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# ANTI-INFLAMMATORY AND PREBIOTIC POTENTIAL OF AGARICUS BISPORUS POLYSACCHARIDES AND THEIR APPLICATION IN SYNBIOTIC ALGINATE MICROCAPSULES

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**Short running title:** *A. bisporus* as an anti-inflammatory and prebiotic agent

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**ABSTRACT**
Edible mushrooms are widely cultivated across the world and have been part of the human diet since ancient times due to their culinary and medicinal properties. Polysaccharides are reported to be the most potent mushroom-derived components with several health benefits. The present investigation was carried out to analyze the anti-inflammatory, prebiotic, and synbiotic potential of polysaccharides isolated from the fruiting bodies of A. bisporus. Polysaccharides (PS) from this mushroom were isolated by hot water extraction, ethanol precipitation, deproteinization, dialysis, and lyophilization. Anti-inflammatory activity was studied by Cyclooxygenase (COX) inhibition assay, and PS showed a significant reduction in COX expression by 55.05%, at a dose of 100µg/ml. Prebiotic potential was studied by probiotic growth stimulation study using Lactobacilli species isolated from curd sample. The isolated Lactobacilli were identified by microscopic, cultural, and biochemical analysis. PS promoted the growth of Lactobacilli in a dose-dependent manner when added to the culture media. The synbiotic potential was studied by incorporating the PS in Sodium alginate microcapsules along with Lactobacilli species, and the drug considerably increased the viability of probiotic species compared to Control group. The present investigation indicates that A. bisporus is a viable choice for developing nutraceuticals with anti-inflammatory, prebiotic, and symbiotic potential.

## Introduction

The human microbiota, contributing over 150 times more genetic information than the entire human genome, plays a significant role in human health and is referred to as the "hidden organ' of our body. Among them, the gut microbiota is most important, contributing to various functions such as resistance to pathogens, immune modulation, vitamin production, and food fermentation, thereby helping maintain body homeostasis (Hillman et al., 2017). Mounting research in this field over the past few decades has confirmed the association of gut microbiota in the development of ailments, including diabetes, carcinoma, cardiovascular diseases, inflammatory bowel disease, neurological

disorders, and chronic kidney and liver diseases. Thus, maintaining a healthy gut microbiota is vital for disease prevention and treatment (Hou et al., 2022). Probiotics, prebiotics, and synbiotics (a combination of probiotics and prebiotics) can manipulate the gut microbiota, leading to increased immunity, reduced inflammation, and decreased pathogen invasion and colonization (El-Maradny et al., 2025).

Probiotics are bacteria with beneficial health benefits if taken in proper amounts. They are rich in curd and yogurt, and are administered as food supplements to balance the good gut microbiota. Food components that can act as substrates for probiotic bacteria and travel to the gut unaffected by the digestive enzymes until they are acted upon by the intestinal microorganisms are known as prebiotics. *Bifidobacteria* and *Lactobacilli* species are the major probiotic bacteria in the human intestine influenced by the administration of prebiotics. *Bifidobacteria* are involved in stimulating immunity, restoring normal microflora after antibiotic treatment, Vitamin B production, etc., whereas *Lactobacilli* metabolize carbohydrates to lactic acid, promote lactose digestion in lactose-tolerant patients, and prevent irritable bowel syndrome, inflammatory bowel, diarrhoea, constipation, etc. *Lactobacilli* are the most widely studied probiotic bacteria, and include several useful species like *L. casei, L. rhamnosus, L. acidophilus, L. reuteri, L. fermentum, L. gasseri, L. brevis, L. plantarum, L. delbrueckii, L. johnsonii, L. paracasei, L. helveticus,* etc (Dempsey & Corr, 2022). Extensive research is being carried out on harnessing the *Lactobacillus* strains to improve gut health, as the incidence of colon cancer is increasing. Chronic inflammatory conditions like inflammatory bowel diseases (IBD), ulcerative colitis, Crohn's disease etc., act as predisposing factors for colon cancer. These conditions lead to the development of abnormal cells, which become cancerous over time. Multiple strains of *Lactobacilli* have been reported to improve the natural defense of the host through strengthening the immune, epithelial, and mucus barriers. They play a major role in relieving IBD-related conditions, including colitis-associated colorectal cancer. They promote the production of anti-inflammatory mediators, meanwhile suppressing the key pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, etc. Immune signaling pathways are known to be modulated by *Lactobacilli,* leading to the inhibition of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and thus preventing inflammation (Li et al., 2023; Badpaarva and Kheirandish, 2020).

lushrooms are a less explored source of potential prebiotics. They are rich in non-digestible polysaccharides like glucan, chitin, heteropolysaccharides, etc., which are mainly responsible for their prebiotic potential. Glucans, mannans, lentinan, pleuran, schizophyllan, xylans, galactans, chitin, and hemicellulose are some mushroom-derived polysaccharides with a proven record of prebiotic properties. Lentinan and pleuran are most commonly used mushroom glucans with prebiotic potential, which exert remarkable effects on the intestine. Many mushroom derived polysaccharides have been reported to prevent intestinal ulcers in rats, strengthen the intestinal mucosa and thus reduce susceptibility to inflammation and Inflammatory Bowel Disease (Song et al., 2025).

licroencapsulation is an advanced technology now widely used in the food industry for functional food synthesis. In this technique, probiotic bacteria are encapsulated in a protective material. This process considerably increases the viability of probiotic bacteria during challenging conditions like fermentation, processing, and storage. Moreover, this protective layer helps the probiotic organisms to survive the gastric acids and thus reach the small intestine safely and colonize. Microencapsulation increases the stability of bioactive components and ensures their controlled release in the target site (Arkadiusz et al., 2025). Co-encapsulation of probiotics with prebiotics is more attractive, as it can significantly enhance their viability both inside and outside the human body, and improve their shelf-life during storage. Synthesis of synbionts, consisting of probiotic bacteria co-capsulated with prebiotic mushroom polysaccharides, has been reported to be effective in increasing the probiotic cell viability and shelf life (Michalska et al., 2025).

*garicus bisporus,* commonly called the button mushroom, is an edible basidiomycete with therapeutic potential. It is one of the widely cultivated and consumed mushrooms, rich in nutrients like polysaccharides, proteins, lipids,

minerals, fibers, terpenoids, lectins, vitamins, glycoproteins, fatty acids, and their derivatives. They have been reported to possess medicinal properties like anti-oxidant, anti-inflammatory, antibacterial, anticancer, antidiabetic, hepatoprotective, antihypertensive, and antihypercholesterolemic properties (Usman *et al.*, 2021). The present investigation was conducted to analyze the prebiotic and anti-inflammatory properties of polysaccharides isolated from the fruiting bodies of *Agaricus bisporus.* The study further examined the synbiotic capsule synthesis using probiotic bacteria *Lactobacilli* and the mushroom-derived prebiotic polysaccharide to evaluate the ability of *Agaricus bisporus* to enhance the viability and shelf life of the *Lactobacilli* species.

## 2. Materials and Methods

Fruiting bodies of *Agaricus bisporus* were purchased from the authorised mushroom cultivators in Thrissur, Kerala, India. The specimen was identified as *Agaricus bisporus* at the Department of Botany, St Mary's College (Autonomous), Thrissur, Kerala, India.

### 2.1. Isolation of polysaccharides (PS) from the aqueous extract of *Agaricus bisporus*

The fruiting bodies were cleaned, cut into small pieces, dried, and ground into powder. 200 g of the mushroom powder was defatted in a Soxhlet apparatus, using Petroleum ether for 7-8 hours. Then, it was subjected to hot water extraction at 100°C in a water bath for 7-8 hours, filtered using Whatman no. 1 filter paper, and concentrated to half the volume. The concentrated aqueous solution was precipitated with 5X volume of chilled ethanol and kept in the refrigerator at 4 $^0$C for 48 hours. It was then centrifuged for 20 minutes at 15000 rpm. The precipitate thus received was deproteinized by the Sevag method, dialyzed, lyophilized, and kept at 4 $^o$C until further use. Qualitative and quantitative estimation of polysaccharides (PS) isolated was done by the Anthrone method (Yemm & Wills, 1954), using glucose as the standard.

### 2.2. Anti-Inflammatory activity of PS of *Agaricus bisporus*

The RAW 264.7 (macrophage) cell line purchased from the National Center for Cell Sciences (NCCS), Pune, India, was maintained in Dulbecco's modified Eagle medium DMEM (Sigma Aldrich, USA). The cell line was cultivated in a 25 cm$^2$ tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany), and the antibiotic solution containing: Penicillin (100 U/ml), Streptomycin (100 µg/ml). ml) and Amphotericin B (2.5 ug/ml). Cultured cell lines were maintained at 37 °C in a humidified 5% CO2 incubator (NBS Eppendorf, Germany). Cells were grown to 60% confluence, activated with 1 µl lipopolysaccharide (LPS: 1 µg/ml). LPS-stimulated cells were exposed to different concentrations of PS (25, 50, 100 µg/ml) and incubated for 24 h. After incubation, the anti-inflammatory assay was performed using the cell lysate.

#### 2.2.1. Cyclooxygenase (COX) activity

100 µL of cell lysate was incubated with Tris-HCl buffer (pH 8), glutathione 5 mM/L, and hemoglobin 5 mM/L for 1 min at 25°C. The reaction was initiated by the addition of arachidonic acid (200 mM/L) and terminated after 20 min of incubation at 37°C by the addition of 200 µL of 10% trichloroacetic acid in 1 N hydrochloric acid. After centrifugation and addition of 200 µL of 1% thiobarbiturate, the tubes were boiled for 20 min. After cooling, the tubes were centrifuged for three minutes, and COX activity was determined by reading the absorbance at 632 nm. Diclofenac was used as the standard. The percentage inhibition of enzyme was calculated as,

% inhibition= ((Absorbance of Control-Absorbance of test) / Absorbance of Control) ×100

### 2.3. Prebiotic potential of *A. bisporus*

#### 2.3.1. Isolation of Probiotic *Lactobacillus spp.*

Probiotic *Lactobacilli* species were isolated from the curd sample through serial dilution

and the spread plate technique. The inoculated Nutrient Agar plates were incubated at 37 $^0$C for 24-48 hours. After incubation, individual colonies suspected as *Lactobacillus* species were selected and purified by streaking on the selective media de Man Rogosa Sharpe Agar (MRS) plates. The inoculated MRS plates were incubated for 24-48 hours at 37 °C.

**2.3.2. Identification of *Lactobacillus spp.***

Morphological analysis and standard biochemical tests were performed to identify the isolated microorganisms. Gram staining, motility test using Semi-solid agar (agar conc. of 0.5%), standard biochemical assays including Indole, Methyl Red, Voges-Proskauer, Citrate Utilisation (IMViC), Catalase, and Oxidase tests were conducted. These tests were performed following the standard laboratory procedures.

**2.3.3. Confirmation of *Lactobacillus spp.* by Salt Tolerance Test**

The test was conducted to determine the tolerance of the isolated organism to high salinity. To determine salt tolerance, MRS broth supplemented with different concentrations of NaCl (1–8%) was prepared and sterilized at 121°C for 15 minutes. The overnight culture of the test organism (5 µl) was inoculated into all test tubes and incubated at 37 °C for 24 h. After incubation, the optical density of the culture was measured spectrophotometrically at 600 nm and compared with the Control.

**2.3.4. Probiotic growth stimulation by PS of *A. bisporus***

100 µl of 3.0 × $10^8$ Cells/mL of *Lactobacilli* culture was added to the tubes containing 5ml of sterile MRS broth. Different concentrations of PS (100 µg, 250 µg, 500 µg, 750 µg and 1000 µg) were added to each tube of *Lactobacilli* inoculated MRS broth. One tube without PS served as the Control. The tubes were incubated at 37 °C for 24 h, and after incubation, the optical density was measured at 600 nm using a UV-Spectrophotometer.

**2.4. Synthesis of Synbiotic Microcapsules**

The isolated probiotic *Lactobacilli* were cultivated in 100 ml of sterile MRS broth and incubated at 37 °C for 48 h. After incubation, the culture was centrifuged at 6300 rpm for 10 minutes at 4°C. The supernatant was discarded, and the cell pellet was resuspended in sterile normal saline.

Synbiotic microcapsules were prepared using Calcium chloride and Sodium alginate. Calcium chloride (4%) was prepared, and pH was adjusted to 6 using glacial acetic acid. The prepared Calcium chloride solution was cooled in the refrigerator for 1 hour. 3% Sodium alginate was prepared by adding 3g of Sodium alginate in 100 ml of distilled water and stirring at 100 °C in a water bath until a clear solution was obtained. Synbiotic microcapsules were prepared by mixing a bacterial suspension: *Agaricus bisporus*: Sodium alginate in a ratio of 1:1:10. A control sample was also prepared without the addition of polysaccharide. The mixture was added dropwise to the Calcium chloride solution for microcapsule formation and left in the solution for 30 min for hardening. Beads were separated using a sieve, washed twice with saline, and stored in air-tight containers in the refrigerator. Microcapsules prepared without PS were taken as the Control (Moumita et al., 2017).

**2.4.1. Effect of PS on viability of *Lactobacilli* in Synbiotic Microcapsules**

The symbiotic microcapsules prepared were stored in separate air-tight containers at 4$^o$ C for 7 days. The viability of *Lactobacilli* in microcapsules was analysed on days 1 to 7. The synbiotic microcapsules (0.5 g) were dissolved in 10 ml of tri-sodium citrate (3%) solution. 100 µl of the dissolved solution was spread on MRS agar plates, incubated at 37°C for 48 hours, and the colony-forming units per ml (CFU/ml) were calculated (Sahoo et al., 2018).

CFU/ml = (Number of colonies X Dilution factor) / Volume of sample plated

**2.5. Statistical Analysis**

All data presented in this study are from triplicate analysis, and the values are expressed as Mean ± SD.

## 3. Results and Discussion

Fruiting bodies(1kg) of *A.bisporus* were sliced, dried in the shade for 2-3 days, and powdered using a mixer grinder. From 1kg of *A.bisporus* fruiting body, 56.67g of dry powder was obtained (Fig. 1 &2).

**Fig. 1.** Fresh and sliced fruiting bodies of *A.bisporus*

**Fig. 2.** Dried and powdered fruiting bodies of *A. bisporus*

### 3.1. Isolation of polysaccharides (PS) from the aqueous extract of *Agaricus bisporus*

Carbohydrates form one of the major components present in mushrooms, and usually, 35-70% of their dry weight consists of carbohydrates. These carbohydrates are mainly the polysaccharides, like β or α-glucans, chitins, glycogens, monosaccharides, or disaccharides (Sławińska et al., 2020; Maity et al., 2021). The present investigation was focused on the isolation and quantitative as well as qualitative estimation of polysaccharides from *Agaricus bisporus.* The dried powder of *A. bisporus* was defatted using petroleum ether. Polysaccharides present in *A. bisporus* were isolated by hot water extraction, filtration, ethanol precipitation, deproteinization, dialysis, and freeze-drying. The yield of crude polysaccharides from the dried and powdered fruiting bodies was 4.56 % (4.56g/100g). Polysaccharides present in the obtained material were analysed quantitatively and qualitatively by the Anthrone method. On both quantitative and qualitative analysis, the isolated compound produced a bluish green color, characteristic of polysaccharides. In this study, the quantitative estimation showed the presence of 20.32 % of polysaccharides in *A. bisporus*. ie, 203.21 mg of polysaccharide was present in 1 gram of the dry powder of *A.bisporus.* This value coincides with the analysis of Khalil and Lukasiewicz (2024), where the quantity of PS from *A. bisporus* is reported to fluctuate between 122.10 and 208.24 milligrams per gram dry weight of the mushroom. The authors have also remarked that the variations in the carbohydrate yield are dependent on many parameters, such as extraction time, temperature, and liquid/solid ratio.

The most convenient method for carbohydrate isolation from the mushrooms is hot water extraction (HWE) and has been the most widely used separation technique in laboratories and industries (Drakhshan et al., 2024). Other methods, including enzymatic, acid/ alkali, ultrasound, or microwave extraction methods, could be employed for obtaining polysaccharides from mushrooms. However, these methods demand high economic and energy input and should be followed by purification to remove the unnecessary byproducts, such as enzymes, salts, etc. The major advantage of

the HWE method is that the resulting extract can be directly used for food production without additional purification procedures, and it is a simple method without high economic or energy inputs (Khalil and Lukasiewicz, 2024).

### 3.2. Anti-inflammatory activity of *A.bisporus* polysaccharide

Anti-inflammatory activity of *A.bisporus* polysaccharide was tested by the cyclooxygenase (COX) inhibition activity. The PS of *A.bisporus* showed anti-inflammatory activity in a dose-related manner, and at a concentration of 50µg and 100µg, the drug showed 39.66 and 56.46% of COX inhibition, respectively (Fig. 3).

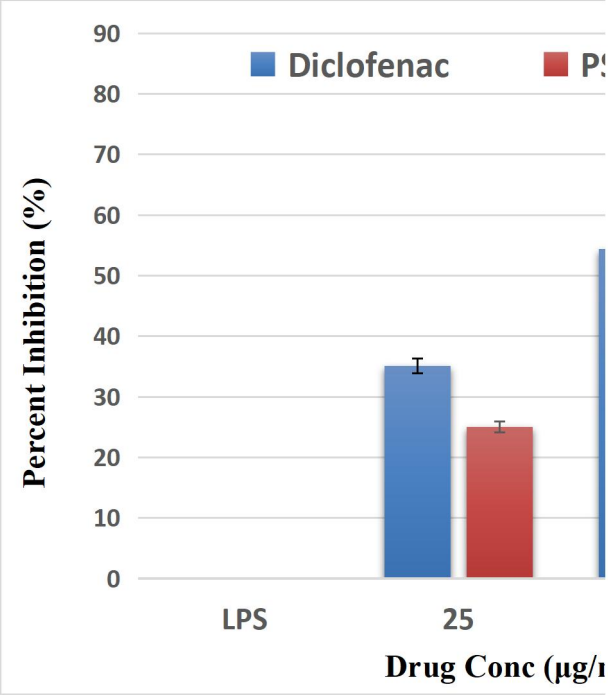



**Fig. 3.** COX inhibition assay of PS from *A. bisporus.* Values are ± SD, n =3.

In inflammatory pathways, COX-2 is a major mediator, and an elevation in its expression is associated with several malignancy conditions in humans. The overexpression and upregulation of COX-2 contribute to the physiological conditions, including inflammation, increased cell growth, and uncontrolled cell proliferation, apoptosis, metastasis, angiogenesis, etc., adversely affecting the body's homeostasis and ultimately leading to cancer (Gandhi et al., 2017). In vitro studies show that the extracts of *A. bisporus* could reduce the expression of COX-2, prostaglandin F2α receptor, and IL-6 in the Caco-2 colon epithelial cells activated with lipopolysaccharide (LPS) and tumor necrosis factor (TNF)-α (Muszynska et al., 2018). Anti-inflammatory and analgesic properties have been reported to be possessed by the polysaccharides isolated from *A.bisporus,* like fucogalactan, by inhibition of total cell migration and peritoneal capillary permeability (Komura et al., 2010). Ruthes et al., (2013) also reported the anti-inflammatory potential of fucogalactan, a polysaccharide isolated from *A.bisporus,* due to its ability to downregulate the expression of proinflammatory enzymes like iNOS and COX-2.

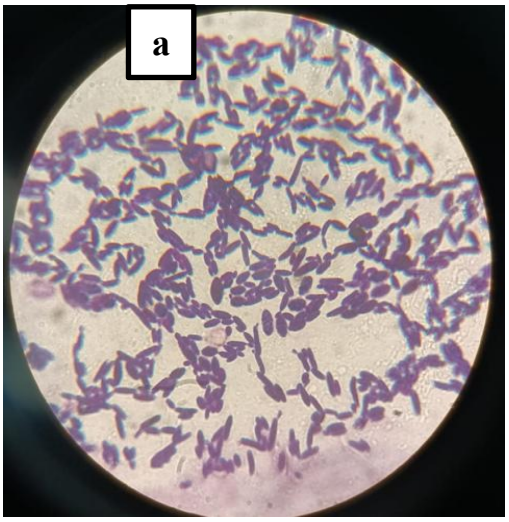
### 3.3. Prebiotic potential of *A. bisporus*

#### 3.3.1. Isolation and identification of Probiotic Bacteria

On incubation at 37°C for 24-48 hours, individual bacterial colonies were formed on the Nutrient agar plates inoculated with serially diluted curd samples. Small, circular, convex, opaque, smooth colonies with an entire margin, milky white in color, and no pigment production, which are characteristic features of *Lactobacilli,* were subcultured onto the MRS agar, the selective media for *Lactobacilli spp.* (Fig. 4). On MRS agar, pin-headed, round, shiny colonies, milky-white to cream-colored, were formed, confirming the isolated organisms as *Lactobacillus* species. Further, these colonies were pure cultured on MRS agar for morphological and biochemical analysis. On Gram staining, the isolated organisms appeared as Gram-positive bacilli in chains and as single cells. In the motility test using semi-solid agar, bacterial growth was only along the line of stab inoculation, indicating that the isolated organism is non-motile, another characteristic feature of *Lactobacilli* (Fig. 5). In the Standard Biochemical tests performed, the isolates showed negative

results in IMViC, Catalase, and Oxidase tests. The microscopic study, colony morphology, motility test, and biochemical results revealed the features characteristic of *Lactobacillus* species, indicating that the isolated organisms from the curd are probiotic *Lactobacillus.*

**Fig. 4.** Growth of *Lactobacilli* on the selective media MRS agar



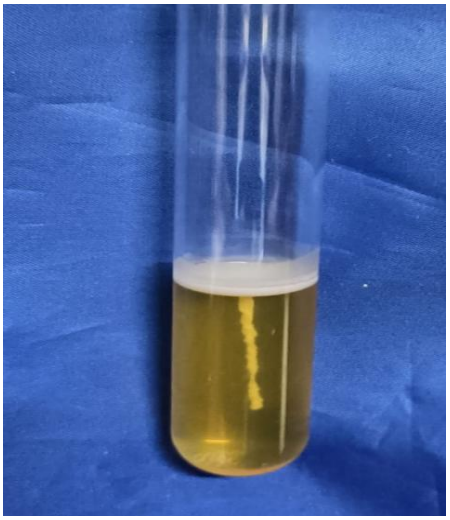



**Fig. 5.** (a) Gram staining of *Lactobacilli* (b) Motility test using semisolid agar

**3.3.2. Salt Tolerance Test of *Lactobacillus***

Salt tolerance is one of the specific identification tests for *Lactobacillus* spp. Salt tolerance of *Lactobacillus* was checked by incorporating 1-8% NaCl in MRS broth, followed by incubation at 37°C for 24 hours. The OD after incubation showed that the isolated strain could tolerate NaCl up to a concentration of 1-6% and further increase in NaCl concentration considerably inhibited the growth of the organisms (Fig. 6). Up to 5% NaCl concentration, the isolates showed significant growth. NaCl has an inhibitory action on many bacteria. However, probiotic organisms must withstand high salt concentrations in the gut (Chen et al., 2022). Hence, the salt tolerance test not only confirms the organism as *Lactobacilli,* but it also shows the probiotic potential of the isolated organisms.

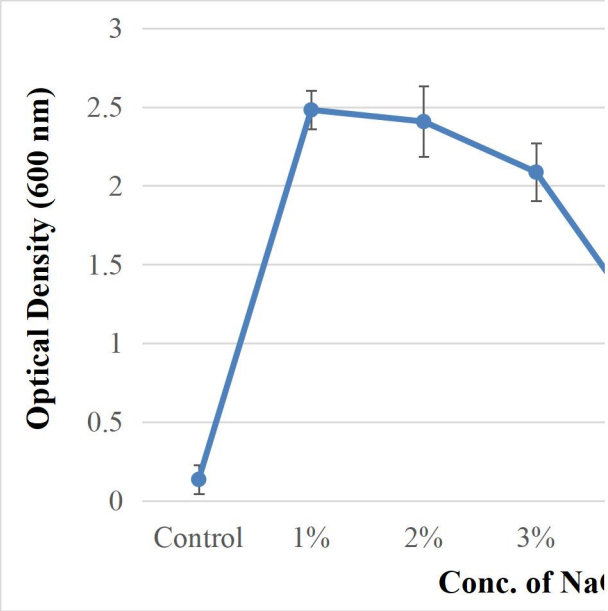



**Fig. 6.** Salt tolerance test of *Lactobacilli* species using different concentrations of NaCl. Values are ± SD, n =3.

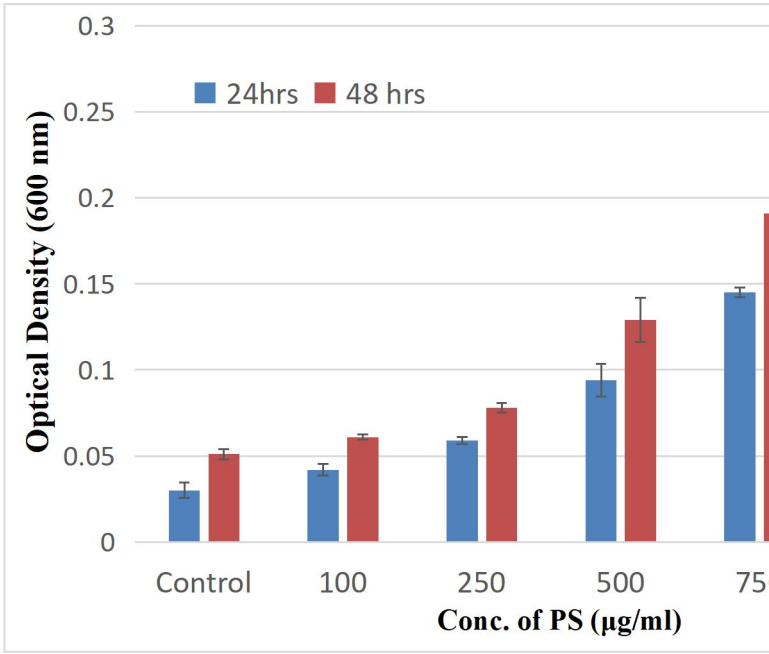



**Fig. 7.** Growth stimulation of *Lactobacilli* using different concentrations of PS. Values are ± SD, n =3.

### 3.3.3. Growth stimulation of Probiotic *Lactobacilli spp.* by PS of *A. bisporus*

Prebiotic potential of *A.bisporus* polysaccharide was studied by incorporating different concentrations of mushroom polysaccharides in MRS broth, followed by the addition of an equal volume(5µL) of an overnight culture of isolated *Lactobacillus spp.* All the inoculated tubes were incubated at 37°C for 24 hours, and the optical density was measured spectrophotometrically at 600nm. OD obtained after 24 hours showed an increase in the growth of probiotic *Lactobacillus,* and the higher growth was achieved with increasing concentrations of polysaccharides (Fig. 7). Results indicated that supplementation of MRS broth with PS of *A.bisporus* stimulated the growth of the probiotic *Lactobacilli,* indicating a positive impact of PS on the probiotic organisms.

### 3.3.4. Efficacy of PS of *A. bisporus* on viability of *Lactobacilli* in Synbiotic Microcapsules

Microcapsules were synthesized using cell pellets of *Lactobacillus*, *Agaricus bisporus* polysaccharide, and sodium alginate. The synthesized microcapsules were bead-shaped, white in colour, and were stored in the refrigerator. To determine the viability of *Lactobacillus* within the microcapsules, the samples were plated on MRS agar on days 1 and 7. The microcapsules containing *A.bisporus* polysaccharide and the control without the PS were cultured using the spread plate technique, followed by incubation at 37°C for 24 hours, and the CFU/ml was calculated. On day 1, both the control and *A.bisporus* polysaccharide-containing microcapsules showed colonies that were "Too Numerous to Count (TNTC)". On the consecutive days, the results showed that the viability of *Lactobacillus* was higher in microscapsules containing *A.bisporus* polysaccharide, with a CFU/ml of $2.72x10^2$ on day 7. In the control, CFU/ml was $1.96x10^2$, indicating lower viability of *Lactobacillus* compared to

the microcapsules containing PS of *A.bisporus* (Fig. 8). These results confirm the prebiotic potential of polysaccharides from *A. bisporus* and their usefulness in synthesizing synbiotic microcapsules.

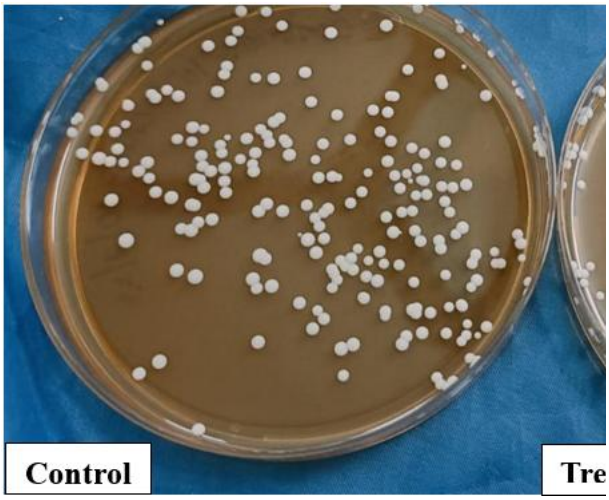



**Figure 8.** Viability of *Lactobacillus* from microcapsules on MRS agar plate on day 7.

The Sodium alginate encapsulation and the prebiotic potential of PS might have worked synergistically to enhance the viability of *Lactobacilli.* Microencapsulation has been reported to augment the viability of probiotics like *Bifidobacterium* and *Lactobacillus* species by up to 80 to 95% (Prakash et al., 2011). Microencapsulation with alginate, in the presence of prebiotics like oligofructose and inulin, improved the heat tolerance, gastrointestinal tolerance, as well as stability during the storage of probiotic bacteria (Fritzen-Freire et al., 2012).

Probiotic bacteria have enormous health benefits if they are delivered in the gut in the appropriate amounts. But their pharmaceutical applications are very limited, as they are highly vulnerable to the enzymes like lysozyme in the mouth, other digestive enzymes, and harsh conditions in the upper digestive tract, acidic pH of the stomach, bile salt, and oxidative stress. The development of a probiotic delivery system, such as encapsulation in protective materials like alginate, carrageenan, gum arabic, pectin, starch derivatives, gellan, xanthan, and animal proteins, is an attractive strategy to enhance the viability, stability, and bioavailability of probiotics during gastrointestinal transit as well as during food processing and storage. Alginate has been widely applied for this process due to its unique physicochemical properties, like simple structure, simple raw materials, ease of processing, minimum toxicity, and fast gel matrix formation around the probiotic organisms (Wang et al., 2022; Yash et al., 2023). Studies have shown that the probiotic organisms *Limosilactobacillus reuteri* and *Lactobacillus salivarius*, when encapsulated in a chitosan-coated alginate–inulin matrix, survive gastrointestinal transit better than free bacterial cells (Parsana et al., 2023).

The present investigation revealed the significant prebiotic potential of polysaccharides from *Agaricus bisporus* on probiotic *Lactobacilli* when added to the bacterial culture and incorporated into alginate microcapsules. These results agree with the existing reports on the prebiotic activity of *A. bisporus.* Fortification of yogurt with this mushroom has been shown to increase its nutritional value by elevation in protein, fibres, amino acids, ash, minerals, vitamins like riboflavin, niacin, B12, B1, biotin, folate etc. Fortification was also found to increase the total count of probiotic organisms *S. themophilus, L. delbrueckii spp. bulgaricus, L. acidophilus, Bifidobacterium spp*, which were added to the yogurt as starter cultures (Dardiry et al., 2015). In another study, the in vitro prebiotic activity of different concentrations of *A. bisporus* polysaccharides (0.25%, 0.5%, 1%, 2%) has been reported to promote the growth of *L. acidophilus, L. plantarum*, and *E. coli*. However, very high concentrations of polysaccharides showed an inhibitory effect on the proliferation of test organisms (Türsen et al.,2022). Both the promotion of probiotic bacterial growth and the inhibition of pathogenic bacteria are considered under the prebiotic potential of a drug under study. The addition of 5 mg/ml of PS extract of *A.bisporus* was reported to stimulate the growth of probiotic *lactobacilli* strains such

as *L. acidophilus, L. pentosus, L. plantarum, and L. paracasei.* Cell-free supernatants of these probiotic organisms enriched with the PSs also strongly inhibited the growth of pathogens, including *MRSA, S. aureus, E. coli, S. dysenteriae,* and most notably, *L. monocytogenes. Lactobacilli* exhibit antibacterial effects against pathogens through environmental acidification as well as bacteriocin production. The study suggests that by acting as a prebiotic, PS derived from mushrooms profoundly enhanced the growth of *LAB,* resulting in the production of higher amounts of antibacterial agents against pathogenic strains (El-Maradny et al., 2025).

## 4. Conclusion

*Agaricus bisporus,* commonly known as the button mushroom, is widely cultivated and consumed worldwide for its culinary properties and pharmaceutical value. Among the variable components present in it, polysaccharides are the most potent ingredient with multiple health benefits. In this study, we isolated the polysaccharides from the hot water extract of *A. bisporus* to analyze their anti-inflammatory and prebiotic potential. PS from *A. bisporus* showed significant anti-inflammatory activity by downregulating the expression of the pro-inflammatory mediator COX in the in vitro studies. In the prebiotic analysis, the PS showed promising activity in a dose-dependent manner. The compound significantly promoted the proliferation of probiotic *Lactobacilli* species when supplemented in the MRS culture broth. The PS also enhanced the viability of the *Lactobacilli* when incorporated into Sodium alginate microcapsules. Prebiotic components stimulate the growth of probiotic gut microflora, which has become a hot spot of research in promoting human health. Study suggests that the incorporation of this mushroom in the normal diet may lead to a healthy gut and prevent colonization of pathogens. As the drug also possesses anti-inflammatory potential, its administration would prevent the development of inflammatory conditions in the colon that may lead to carcinoma. Microencapsulation of probiotic bacteria with PS was also found to increase the survival and viability of the *Lactobacilli* and hence could be effectively used to deliver the probiotic bacteria at the target site without being destroyed by the harsh conditions of the GI tract. Such microcapsules can be added to the food items to promote the beneficial gut microbiota. This study demonstrates that the prebiotic, synbiotic, and anti-inflammatory potential of *A. bisporus* polysaccharides can be harnessed to modulate the gut microflora, thereby reducing inflammation, limiting pathogen colonization, and enhancing immunity. Thus, *A. bisporus* could be a valuable resource for developing nutraceuticals with multiple health benefits.

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