevaluasi aktivitas dan indeks prebiotic serta aktivitas dan indeks enzimatik dari jamur grigit (*Schizophyllum commune*) melalui karakterisasi ekstrak serta bagian miseliumnya. Ekstrak disiapkan melalui maserasi air selama 24 jam, kemudian diuji kemampuan prebiotiknya terhadap bakteri *Lactobacillus casei* serta penghambatannya terhadap *Escherichia coli*. Inulin digunakan sebagai kontrol positif untuk membandingkan efektivitas ekstrak dalam mendukung pertumbuhan bakteri menguntungkan dan menekan bakteri patogen. Bagian miselium dikultur secara terkontrol dan dianalisis aktivitas amilase, selulase, serta protease menggunakan prosedur enzimatik standar yang lazim diterapkan dalam penelitian biokimia. Hasil penelitian menunjukkan bahwa ekstrak jamur mampu meningkatkan pertumbuhan *L. casei* secara signifikan dan memperlihatkan kemampuan menghambat pertumbuhan *E. coli*. Sebagai refleksi mendukung pertumbuhan bakteri *L.casei* dan menghambat *E.coli* menghasilkan indeks prebiotik ekstrak jamur grigit yang pada studi ini lebih tinggi dibandingkan inulin. Analisis miselium jamur grigit mengonfirmasi adanya aktivitas amilase, selulase, dan protease dengan nilai yang konsisten serta dapat direproduksi, walaupun aktivitasnya masih lebih rendah daripada enzim komersial yang digunakan sebagai pembanding dalam penelitian ini. Temuan ini mengindikasikan bahwa komponen bioaktif jamur grigit memiliki kapasitas yang relevan untuk aplikasi berbasis mikroba. Profil bioaktivitas yang diperoleh dari studi ini memperkuat potensi jamur grigit sebagai sumber senyawa prebiotik alami dan sebagai penghasil enzim multifungsi yang dapat dimanfaatkan dalam berbagai proses bioteknologi pangan. Hasil ini membuka peluang pengembangan formulasi inovatif yang tidak hanya mendukung pertumbuhan bakteri probiotik, tetapi juga menyediakan aktivitas enzimatik tambahan untuk meningkatkan efisiensi biokonversi pada berbagai aplikasi industri.

Katakunci: Aktivitas Enzimatik, *Escherichia Coli*, Jamur Konsumsi, *Lactobacillus Casei*, Prebiotik, *Schizophyllum Commune*.

Abstract: *This study aims to evaluate the prebiotic activity and index as well as the enzymatic activity and index of the split gill mushroom (Schizophyllum commune) through the characterization of the extract and its mycelial part. The extract was prepared through water maceration for 24 hours, then tested for its prebiotic ability against Lactobacillus casei bacteria and its inhibition against Escherichia coli. Inulin was used as a positive control to compare the effectiveness of the extract in supporting the growth of beneficial bacteria and suppressing pathogenic bacteria. The mycelial portion was grown under controlled conditions and tested for amylase, cellulase, and protease activity using fundamental enzymatic techniques commonly used in biochemical research. The results showed that the mushroom extract of split gill was able to significantly increase the growth of L. casei and showed the ability to inhibit the growth of E. coli. As a reflection of supporting the growth of L. casei bacteria and inhibiting E. coli, the prebiotic index of split gill mushroom extract in this study was higher than inulin. Analysis of the split gill mushroom mycelium confirmed the presence of amylase, cellulase, and protease activities with consistent and reproducible values, although the activities were still lower than those of the commercial enzymes used as a comparison in this study. These findings indicate that the bioactive components of split gill mushrooms have relevant capacity for microbial-based applications. The bioactivity profile obtained from this study strengthens the potential of split gill mushrooms as a source of natural prebiotic compounds and as a producer of multifunctional enzymes that can be utilized in various food biotechnology processes. These results open opportunities for the development of innovative formulations that not only support the growth of probiotic bacteria but also provide additional enzymatic activity to improve bioconversion efficiency in various industrial applications.*

Keywords: *Edible Mushroom, Enzymatic Activity, Escherichia Coli, Lactobacillus Casei, Prebiotic, Schizophyllum Commune*

Introduction

Split gill mushrooms (*Schizophyllum commune*) are a rich biodiversity of edible mushrooms that have not been cultivated and utilized properly. This fungus is usually found at the start of the rainy season and can live in soil or wood at fairly humid air temperatures (Khomariyah, 2018). *Basidiomycota* mushroom is a widely studied that produces β-glucan, phenolic compounds, and bioactive metabolites with high immunomodulatory and antioxidant activities (Mirónczuk-Chodakowska et al., 2021). Split gill contains bioactive substances like triterpenoids and phenolic compounds. Its cell wall has a special polysaccharide structure called β-glucan, which is cross-linked with chitin to form a triple helix structure that known as a β-1,3-D-glucan polymer with β-1,6-D branches, and it functions as a prebiotic by specifically promoting the growth of probiotic bacteria (Saetang et al., 2023; Lee and Ki, 2020). The split gill biological characteristics make it an important natural resource for a number of industries, including the pharmacological and medical fields, as well as for traditional and alternative diets around Asia (Chutimanukul et al., 2025). This mushroom is a traditional edible and medicinal mushroom that has received attention in modern research due to its bioactive polysaccharide content, which has immunomodulatory, anti-inflammatory, and beneficial metabolic effects in animal models and in vitro experiments (Kumar et al., 2025). β-Glucan is a non-starch polysaccharide that cannot be digested by human digestive enzymes but can be fermented by colonic microbiota, especially *Bifidobacterium* and *Lactobacillus* groups (Vetvicka et al., 2019). Fermentation of β-glucan produces short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, which play an important role in maintaining gut health, lowering colonic pH, and improving the integrity of the intestinal epithelial barrier (Wu et al., 2025). β-glucans meet the main criteria for prebiotics as defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP), namely compounds that selectively stimulate the growth of beneficial microbes and provide health benefits to the host (Gibson et al., 2017). Recent research also shows that β-glucans from the split gill have potential prebiotic activity because its can increase the growth of probiotic microbes and exhibit anti-inflammatory effects (Mirónczuk-Chodakowska et al., 2021; Le et al., 2020). Therefore, fungal β-glucans are a promising source of natural prebiotics for functional food applications and gut health.

Additionally, the mushroom is known to produce a number of extracellular enzymes and secondary metabolites, including lignin peroxidase, xylanase, and cellulase, which are essential for the breakdown of lignocellulosic biomass. Research into fungal enzymatic activity not only provides fundamental understanding of the physiological and ecological mechanisms of fungi but also opens up opportunities for broad applications in industrial enzyme production, functional foods, and sustainable waste management. Fungi produce a variety of extracellular enzymes—such as cellulases, xylanases, ligninase, amylases, and proteases—that can decompose complex compounds like lignocellulose into simple sugars and high-value organic compounds (El-Gendi et al., 2022). This enzymatic activity is fundamental to the bioconversion of biomass to produce bioethanol, bioplastics, and bio-based chemicals, while reducing the need for conventional, environmentally unfriendly chemical processes (Civzele et al., 2023). In food biotechnology, fungal enzymes are utilized to increase nutritional value, improve texture, and produce distinctive flavor and aroma compounds in fermented products. Research into fungal enzymatic activity supports the development of green biotechnology because fungal enzymes are environmentally friendly, work under mild conditions, and can replace synthetic chemicals in various industrial processes (Gupta et al., 2016). This is also in line with the concept of a circular economy, where agricultural waste or organic residues are converted into high-value products through the activity of fungal enzymes (Hyde et al., 2019). The physiological capabilities of the fungus are mostly determined by its enzymatic activity, specifically in relation to its bioactivity toward digesting substrates and its capacity to break down complex polysaccharides. split gill has been reported to produce cellulases (Sornlake et al., 2017; Kumar et al., 2018), xylanases (Gautam et al., 2018), pectinases (Zhu et al., 2016; Mehmood et al., 2018), lipases (Kam et al., 2016), laccases (Kumar et al., 2015), manganese peroxidase, and lignin peroxidase (Asgher et al., 2016). In previous research, split gill mushroom extract was able to tenderize beef, which was thought to be due to the activity of the protease enzyme (Sopandi et al., 2025). The prebiotic index indicates chemical diversity, which differs in molecular size, type of glycosidic bond, and branching pattern that determine the location of fermentation, degradation rate, and selectivity towards the gut microbiome (Zeng et al., 2023). Different prebiotics can have different effects on the composition of the gut microbiota and its metabolic profile (Fu et al., 2025). Meanwhile, enzymatic index of fungi are dynamic properties influenced primarily by the fungal strain/species, the substrate composition (nutrients), and the surrounding environmental conditions such as temperature, pH, and water activity (Drozłowska, 2019).

Evaluation of the enzymatic index can provide an overview of the fungus' metabolic capacity and adaptability to the bioconversion environment, while the prebiotic index reflects its potential to support gut microbial balance. The combination of these two parameters is expected to provide a comprehensive understanding of the dual role of split gill as a biotechnological agent and a natural functional food. Therefore, this study aims to determine the prebiotic and enzymatic index of split gill through a laboratory experimental approach.

Method

The study was conducted in two stages: the first measured the prebiotic index, and the second measured the enzymatic index. The first stage used a completely randomized design (CRD) with seven treatments: P1, commercial inulin at a concentration of 0.5% as a positive control; P2, mushroom extract at 0.5%; P3, mushroom extract at 1.0%; P4, mushroom extract at 1.5%; P5, mushroom extract at 2.0%; and P6, mushroom extract at 2.5%. The negative controls (P0) used deMan Ragosa Sharpay (MRS) media for *Lactobacillus casei* and Trypton Soy for *Escherichia coli*. The second stage of the study was designed experimentally using inferential statistics to determine enzyme activity using the Student's t-test.

Mushroom extraction

A 500 g of fresh split gill mushrooms purchased from collectors in South Bengkulu Regency, Bengkulu Province, Indonesia, were cleaned under running water to remove dirt, cut into 1x1 cm pieces, dried, ground, and sieved to pass a 60-mesh sieve. The split gill mushroom powder was placed in an Erlenmeyer flask, added with 1000 mL of distilled water for maceration, and placed on a shaker at 150 rpm for 24 hours. After maceration, the mixture was filtered using filter paper, and the filtrate was concentrated using a vacuum rotary evaporator.

Bacterial preparation and inoculation

One loop of *L. casei* and *E. coli* bacterial colonies was each suspended in sterile distilled water, and bacterial cell counts were performed using the McFarland 0.5 standard at 625 nm to determine the initial bacterial count. A total of 1,100 mL of MRS and 1,400 mL of TS media were divided into 20 mL each and placed into Erlenmeyer flasks, with 50 mL per flask. All media were sterilized by autoclaving at 121°C and 1 atmosphere pressure for 15 minutes. After cooling, the MRS and TS media were divided into 7 parts, each with 0.5% inulin (P1) as a positive control, and 0.5% (P2), 1.0% (P3), 1.5% (P4), 2.0% (P4), and 2.5% (P6) of split gill mushroom extract. One part was not given additional inulin and/or split gill mushroom extract used as a negative control (P0). Next, 1 mL of *L. casei* bacterial suspension (9.0×108/mL) was added to the Erlenmeyer flask containing MRS media and *E. coli* (9.0×108 /mL) was added to the TS media. All flasks were placed in a shaker for 24 hours at 37°C and a rotation speed of 60 rpm. After incubation, 1 mL from each Erlenmeyer flask was transferred to a test tube containing 9 mL of phosphate buffer and serially diluted. From each dilution, 0.1 mL was taken with a micropippet and spread onto nutrient agar. The inoculated medium was incubated for 24 hours at 37°C. The number of bacteria on the agar medium was counted using a colony counter.

Prebiotic activity

Prebiotic activity is calculated by comparing the number of *L. casei* bacteria (probiotic) with the number of *E. coli* bacteria (pathogen) growth bacteria using the formula:

$$Prebiotic\ Activity\ = \frac{(\log P24 - \log P0)\ extract}{(\log P24 - \log P0)\ glucose} - \frac{(\log E24 - \log E0)\ extract}{(\log E24 - \log E0)\ glucose}$$

$\log P24$ = Number of probiotic bacteria after 24 hours incubation; $\log P0$ = Initial number of probiotic bacteria; $\log E24$ = Number of pathogenic bacteria after 24 hours incubation; $\log E0$ = Initial number of pathogenic bacteria

Prebiotic Index

The prebiotic index is calculated based on the ratio of probiotic growth in the presence of the prebiotic to the growth of the probiotic in the presence of a control carbohydrate (Palframan et al. 2003). A prebiotic index greater than 1 indicates that the carbohydrate has a positive effect on probiotic growth. A prebiotic index closer to 1 indicates low effectiveness of the carbohydrate being evaluated (Figueroa-González et al. 2019). The prebiotic index is calculated using the formula:

$$Prebiotic\ Index\ = \frac{(\log P24 - \log P0)\ extract}{(\log P24 - \log P0)\ glucose}$$

$\log P24$ = Number of probiotic bacteria after 24 hours incubation; $\log P0$ = Number of probiotic bacteria before incubation.

Enzymatic Index

The split gill fruiting bodies attached to wood were harvested and sliced into 1x1 cm pieces. Three slices of split gill were aseptically placed on sterile PDA media and incubated at 25-30ºC for 96 hours. Amylase enzyme activity was observed using a qualitative iodine staining method added to a medium containing 0.5% starch (Xiao et al., 2006). The split gill mycelium cultured on PDA media was streaked and spread on sterile Starch Agar (0.5% starch medium) in a Petri dish and incubated at 32ºC for 48 hours. After the incubation period, the split gill fungal isolates were treated with three drops of iodine, and the diameter of the clear zone formed around the colonies was measured. Observation of cellulase enzyme activity was carried out according to the congo red staining method which can bind to cellulose molecules and produce a red complex. The split gill mycelium growing on PDA media was streaked and spread on Carboxy Methyl Cellulose (CMC) medium in a petri dish and incubated at 32ºC for 48 hours. After the incubation period, split gill fungal isolates grown on CMC medium were flooded with congo red for 15 minutes. The congo red dye was rinsed using 1 M NaCl, and the diameter of the clear zone formed around the colony was measured. Observation of protease enzyme activity was carried out according to the method of using SMA (Skim Milk Agar) media. The split gill mycelium growing on sterile PDA media in a petri dish was streaked and spread on SMA medium and incubated at 25-30 ºC for 48 hours. After the incubation period, the diameter of the clear zone in the media around the colony was measured. The enzymatic index was calculated using the formula of Kaur and Phutela (2016):

$$Enzymatic\ index = \frac{\text{Clear zone diameter (mm)}}{\text{Colony diameter (mm)}}$$

Data Analysis

Prebiotic activity and index data were analyzed using one-way analysis of variance at a significance level of 0.05 in accordance with a completely randomized experimental design (CRD). Data from bacterial count observations were first transformed into logarithms before analysis of variance. Further testing was conducted using the Tukey HSD test at a significance level of 0.05 to determine differences between treatments. The research data for the activity and index of each enzyme were analyzed using the Student's t-test at a significance level of 0.05.

Results and Discussion

The results of this study (Figure 1) show that the number of *L. casei* bacteria at all concentrations of split gill mushroom extract tested was significantly (P<0.05) higher than 0.5% inulin and the initial bacterial count. The highest number of *L. casei* bacteria was obtained at a concentration of 2.5% split gill mushroom extract. The results of this study (Figure 1) also show that the number of *E. coli* bacteria at all concentrations of split gill mushroom extract was significantly (P<0.05) lower than 0.5% inulin and the initial bacterial count. The lowest number of *E. coli* bacteria was obtained at a concentration of 2.5% split gill mushroom extract. The number of *E. coli* bacteria was significantly (P<0.05) lower compared to *L. casei* at each concentration of split gill mushroom extract. Figure 2 showed that the prebiotic index at all tested concentrations of split gill mushroom extract was significantly (P<0.05) higher than that of 0.5% inulin. The highest prebiotic index was obtained at a concentration of 2.5%.

The increase in *L. casei* bacterial growth and the inhibition of *E. coli* growth by split gill mushroom extract are thought to be due to the activity of prebiotic components and secondary metabolites that have antibacterial properties. The prebiotic polysaccharide components, especially β-glucan (schizophyllan) in split gill mushroom, can be fermented by lactic acid bacteria (LAB) such as *L. casei*, providing a usable carbon source that encourages their growth and activity (Mirónczuk-Chodakowska et al. 2021). The inhibition of E. coli growth is also thought to be caused by the antimicrobial components in split gill mushroom mushroom extract. The split gill mushroom mushrooms contain phenolics, terpenoids, proteins/polypeptides or other secondary metabolites that show direct antibacterial activity against Gram-negative bacteria including *E. coli* (Acanto and Cuaderes 2021). This study also show that *L. casei* growth increased with increasing concentration of split gill mushroom fungus extract, indicating that up to a concentration of 2.5%, the split gill mushroom fungus extract provides a complex carbon source (β-glucan, heteropolysaccharides) that can be fermented by *L. casei*. This increases the growth rate and metabolic activity, because these polysaccharides function as natural prebiotics (Mirónczuk-Chodakowska et al. 2021; Vu et al. 2022). The inhibition of *E. coli* increased with increasing extract concentration, presumably due to the direct inhibitory effect of the high content of phenolic and terpenoid compounds that can damage the integrity of the Gram-negative outer membrane, causing leakage of cell contents and death (Huang et al. 2022). This study is in accordance with several studies that have reported that split gill mushroom extract can increase the population of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* (Saetang et al., 2023; Chen et al., 2023).

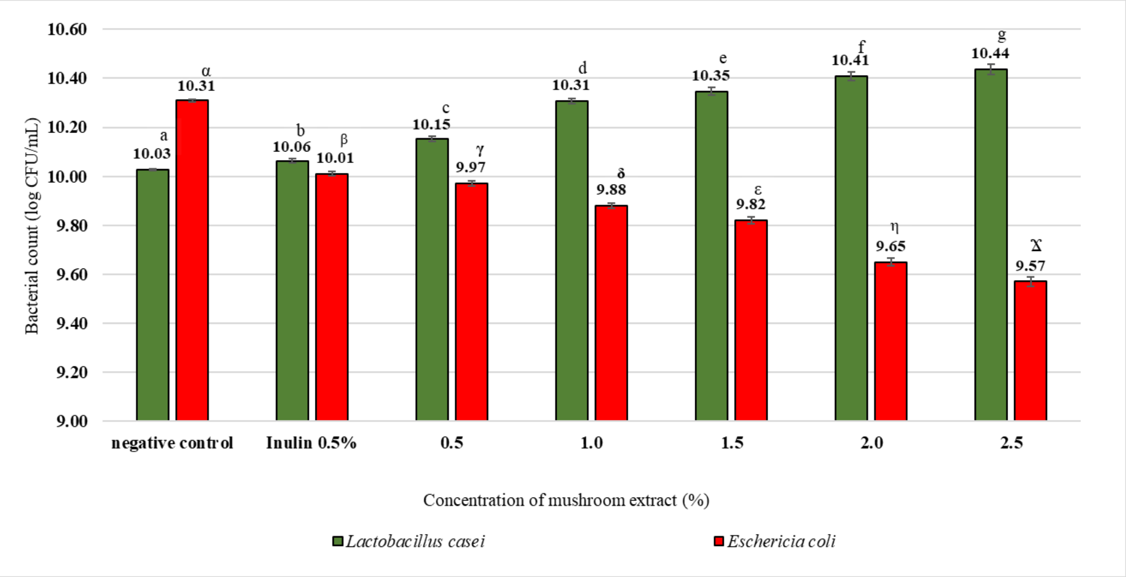

Figure 1. Number of *Lactobacillus casei* and *Eschericia coli* bacteria in MRS media supplemented with several concentrations of split gill mushroom extract and 0.5% inulin.

The split gill mushroom mushroom extract was able to increase the growth of *L. casei* and simultaneously inhibit the growth of *E. coli* more strongly than inulin. This phenomenon is thought to be due to the effect of the higher complexity of the fungal polysaccharide structure. The main fungal polysaccharide, namely β-(1→3)(1→6)-glucan (schizophyllan), has a branched structure that allows it to be fermented more efficiently by L. casei, producing short-chain fatty acids (SCFA) and lactic acid in greater quantities than inulin fermentation which only consists of β-(2→1)-fructose chains (Mirónczuk-Chodakowska et al., 2021; Vu et al., 2022). This branched structure allows more efficient enzymatic access by fermentative bacteria compared to the linear fructans in inulin, thereby accelerating the growth rate and metabolic activity of beneficial bacteria including *L. casei* (El Khoury et al., 2011). The presence of additional bioactive compounds in mushroom extracts, including lectin and hydrophobin proteins, is thought to provide a synergistic effect on colonization and probiotic activity. The split gill mushroom extract contains phenolic compounds, terpenoids, and bioactive peptides that have direct antibacterial activity (Huang et al., 2022; Mirfat et al., 2014). The combination of prebiotic and antibacterial properties makes the mushroom extract work synergistically, increasing the selectivity of beneficial bacterial growth and suppressing pathogenic microbes, because it supported the growth of *L. casei* and inhibited the growth of *E. coli* more than inulin, the extract from split gill mushrooms had a higher prebiotic index than inulin.

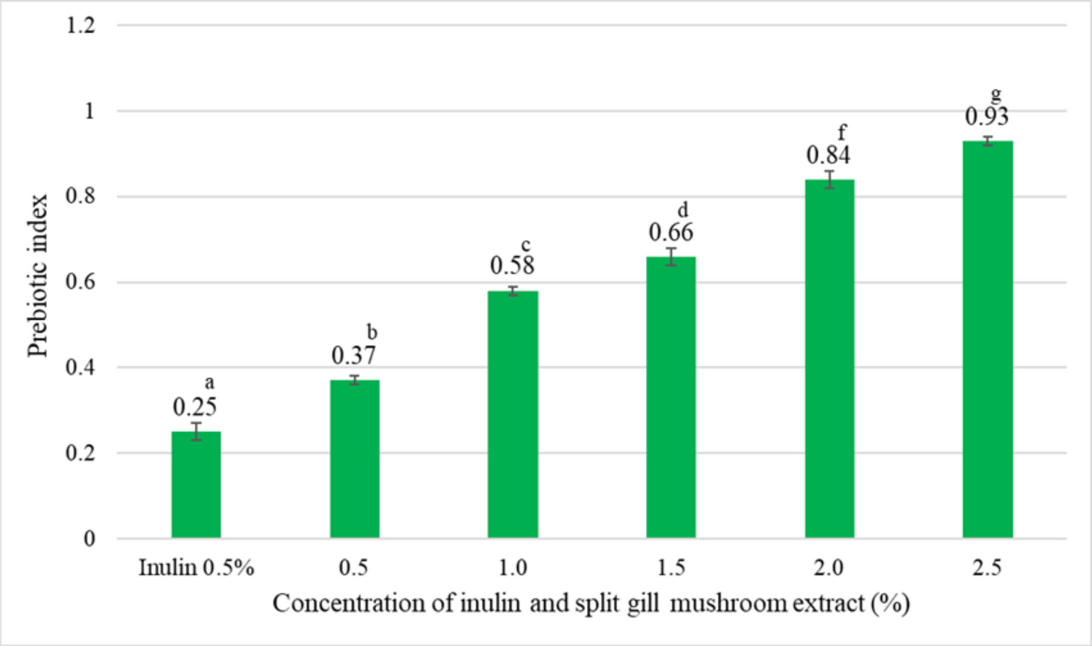

Figure 2. Prebiotic index of split gill mushroom extract against *Lactobacillus casei* and *Escherichia coli* bacteria.

The results (Figure 3) show clear zones in the amylase, cellulase, and protease enzyme tests. The activity of the amylase, cellulase, and protease enzymes (Figure 4) from split gill mushroom mycelium was significantly (P<0.05) lower than that of the commercial enzyme. Meanwhile, the cellulase enzyme activity of split gill mushroom mycelium was significantly (P<0.05) higher than amylase and protease.

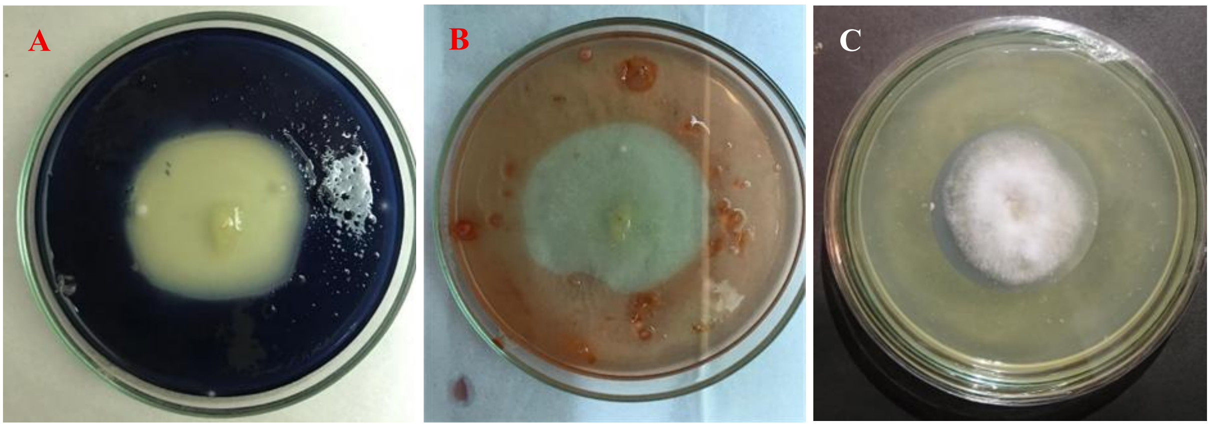

Figure 3. Clear zones formed by a) amylase, b) cellulase, and c) protease enzyme activity from mycelium of split gill mushroom

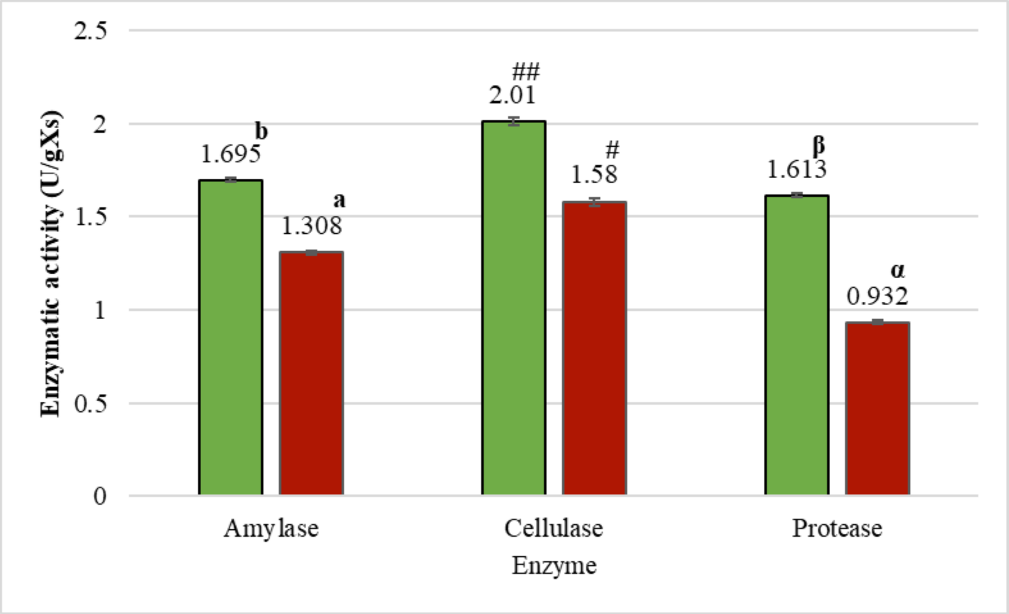

Figure 4. Enzymatic activity of amylase, cellulase and protease of split gill mycelium and commercial enzymes

The activity of amylase, cellulase, and protease enzymes produced from fungal mycelium is generally lower than that of commercial enzymes. This is due to several factors, primarily because the enzyme extract obtained from mycelium is still a crude extract containing various non-enzymatic proteins, secondary metabolites, and inhibitory compounds that can reduce specific enzyme activity (Haneef et al., 2017). Furthermore, mycelial culture conditions are often suboptimal for inducing the production of certain enzymes due to the absence of inducing substrates such as starch, cellulose, or protein in the medium (Gusakov, 2011). Natural enzymes from mycelium also generally have lower stability to temperature and pH compared to commercial enzymes that have been produced from thermotolerant strains and formulated to be more stable (Sharma et al., 2019; Turner et al., 2007). The relatively low enzyme activity of mycelium indicates the need for purification processes and optimization of fermentation conditions to increase the enzymatic potential of fungi as a source of industrial biocatalysts (Raveendran et al., 2018).

The cellulase enzyme activity of the split gill fungus mycelium is higher than its amylase and protease activities. This is due to the physiological ability of the fungus as a white rot fungus that naturally plays a role in lignocellulose degradation, so that the genes encoding cellulolytic enzymes are expressed more dominantly than other hydrolytic enzymes (Rytioja et al., 2014). The cellulase enzyme is also produced semi-constitutively because it plays an important role in the degradation of β-glucan and structural polysaccharides in the fungal cell wall itself. Meanwhile, higher amylase activity compared to protease activity indicates that split gill prioritizes the utilization of polysaccharides as the primary carbon and energy source, while protease synthesis tends to be suppressed by nitrogen availability in the medium (Singh et al., 2021). This pattern reflects the metabolic adaptation of split gill fungi to lignocellulosic substrates in nature.

The split gill mycelium in this study exhibited α-amylase activity, suggesting the presence of hydrolytic enzymes actively secreted during vegetative growth. In contrast, Wunjuntuk et al. (2022) reported anti-α-amylase activity in the hexane extract of the mushroom's fruiting body. This difference can be attributed to both the nature of the solvent and the fungal material used. Mycelial biomass mainly produces primary metabolites, including extracellular enzymes for substrate degradation, whereas the fruiting body accumulates secondary metabolites such as terpenoids or phenolics that may inhibit α-amylase. Therefore, the enzymatic and inhibitory properties observed in different studies highlight distinct metabolic profiles between vegetative and reproductive phases of split gill. For cellulase enzyme activity, this study is in line with Ohm et al. (2010), who reported that split gill fungus showed pectinase, hemicellulase, and cellulase enzyme activity and could degrade cellulose and lignin. The protease enzyme activity in this study is also consistent with Wongaem et al. (2020), who reported that the split-gill naturally releases protease enzymes into the surrounding environment as a fundamental part of its feeding process, which is known as extracellular digestion, in which the fungus breaks down complex organic matter for nutrient absorption.

Conclusion

Split gill mushroom (*Schizophyllum commune*) extract showed higher prebiotic activity than inulin, as evidenced by increased growth of *Lactobacillus casei* and inhibition of *Escherichia coli* growth with a significantly higher prebiotic index value than inulin. Meanwhile, the fungal mycelium produced amylase, cellulase, and protease activities, although still lower compared to commercial enzymes. These results indicate that split gill mushroom has dual potential as a source of natural prebiotic compounds and a producer of multifunctional enzymes that are prospective for development in functional food applications and microbial biotechnology. The split gill mycelium is a source of active enzymes that is worthy of exploration in biotechnology applications and enzymatic research. An important implication of this study is that split gill mushroom extract can be considered as an alternative raw material for functional food formulations that support gut health and as a bioactive component that strengthens the stability of probiotic microbiota. Furthermore, the presence of hydrolytic enzymes in the mycelium opens up opportunities for its use in bioconversion processes, lignocellulosic substrate degradation, controlled fermentation, or increasing the efficiency of enzymatic processes in industry. For further research, it is recommended to conduct structural characterization of prebiotic polysaccharides, identification of specific bioactive components, optimization of culture conditions to increase enzyme production, and in vivo studies of its prebiotic effectiveness in animal models. Practically, split gill extract and mycelium have the potential to be formulated into food products, microbiota supplements, or enzymatic preparations after undergoing composition standardization and a more comprehensive safety evaluation.

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