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Research Article

Lentinus squarrosulus Mont. selectively stimulates *Lactobacillus plantarum* and *Lactobacillus pentosus* growth and inhibits pathogenic bacteria: A promising prebiotic potential

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Abstract

Prebiotics are agents that can selectively stimulate the growth of beneficial microorganisms (probiotics). Mushrooms have been reported as a significant source of prebiotics. This study investigates the prebiotic potential of *Lentinus squarrosulus* Mont. Fruiting bodies of *L. squarrosulus* were lyophilized, pulverized, and extracted with chloroform/methanol (1:1) and distilled water. The percentage growth stimulation effect of the mushroom extracts on *Lactobacillus plantarum* AO11 and *Lactobacillus pentosus* A4c, as well as their activity against pathogenic bacteria, including enteropathogenic *Escherichia coli* EPEC 034A, *Salmonella typhi* ATCC 14028, and *Staphylococcus aureus* ATCC 25923, was determined using standard methods. Inulin and ciprofloxacin served as controls. Chemical composition of the mushroom extract was identified using Gas Chromatography-Mass Spectrometry (GC-MS). Culture media supplemented with 20 mg/ml of the aqueous extract of *L. squarrosulus* produced a 74% growth stimulation of *Lactobacillus plantarum*, which is comparable to that of inulin (75%), a commercial prebiotic. The zone of inhibition of the supernatant of *Lactobacillus* species cultured in media supplemented with mushroom extracts, inulin, or ciprofloxacin against *E. coli*, *S. typhi*, and *S. aureus* ranged from 10 to 23.5 mm. The supernatant of *Lactobacillus plantarum* AO11 and *Lactobacillus pentosus* A4c cultured in media supplemented with the CME (26-57%), inulin (40-54%), or ciprofloxacin (54-59%) showed greater activity against pathogenic organisms than the supernatant of extract-free culture media. Notable compounds in the aqueous extract include 9,12-octadecadienoic acid (Z, Z methyl ester), n-hexadecanoic acid, and oleic acid. *Lentinus squarrosulus* stimulates *Lactobacillus plantarum* AO11 and *Lactobacillus pentosus* A4c. growth and inhibits pathogenic organisms. Its usefulness in promoting gut health could be further explored.

Keywords: *Lentinus squarrosulus*; *Lactobacillus plantarum*; *Lactobacillus pentosus*; prebiotics

Introduction

Over the years, researchers have established the contribution of intestinal flora to human health by enhancing food nutrient metabolism in the host cell and producing life-enhancing functional substances, such as vitamins, amino acids, and short-chain fatty acids, among many other beneficial functions [1,2]. However, to avoid diseased states such as obesity and diabetes, which may result from an imbalance in the intestinal flora, there is a need for the adequate presence of probiotics, which are

beneficial microorganisms that maintain and enhance balance in the intestinal flora as well as inhibit the growth of pathogenic bacteria within the host's intestine [3,4].

Probiotics are living organisms; therefore, there is a need to maintain their viability, functionality, and promote their reproduction within the host. All these are enhanced by prebiotics. Prebiotics are often referred to as substances that are indigestible and unabsorbed by the host, but promote the growth and survival of intestinal flora [5]. In past years, the term "prebiotics" was limited to oligosaccharide carbohydrates [6]. However, advancements in science have clarified that prebiotics are not limited to oligosaccharide carbohydrates but also extend to non-carbohydrates, as long as they meet the criteria of passing through the large intestine without being digested in the upper GI tract, and thus becoming accessible to probiotics [7,8].

Mushrooms have been identified over the years as a nutritious food rich in dietary fibre, whose consumption has been linked to the amelioration of diseases such as obesity, cardiovascular disorders, and diabetes, resulting from their high potential to improve the gut microbiota by acting as prebiotics [8,9]. *Lentinus squarrosulus* is an edible mushroom belonging to Polyporales [10]. Due to its abundance of valuable components, including dietary fiber, protein, low-fat carbohydrates, and various polysaccharides, it has a range of medicinal uses [11,12]. Since prebiotics are often recommended in supplement form to patients taking antibiotics, the goal is to repopulate the colon with desirable bacteria after the antibiotics have eliminated both beneficial and undesirable bacteria. This study is therefore directed at evaluating the effect of *Lentinus squarrosulus* on the growth of two different species of *Lactobacillus* with an interest in their usefulness as prebiotics.

Materials and Methods

Reagents and chemicals

Methanol, chloroform, distilled water, dimethyl sulphoxide (DMSO), microorganisms [*Lactobacillus plantarum* AO11 and *Lactobacillus pentosus* A4c, enteropathogenic, *Eschericshia coli* EPEC 034A, *Salmonella typhi* ATCC 14028 and *Staphylococcus aureus* ATCC 25923], methylated spirit, normal saline, *p*-iodonitrotetrazolium dye, Man Rogosa and Sharpe (MRS) agar, MRS broth, Tryptone Soya Broth (Biomark), Mannitol salt agar (Hi media), inulin, ciprofloxacin, Nutrient Agar, Muller Hinton agar, *Salmonella shigella* agar. All other reagents and chemicals used were of quality analytical grade procured from commercially available sources.

Collection and identification of Mushrooms

Mushroom samples believed to be *L. squarrosulus* were meticulously collected in the vibrant surroundings of the University of Ibadan in Ibadan, Oyo State, Nigeria (Figure 1). Their identification was rigorously conducted using detailed morphological characteristics from a reliable mushroom database, in conjunction with advanced molecular biology tools [11].

Figure 1. Picture of *L. squarrosulus*.

Extraction of L. squarrosulus fruiting body

The fruiting bodies of mushroom samples were collected, freeze-dried, and ground into a fine powder. A 250 gm portion of this powder was then expertly extracted through maceration using a potent solvent mixture of methanol and chloroform (1:1) for 24 h in a sonicator, ensuring thorough extraction of active compounds. Following this, the mixture was carefully filtered, with the filtrate concentrated using a rotary evaporator under controlled temperature and pressure to preserve the extract's integrity. The resulting residue was meticulously dried and re-soaked in distilled water, which was subsequently heated in a water bath at 70 °C for 3 h. This process was followed by an additional filtration to refine the extract. Finally, the filtrate was concentrated once more using a rotary evaporator

to yield a high-quality aqueous extract of *L. squarrosulus*, from which the percentage yield was calculated, demonstrating the efficacy of the extraction process.

Microbiological procedures

Sources of microorganisms and preparation of standard inoculum

The microbes utilized in this research were sourced from Pharmaceutical Microbiology Department at the University of Ibadan. We cultivated pure cultures of *Lactobacillus plantarum* AO11 and *Lactobacillus pentosus* A4c on De Man, Rogosa, and Sharpe (MRS) agar under controlled anaerobic conditions at a temperature of 30 °C. In parallel, we cultured Enteropathogenic *Escherichia coli* EPEC 034A, *Salmonella typhi* ATCC14028, and *Staphylococcus aureus* ATCC 25923 on Nutrient agar at 35 °C. Each culture was meticulously incubated for 24 h to ensure robust growth for our screening procedure.

Evaluation of probiotic growth stimulation effect of L. squarrosulus extracts

Lactobacillus plantarum AO11and *Lactobacillus pentosus* A4c strains were streaked on an MRS agar plate and incubated in an anaerobic environment at 37 °C for 24 h. A lactobacillus cell suspension equilibrated to a 0.5 McFarland standard was used for the assay. Discrete colonies of each lactobacillus strain were cultured separately in MRS broth supplemented with the mushroom extract (10 and 20 mg/ml) at 37 °C for 24 h under anaerobic conditions. Inulin, a commercial prebiotic, served as the positive control. Also, 0.1% DMSO served as a negative control. After 24 h of incubation in an anaerobic jar, the cultures were quantified by measuring the optical cell density using spectrophotometry at 620 nm and the spread-out method [13]. The assay was carried out in duplicate and repeated twice.

Evaluation of pathogenic growth inhibition of supernatant of Lactobacillus spp. cultured in media supplemented with chloroform/ethanol and aqueous extracts of Lentinus squarrosulus

The assay was based on the agar diffusion method as described by Rousseau *et al*. [14]. The pathogenic bacterial suspensions (*E. coli*, *S. typhi*, and *S. aureus*) were equilibrated to a 0.5 McFarland standard, and the inoculum of each bacterial suspension was spread on the surface of Muller-Hinton agar plates. The plate was incubated at room temperature for 5 minutes to allow the bacterial suspension to acclimate to the agar. A sterile cork borer (8 mm diameter) was used to drill holes in the plate, which were labelled appropriately. Thereafter, cultured *Lactobacillus* was treated with 10, 20 mg/ml of mushroom extract, inulin (20 mg/ml), ciprofloxacin (10 µg/ml), or 0.1% DMSO and centrifuged at 10,000 rpm at 4 °C for 15 min. One milliliter (1 ml) of the supernatant from the cultured *Lactobacillus* was dispensed into each of the labeled holes bored on the already prepared plates. The plates were then incubated at 37 °C for 24 h. The zone of inhibition around each hole was measured along two axes, and the mean was then obtained (the experiment was carried out in triplicate and repeated twice).

Chemical Composition of the aqueous extract of Lentinus squarrosulus

The chemical composition of *Lentinus squarrosulus* was identified using gas chromatography-mass spectrometry, as previously reported by our team, Abiodun and co-workers [11]. The identification process was thorough, involving the use of the Agilent Technologies 7890 GC system coupled with an Agilent Technologies 5975 MSD (Mass Spectrometry Detector). The carrier gases used for Gas Chromatography-Mass Spectrometry (GC-MS) were hydrogen and helium, respectively. Successful separation was achieved by the use of a Restek fused capillary column (Rxi-5MS, 30 m (length) x 0.25 mm (internal diameter), 0.25 m (film thickness). The percentage content of the components was determined based on the difference in retention time. The compounds were identified by correlating each mass spectral peak representing a compound with the NIST and NISTREP mass spectra libraries of the GC-MS data system.

Statistical analysis

The impact of mushroom extract on probiotic growth stimulation was quantified as a percentage. The results were clearly presented as the mean plus the standard error of the mean (SEM), and a rigorous analysis was conducted using the Mann-Whitney U test. A p-value of less than 0.05 was

deemed statistically significant, underscoring the reliable efficacy of the mushroom extract in promoting probiotic growth.

Results

Percentage yield of the mushroom extracts

The percentage yields of the chloroform/methanol and aqueous extracts of the mushroom sample were 4.51% and 10.61%, respectively.

Prebiotics activity of Lentinus squarrosulus extract on Lactobacillus spp

The percentage growth stimulation of chloroform/methanol and aqueous extract of the mushroom *L. squarrolusus* at 10 and 20 mg/ml on the two probiotics tested (*L. plantarum* AO11 and *L. pentosus* A4c) ranged from 45.23 ± 1.65 to 73.98 ± 1.3% (Table 1). Inulin, a standard control, produced between 60.54 ± 0.45 and 73.98 ± 1.37% at the same concentration. Interestingly, the percentage growth stimulation of aqueous extract of *L. plantarum* AO11 and *L. pentosus* A4c was comparable to inulin (Table 1).

Table 1. Growth Stimulation Mushrooms extracts on *Lactobacillus* species.

Mushroom Extracts	**Mean ± SEM Percentage growth @ 10 mg/ml**		**Mean ± SEM Percentage growth @ 20 mg/ml**	
	L. plantarum AO11	*L. pentosus* A4c	*L. plantarum* AO11	*L. pentosus* A4c
Lentinus squarosulus (CME)	52.46 ± 0.8*	45.23 ± 1.65*	66.44 ± 0.29*	52.90 ± 0.86*
Lentinus squarosulus (AE)	62.41 ± 1.43	59.31 ± 1.78	73.98 ± 1.37	70.01 ± 1.05
Inulin (standard prebiotics)	60.54 ± 0.45	66.44 ± 0.29	74.98 ± 0.22	72.13 ± 1.06

CME - chloroform/methanol extract, AE- Aqueous extract, *inulin vs mushroom extracts $p<0.05$, n=2 (The assay was carried out in duplicate and repeated twice).

Pathogenic growth inhibition

Zone of inhibition of the supernatant of probiotics (*L. plantarum* AO11 and *L. pentosus* A4c) cultured in media supplemented with chloroform/ethanol, aqueous mushroom extracts, inulin, and Ciprofloxacin against *E. coli*, *S. typhi*, and *S. aureus* ranged from 10-23.5 mm (Figure 2). *E. coli* was more susceptible than the two other pathogenic organisms (Figure 2). Ciprofloxacin, a standard antibacterial agent, produced the highest inhibitory activity against the three microorganisms. In addition, the supernatant of probiotics *L. plantarum and L. pentosus* cultured in media supplemented with 20 mg/ml chloroform/methanol extract of the mushroom produced a higher inhibition of *E. coli* and *S. aureus* than inulin and aqueous extract (Figure 2; $p<0.005$). In contrast, the supernatant of probiotics *L. plantarum and L. pentosus* cultured in media supplemented with inulin or Ciprofloxacin significantly inhibited *S. typhi* than other treatments (Figure 2). The supernatant of lactobacillus species cultured in media supplemented with the CME (26-57%), inulin (40-54%) or Ciprofloxacin (54-59%) were more active against pathogenic organisms than the supernatant of extract-free culture media**.**

GC-MS Analysis of the aqueous extract of Lentinus squarrosulus

The GC-MS chromatogram was used to identify the chemical components of the aqueous extract of Lentinus squarosulus. Nine peaks, corresponding to the presence of nine chemical compounds based on their retention times, peak areas, and mass spectral fragmentation patterns, were identified (Table 2). Using the National Institute of Standards and Technology (NIST) library, the identified compounds, along with their mass spectra and structures, are shown in Figure 3-8. The relative abundance of the compounds ranged from 0.89 to 50.30%. The most abundant compound is 9, 12- octadecadienoic acid (Z, Z) (50.3%), followed by n-Hexadecanoic acid (22.2%), 11- octadecadienoic, methyl ester (16.02%), 9, 12- octadecadienoic acid (Z, Z methyl ester) 9.04%, Oleic acid (8.12%) (Figure 4 -8).

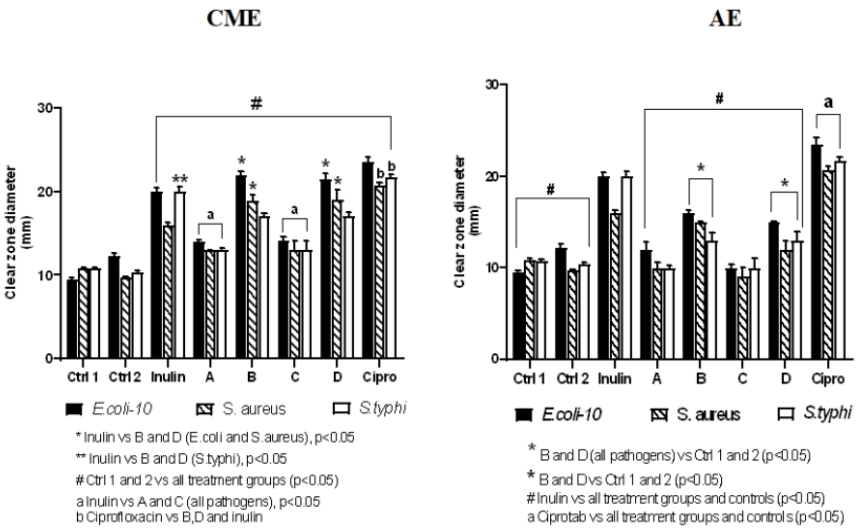

Figure 2. Zone of inhibition of E. coli, S. typhi and S. aureusby supernatant of probiotics Lactobacillus plantarum AO11 and Lactobacillus pentosus A4c cultured in media supplemented with chloroform/ethanol (CME) and aqueous (AE) mushroom extracts. Ctrt 1 and 2 - control (supernatant of probiotics L. plantarum and L. pentosus without mushroom extract), A and B - supernatant of L. plantarum cultured in 10 and 20 mg/mL mushroom extract, C and D - supernatant of L. pentosus cultured in 10 and 20 mg/mL mushroom extract. Cipro - Ciprofloxacin. Bar indicates Mean and S.E.M.

Table 2. Chemical components of the aqueous extract of *Lentinus squarrosulus.*

Peak no.	Chemical compounds	Retention time (mins)	Abundance (%)	M+1
1	Pentadecanoic acid	14.02	1.46	242
2	Hexadecanoic acid, methyl ester	14.57	2.84	270
3	n-Hexadecanoic acid	14.99	22.20	256
4	9,12-octadecadienoic acid (Z,Z methyl ester)	15.95	9.04	294
5	11- octadecadienoic, methyl ester	16.02	1.40	296
6	9, 12- octadecadienoic acid (Z,Z)	16.41	50.30	280
7	Oleic acid	16.44	8.12	280
8	Octadecanoic acid	16.59	3.78	284
9	3-(4 methoxyphenyl) quinolin-4-ol	20.98	0.89	251

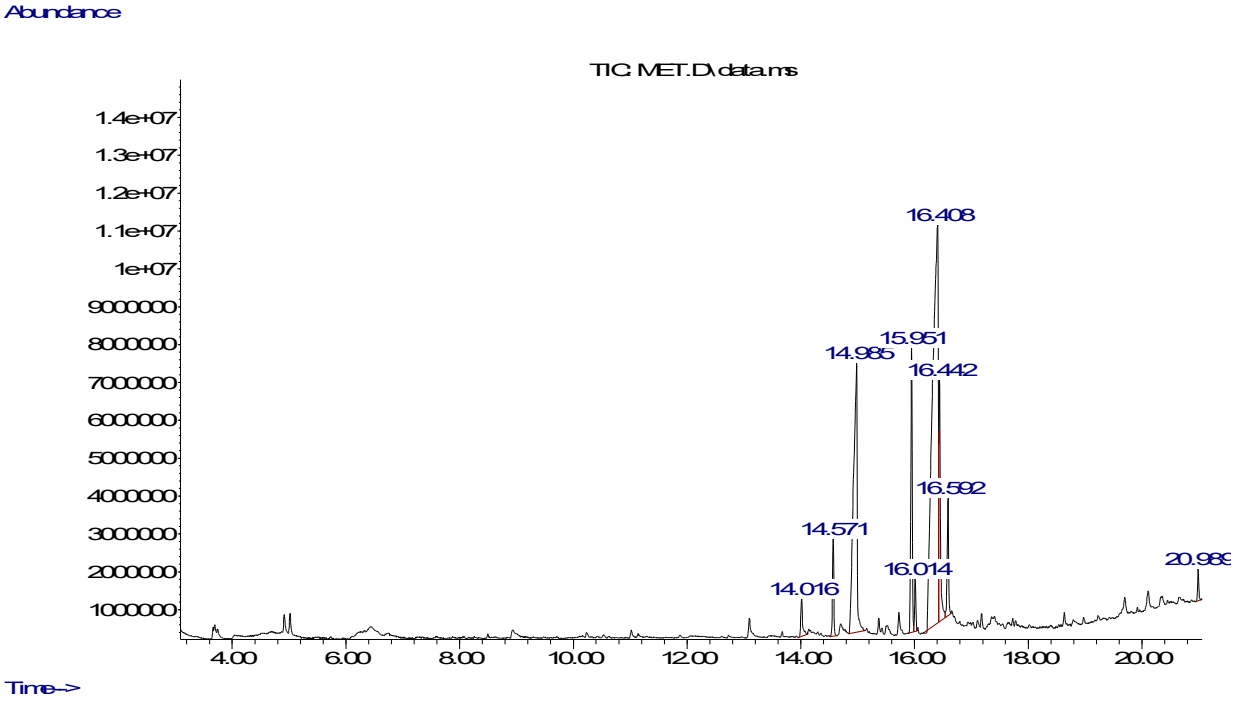

Figure 3. A Gas chromatogram of Lentinus squarosulus showing a plot of intensity versus retention time (Minutes) [11].

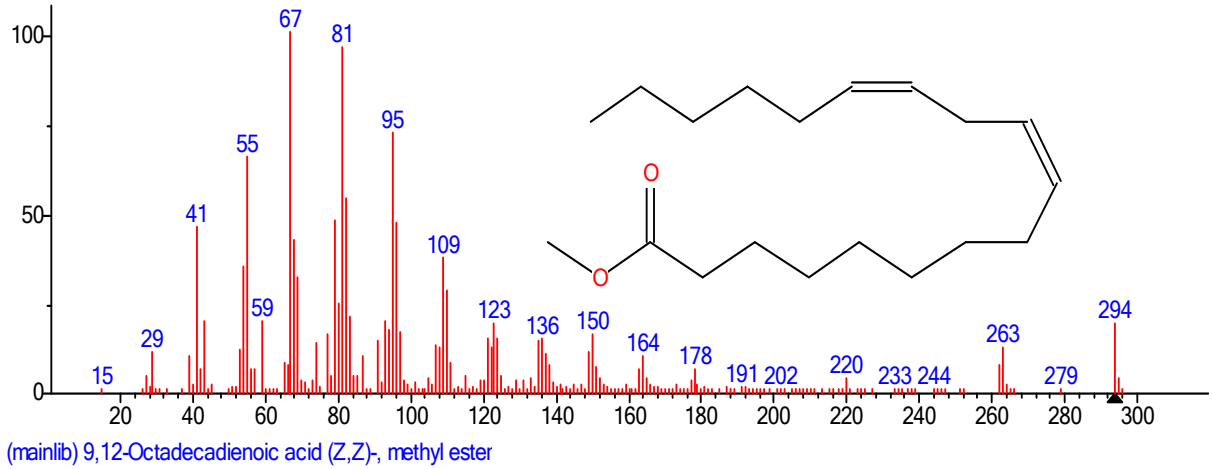

Figure 4. Mass spectrum of peak 9,12- Octadecadienoic acid (Z, Z)- methyl ester, a plot relative abundance versus mass of charge ratio.

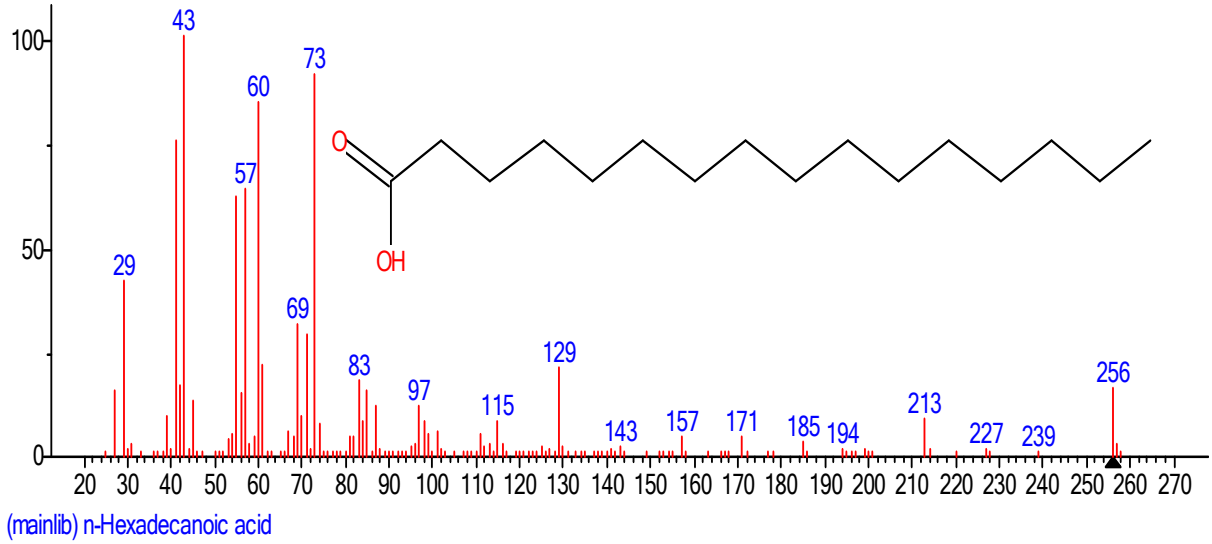

Figure 5. Mass spectrum of peak n-Hexadecanoic acid, a plot relative abundance versus mass of charge ratio.

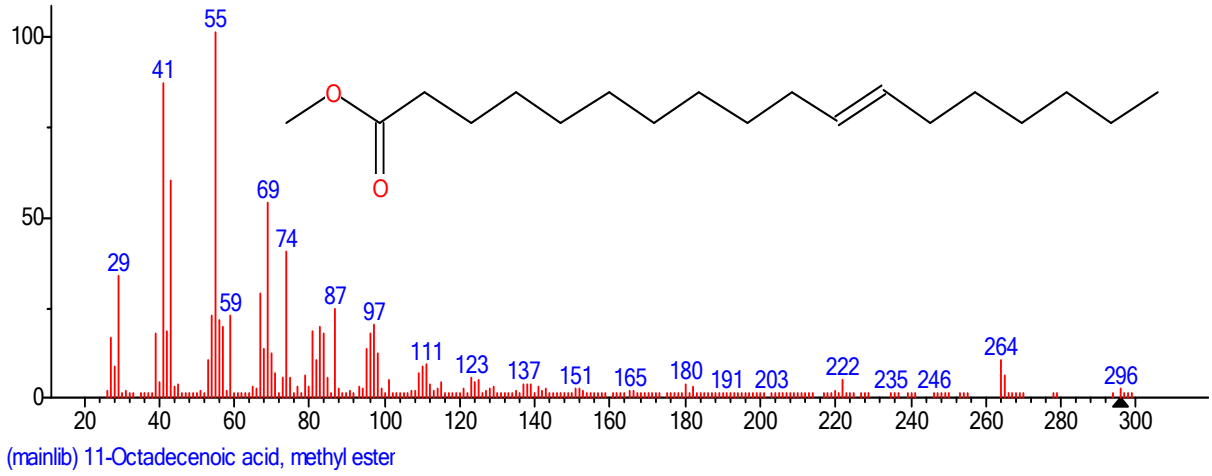

Figure 6. Mass spectrum of peak 11- Octadecenoic acid, methyl ester a plot relative abundance versus mass of charge ratio.

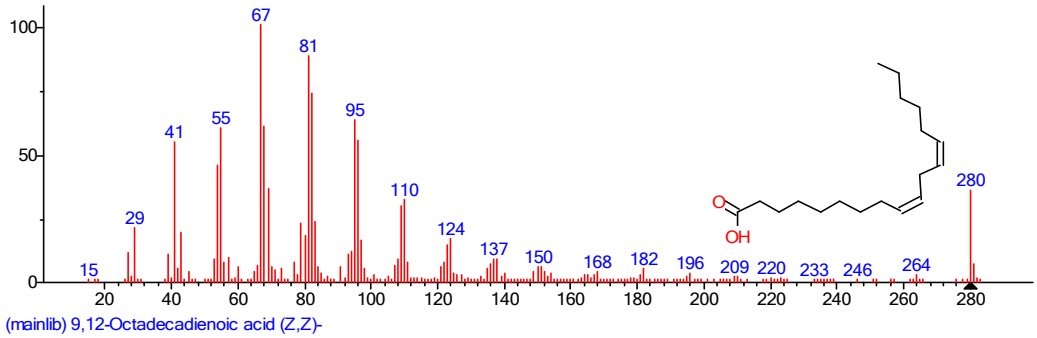

Figure 7. Mass spectrum of peak 9,12 - octadecadienoic acid, (Z, Z)- a plot relative abundance versus mass of charge ratio.

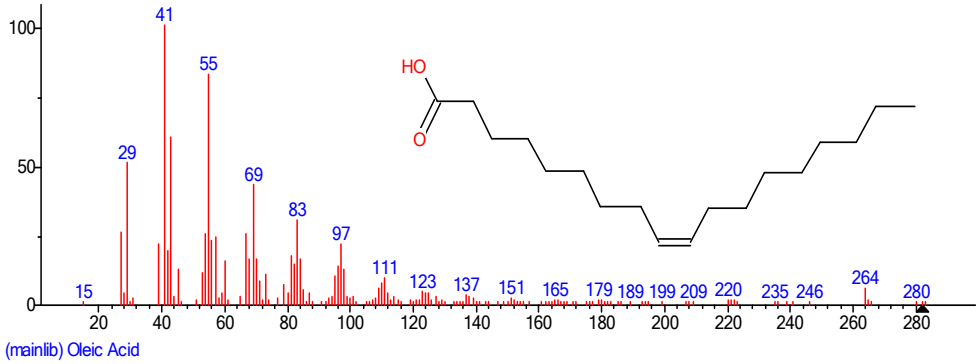

Figure 8. Mass spectrum of peak Oleic acid, a plot relative abundance versus mass of charge ratio.

Discussion

Mushrooms have long been recognized as a type of fungi with high nutritional value [15]. They contain significant amounts of dietary fiber and polysaccharides, which positively affect the gut microbiota. This is considered beneficial for various therapeutic functions such as managing obesity and having immunomodulatory properties [11,16]. Prebiotics are agents that can selectively stimulate the growth of beneficial microorganisms (probiotics). Mushrooms have been reported as important sources of prebiotics [17]. *Lentinus squarrosulus* is a type of wild mushroom that is edible and fibre-rich. It has the potential to be a prebiotic, which means it can enhance the growth of beneficial bacteria in the gut. However, it is important to investigate whether the extract of *L. squarrosulus* can promote the growth of harmful bacteria that can harm the host. Therefore, this study reports the prebiotic potential of *L. squarrosulus* extract and confirms that it has no adverse effects on the gut microbiome.

Findings from this study showed that extraction with water yielded a higher yield than extraction with organic solvents. The high percentage yield of the aqueous extract may be due to its ability to dissolve more components, which could be attributed to the different solubility properties of compounds in the mushroom. Growth-enhancing effect on beneficial bacteria is a major property of prebiotics. In this study, a significant increase in the growth of the two Lactobacillus strains was observed when cultured in *L. squarrosulus-supplemented* media. A higher concentration of *L. squarrosulus* extracts showed a higher growth stimulation effect. There have been few studies on the prebiotic activity of mushroom and their polysaccharide constituents [17]. A study reported that polysaccharide components of mushrooms can act as prebiotics, thus enhancing the growth of probiotics [17].

Both the methanol-chloroform and aqueous extracts of *L. squarrosulus* have good prebiotic functions. However, the growth stimulation effect on the lactobacillus species was higher in the aqueous extract than in the organic extract, and this beneficial effect is comparable to that of inulin, a commercially available prebiotic, in this study. Higher activity of the aqueous extract might be a result of the extraction of more polar constituents in the mushroom. This could be due to the presence of polysaccharides and other components in the aqueous extract. Furthermore, a recent small study in

humans revealed that both *L. squarrosulus* and its resistant hydrolysate promoted the growth of selected probiotic bacteria, especially Bifidobacterium strains. Evaluating the fecal microbiota of the volunteers, the resistant hydroxylate stimulated their fecal microbiota by decreasing the ratio of Firmicutes to Bacteroidetes, ranging from 1.3 to 8.2 times [18].

The supernatant of probiotics cultured in *L. squarrosulus* extract exhibited a varying degree of inhibition against the growth of pathogenic organisms, including *E. coli, S. typhi,* and *S. aureus*. Antimicrobial activity of *L. squarrosulus* has been previously reported [19,20]. The testing of the inhibitory potential of the supernatant from cultured probiotics in media supplemented with *L. squarrosulus* extracts against pathogenic microorganisms is novel. The inhibitory potential of the supernatant cannot be attributed to the effect of the mushroom alone. This is because the previously reported antimicrobial activity of *L. squarrosulus* in the literature was moderate [21]. The high inhibitory activity reported in this study might be a combination of the effect of the secretion from the lactobacillus species (probiotics) in culture and the mushroom extract. Ayimbila and coworkers reported that fermentation of resistant hydrosylate from *L. squarrosulus e*nriched the colonic content of branch-chain fatty acids (BCFA) and short-chain fatty acids (SCFA). Likewise, propionic and butyric acids, as well as acetic and butyric acids, were abundant in the volunteers [18]. In addition, Lactobacillus spp. have been shown to produce SCFA such as acetic, propionic, and butyric acids in cultures [22]. A previous report on the *in vitro* antimicrobial activity of SCFAs revealed significant suppression of bacterial growth and downregulation of virulence gene expression at higher concentrations in the colon [23]. Thus, the antimicrobial effect of the supernatant from lactobacillus species cultured in media supplemented with mushroom extracts or inulin resulted in an 18-55% increase in zone of inhibition compared to the supernatant from extract-free culture media.

The presence of bioactive compounds, such as fatty acids, polyunsaturated fatty acids, fatty acid esters, and long-chain unsaturated aldehydes, which have been reported to possess antioxidant, antitumor, and antimicrobial activities, among others, reveals L. squarolus as an excellent source of these bioactive compounds [24,25].

Conclusion

This study shows that *Lentinus squarrosulus* has good prebiotic functions. The combination of probiotics and extracts produced remarkable antimicrobial activity. These observed properties suggest that *L. squarrosulus* might have a positive impact on gut microbiota. *L. squarrosulus* appears to be a good candidate for the development of functional foods or supplements with enhanced prebiotic and antimicrobial value. This study presents *L. squarrosulus* as a promising functional product that can improve health and prevent diseases by enhancing gut health.

Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

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References

1. Dai L, Gu Y, Xu J, Guo J, Jiang K, Zhou X, et al. Toward green production of xylooligosaccharides and glucose from sorghum straw biowaste by sequential acidic and enzymatic hydrolysis. Ind Crops Prod. 2022;179:114662.
2. Lian Z, Zhang Q, Xu Y, Zhou X, Jiang K. Biorefinery cascade processing for converting corncob to xylooligosaccharides and glucose by maleic acid pretreatment. Appl Biochem Biotechnol. 2022;194(10):4946-58.
3. Yan B, Huang C, Lai C, Ling Z, Yong Q. Production of prebiotic xylooligosaccharides from industrial-derived xylan residue by organic acid treatment. Carbohydr Polym. 2022;292:119641.

4. Wang X, Zhang P, Zhang X. Probiotics Regulate Gut Microbiota: an effective method to improve immunity. Molecules. 2021;26(19):6076.
5. You S, Ma Y, Yan B, Pei W, Wu Q, Ding C, et al. The promotion mechanism of prebiotics for probiotics: A review. Front Nutr. 2022;9:1000517.
6. Yin X, Cai T, Liu C, Ma Y, Hu J, Jiang J, et al. A novel solvothermal biorefinery for production of lignocellulosic xylooligosaccharides, fermentable sugars and lignin nanoparticles in biphasic system. Carbohydr Polym. 2022;295:119901.
7. Gu J, Thomas-Ahner JM, Riedl KM, Bailey MT, Vodovotz Y, Schwartz SJ, et al. Dietary black raspberries impact the colonic microbiome and phytochemical metabolites in mice. Mol Nutr Food Res. 2019;63(8):e1800636.
8. Kumari K. Mushrooms as source of dietary fiber and its medicinal value: A review article. J. Pharmacogn. Phytochem. 2020;9(2):2075-8.
9. Ma G, Du H, Hu Q, Yang W, Pei F, Xiao H. Health benefits of edible mushroom polysaccharides and associated gut microbiota regulation. Crit Rev Food Sci Nutr. 2022;62(24):6646-3.
10. Mensah DLN, Mary O. Morphological characteristics of mycelia growth of two strains of the indigenous medicinal mushroom, Lentinus squarrosulus Mont. (Singer), on solid media. AJAR. 2014;9(23):1753-60.
11. Abiodun OO, Alege AM, Ezurike PU, Nkumah A, Adelowo O, Oke TA. Lentinus squarrosulus Mont. mushroom: Molecular identification, in vitro anti-diabetic, anti-obesity, and cytotoxicity assessment. Turk J Pharm Sci. 2022;19(6):642-8.
12. Ayimbila F, Keawsompong S. Functional composition and antioxidant property of crude polysaccharides from the fruiting bodies of Lentinus squarrosulus. 3 Biotech. 2021;11(1):7.
13. Siragusa S, Cagno RD, Ercolini D, Minervini F, Gobbetti M, Angelis MD. Taxonomic structure and monitoring of the dominant population of lactic acid bacteria during wheat flour sourdough type1 propagation using Lactobacillus sanfranciscensis starters. Appl Environ Microbiol. 2009;75(4):1099-109.
14. Rousseau V, Lepargneur JP, Roques C, Remaud SM, Paul F. Prebiotic effects of oligosaccharides on selected vaginal lactobacillus and pathogenic microorganisms. Anaerobe. 2005;11(3):145-53.
15. Hamza A, Mylarapu A, Krishna KV, Kumar DS. An insight into the nutritional and medicinal value of edible mushrooms: A natural treasury for human health. J Biotechnol. 2024;381:86-99.
16. Zhao J, Hu Y, Qian C, Hussain M, Liu S, Zhang A, et al. The Interaction between Mushroom Polysaccharides and Gut Microbiota and their Effect on Human Health: A Review. Biology (Basel). 2023;12(1):122.
17. Nowak R, Nowacka-Jechalke N, Juda M, Malm A. The preliminary study of prebiotic potential of Polish wild mushroom polysaccharides: The stimulation effect on Lactobacillus strains growth. Eur J Nutr. 2018;57(4):1511-21.
18. Ayimbila F, Siriwong S, Nakphaichit M, Keawsompong S. In vitro gastrointestinal digestion of Lentinus squarrosulus powder and impact on human fecal microbiota. Sci Rep. 2022;12(1):2655.
19. Ugbogu EA, Akubugwo IE, Ude VC, Gilbert J, Ekeanyanwu B. Toxicological evaluation of phytochemical characterized aqueous extract of wild dried Lentinus squarrosulus (Mont.) mushroom in rats. Toxicol Res. 2019;35(2):181-90.
20. Roy D R, Kandagalla S, MK. Exploring the ethnomycological potential of Lentinus squarrosulus Mont. through GC–MS and chemoinformatics tools. Mycology. 2019;11(1):78-89.
21. Sutthisa W, Chaiyacham P. Antibacterial activity of ethanolic extracts of Lentinous squarrosulus Mont. against human pathogenic bacteria. J Pure Appl Microbiol. 2022.
22. Kang CH, Kim JS, Park HM, Kim S, Paek N. Antioxidant activity and short-chainfatty acid production of lactic acid bacteria isolated from Korean individuals and fermented foods. 3 Biotech. 2021;11(5):217.
23. Kadry AA, El-Antrawy MA, El-Ganiny AM. Impact of short chain fatty acids (SCFAs) on antimicrobial activity of new β-lactam/β-lactamase inhibitor combinations and on virulence of Escherichia coli isolates. J Antibiot. (Tokyo). 2023;76(4):225-35.
24. Adeoye-Isijola MO, Olajuyigbe OO, Jonathan SG, Coopoosamy RM. Bioactive compounds in ethanol extract of Lentinus squarrosulus Mont - a Nigerian medicinal macrofungus. African Journal of Traditional, Complementary and Alternative Medicines. 2018;15(2):42-50.
25. Shegute T, Wasihun Y. Antibacterial activity and phytochemical components of leaf extracts of agave Americana. J Exp Pharmacol. 2020;12:447-54.

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