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Cheese Fermented with Human-Derived *Limosilactobacillus reuteri* DSM 17938 and Mushroom Powders: A Novel Psychobiotic Food with Enhanced Bioactivity and Sensory Acceptability

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Abstract: Fermented foods containing psychobiotics are of growing interest among food scientists. Human-derived *Limosilactobacillus reuteri* DSM 17938, a gut symbiont and potential psychobiotic strain, has been shown to exhibit the following health benefits: anti-inflammation and GABA-production capacity, as well as modulation of pathogen and cancer cell growth. The aim of this research was to develop an acid-coagulated fresh soft quark-type cheese, fermented with *L. reuteri* DSM 17938, with enhanced bioactivity, sensory acceptability, and overall likeability. Psychobiotic-containing cheeses represent the food of a new generation, so it is of great importance to gain the trust of the consumers. To develop a familiar taste, cheese samples were enriched with mushroom powders of *Agaricus bisporus* and *Pleurotus ostreatus*. A high abundance of lactic acid bacteria was maintained in all cheese samples (>log 7.64 CFU/mL), while cheese extracts exhibited cytotoxicity to colon cancer cell line HCT116 (up to 30.96%) in vitro. Additionally, cheese samples provided a favorable medium for the growth of the probiotic *Escherichia coli* Nissle 1917 (>log 7.11 CFU/mL). Sensory evaluation revealed high scores for all samples (up to 97.21% of maximum overall quality). The survey conducted in this study offered insights into consumer willingness to try products containing psychobiotics. This study demonstrates the potential for the successful development of fermented food products with *L. reuteri* DSM 17938, which exhibits all the desired traits that consumers may receive well. Further research is required to explore the potential health benefits of these innovative food products.

Keywords: *Limosilactobacillus reuteri* DSM 17938; acid-coagulated cheeses; psychobiotics; probiotic *Escherichia coli* Nissle 1917; mushroom powders; sensory evaluation; bioactivity; depression

1. Introduction

Over a century ago, Élie Metchnikoff brought beneficial lactic acid bacteria (LAB) into the scientific spotlight. He popularized the idea that LAB can prevent diseases and delay aging through the consumption of fermented dairy products [1]. Today, probiotics—defined as "living microorganisms that confer a health benefit to the host when administered in adequate amounts" [2]—are just one of several terms related to beneficial LAB, while other terms include postbiotics, paraprobiotics, synbiotics, prebiotics, probioceutics, and psychobiotics. Postbiotics refer to the metabolic products or substances secreted by probiotics that have a beneficial effect on the host's health [3], while paraprobiotics are inactivated or non-viable cells or cell extracts of probiotics that can provide health benefits to humans or animals when consumed in adequate amounts [4]. Synbiotics are a combination of probiotics and prebiotics [5,6], where prebiotics are substances that are selectively used by microorganisms to improve the host's health [4,7]. Probioceutics are derivatives of probiotics, such as reuterin from *Limosilactobacillus reuteri*, that can provide protection against a variety of pathogens [4,8]. Psychobiotics were originally defined as "live microorganisms that, when ingested in adequate amounts, produce health benefits in patients suffering from psychiatric illness". Nowadays, psychobiotics also include paraprobiotics, which have been shown to have a positive effect on markers of stress and immune function. They also include prebiotics, such as galacto-oligosaccharides and fructo-oligosaccharides, that increase the viability of probiotic bacteria that have been found to be an important component of the microbiota–gut–brain axis (MGB). Psychobiotics can regulate emotional behavior and are a useful therapeutic adjuvant in the treatment of anxiety and depression [1]. Psychobiotics exert their effects through various mechanisms, including influencing the synthesis of neurotransmitters, such as serotonin (5-hydroxytryptamine 5-HT) and γ-aminobutyric acid (GABA), modulating inflammatory cytokines, and enhancing the stress response through effects on stress hormones and the MGB axis [9].

According to existing evidence, *L. reuteri* can influence the behavior and stress response of the host, as well as the composition and function of the gut microbiota and the communication between the gut and the brain [10]. *L. reuteri* also has various benefits for brain health, such as modulating the immune system, producing vitamin B12, and enhancing antioxidant defense [11,12]. *L. reuteri* DSM 17938 is a human-derived strain that is widely used in different age groups, from infants [13] to elderly people [14]. We have previously shown that *L. reuteri* DSM 17938 can produce GABA, a neurotransmitter with calming effects [15]. Moreover, Brenner et al. [16] have demonstrated that *L. reuteri* DSM 17938 can lower the inflammatory-marker C-reactive protein and suggested that this strain can reduce stress-induced inflammation and its negative impact on psychological and neurological outcomes. Therefore, *L. reuteri* DSM 17938 can be considered a psychobiotic strain. Besides its GABA-producing and immunomodulatory properties, *L. reuteri* DSM 17938 has other mechanisms that may contribute to its psychobiotic effects. For example, *L. reuteri* DSM 17938 can produce reuterin, an antimicrobial compound that can suppress the growth of harmful bacteria in the gut, which may prevent gut imbalance and inflammation related to anxiety and depression [8]. In addition, *L. reuteri* DSM 17938 can alter the gut microbiota by increasing the abundance of beneficial bacteria, such as *Bifidobacterium,* and decreasing the abundance of harmful bacteria, such as *Clostridium*, which may increase the production of short-chain fatty acids and other neuroactive metabolites that affect brain function and mood [17,18]. Furthermore, *L. reuteri* can benefit the host immune system by reducing the expression of pro-inflammatory cytokines, such as TNF and IL-6, and increasing the expression of anti-inflammatory cytokines, such as IL-10 and TGF-β, which may have beneficial effects on brain inflammation and mood [19,20]. Finally, *L. reuteri* DSM 17938 can increase the levels of oxytocin, a neuropeptide involved in social bonding and stress regulation, in the plasma and hypothalamus of mice, which may improve social behavior and anxiety [17,21,22].

The presence of psychobiotics in food, whether naturally occurring or intentionally added, is an active area of research within the field of food science. Fermented foods such as kimchi, which contains the GABA-producing strain *Lentilactobacillus buchneri* MS, and dadih, a West Sumatran fermented buffalo milk containing *Lactiplantibacillus plantarum* Dad-13, are examples of foods containing psychobiotics [23,24]. Additionally, studies have shown that consuming kefir and yogurt with *Lactobacillus gasseri* SBT2055 and *Bifidobacterium longum* SBT2928 may decrease stress levels [25].

In this research, it was postulated that *L. reuteri* DSM 17938 could be easily integrated into a suitable food matrix and incorporated into a daily dietary regimen. Traditional Serbian acid-coagulated fresh soft quark-type cheese was selected as an appropriate carrier for LAB. As *L. reuteri* is recognized as a potential GABA-producing strain, it required a glutamate-rich environment to exhibit psychobiotic activity. Glutamate serves as a substrate for the bacterial enzyme glutamate decarboxylase, which converts it into GABA. Consequently, in this study, cheeses were fortified with powders of oyster mushrooms (*Pleurotus ostreatus* L.) and button mushrooms (*Agaricus bisporus* L.), which are natural sources of glutamate. Thus, the aim of this study was to create cheeses fermented with a psychobiotic strain with health-promoting potential and desirable sensory characteristics. Cheese samples were initially evaluated for bioactivity. The microbiological quality of the cheese, LAB, and *Escherichia* viability were assessed. Additionally, the cytotoxic effect of the prepared product on human HCT116 cells was examined. Subsequently, a sensory evaluation was conducted. Finally, a survey was conducted to estimate consumer interest in products containing psychobiotics and to determine whether there is a general demand for food containing psychobiotics to be appropriately labeled.

2. Materials and Methods

2.1. Materials

Pasteurized cow milk, 2.8% milk fat, and oyster- and button-mushroom powders were obtained from the local market. Flora Danica lyophilized starter culture was purchased from Chr. Hansen (Hørsholm, Denmark). *L. reuteri* and *Escherichia coli* strains 0157:H7, ATCC 35218, and Nissle 1917 were obtained from the Culture Collection of the Department of Microbiology, Faculty of Biology, University of Belgrade, Belgrade, Serbia. The animal rennet was purchased from the Clerici-Sacco Group (Cadorago, Italy). Human colon cancer cell line HCT116 (ATCC CCL-247) was obtained from the Oncology Institute of Vojvodina, Sremska Kamenica, Serbia. De Man–Rogosa–Sharpe broth (MRS broth) and MRS agar were obtained from Torlak (Belgrade, Serbia); Müller–Hinton broth (MHB) and eosin–methylene-blue (EMB) agar were obtained from HiMedia (Mumbai, India). Dulbecco's Modified Eagle's Medium (DMEM), phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), penicillin-streptomycin mixtures, trypsin from the porcine pancreas, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Production of Acid-Coagulated Cheese Samples at Laboratory-Scale Level

Six cheese variants—I-control cheese; II-cheese + *L. reuteri*; III-cheese + button-mushroom (*A. bisporus*) powder; IV-cheese + *L. reuteri* + button-mushroom (*A. bisporus*) powder; V-cheese + oyster-mushroom (*P. ostreatus*) powder; and VI-cheese + *L. reuteri* + oyster-mushroom (*P. ostreatus*) powder—were made with cow's milk (2.8% milk fat), as described Miloradovic et al. [26]. In short, mesophilic aromatic starter culture Flora Danica consists of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, and *Leuconostoc mesenteroides* subsp. *cremoris* (0.002% w/v). Animal rennet supplied by Caglificio Clerici (0.02 g per 10 L) and monosodium glutamate (MSG 1%) were added to both milk lots prior to fermentation. At the same time, *L. reuteri* DSM 17938 (log 8 CFU/mL) was added to only one batch. In that way, prepared milk was subjected to combined acid–rennet coagulation (18 h at 22–24 °C). After 18 h, cheese gel was carefully transferred to a rectangular mold with cotton cloth and pressed for 5 h by approximately 2 kg of weight per kg of cheese. The cheese gels were transferred from molds to plastic containers. Finally, 4.5 g of 0.8% NaCl was added to all cheese samples, while mushroom powders (2%) were separately added. All prepared cheese samples were stored at 4 °C for one day, and after that, used in further research.

2.3. In Situ LAB Viability

The amount of LAB was determined for freshly prepared cheese samples as described by Jovanović et al. [15]. The viability of the LAB was evaluated upon fermentation using the pour plate technique and serial dilutions in phosphate buffer saline (1% PBS). LAB were enumerated using MRS agar under microaerophilic incubation at 37 °C for 48 h. After the plates were counted, the results were presented as the log of the mean number of the colony-forming units (log CFU/mL).

2.4. In Situ *Escherichia coli* Viability

Escherichia viability was investigated as previously described by Jovanović et al. [15] with minor modification. *Escherichia* strains *E. coli* 0157:H7, *E. coli* ATCC 35218, and *E. coli* Nissle 1917 were incubated at 37 °C overnight in MHB. Cheese samples were inoculated with 8 log CFU/mL of *E. coli* strains. After the stabilization of cheese samples (24 h, 4 °C), cheese serial decimal dilutions were prepared in 1% PBS, plated on EMB agar, and incubated for 24 h at 37 °C under aerobic conditions. The results were expressed as log CFU/mL.

2.5. Cytotoxicity

Cytotoxicity assay was carried out with cheese extracts prepared as described by Rafiq et al. [27]. Water-soluble cheese extracts were obtained by lyophilization. The powder obtained by lyophilization was stored in a refrigerator (−20 °C). Before the cytotoxicity test, an appropriate dilution (10 mg/mL) was made with distilled water and filtered (sterile syringe filter, 0.22 µm). Further dilutions, used for cell treatment, were made from the starting stock in DMEM medium. The cytotoxic effect was estimated by employing an MTT assay. Before cytotoxicity testing, HCT116 cells were grown as a single layer in DMEM medium with 10% fetal bovine serum, 1% penicillin/streptomycin mixtures, and 2 mM of L-glutamine. When the cell culture reached 90% confluence, it was collected and placed into 96-well plates with a density of 2×10^4 cells/well and kept overnight at 5% CO_2 and 37 °C. Next, HCT116 cells were treated with diluted extracts at the concentrations of 0.75, 0.5, and 0.25 mg/mL and left for 24 h. After that, the medium with test substances was replaced with MTT (final concentration 0.5 mg/mL) and left for another 3 h. At the end of the MTT treatment, the medium was taken out, and the formazan crystals were dissolved in DMSO. The cell viability was assessed by measuring the absorbance at 570 nm using a microplate reader (Multiskan FC, Thermo Scientific, Shanghai, China).

2.6. Sensory Analysis

Sensory evaluation of cheese samples was carried out as described in Miloradovic et al. [26] and Jovanović et al. [15]. Cheese samples were evaluated in terms of the most prominent sensory attributes, i.e., appearance, texture, odor, and taste. They were scored from minimum (0) to maximum (5) by a sensory panel consisting of seven members, all experienced in dairy product quality judging. Panelists were instructed to cleanse their palates between samples using water and toasted bread. The scores assigned by the panel were weighted with corresponding coefficients of importance (CI), selected according to the influence of each attribute on overall sensory quality. Maximum overall quality was obtained by adding up the scores of each tested sensory attribute per sample, which was previously multiplied by the CI. The evaluation of cheeses was conducted immediately after the samples were taken out from the refrigerator. Samples were presented to the panelists in a random order, labeled with 3-digit codes.

2.7. Consumer and Patient Attitudes towards Psychobiotics: A Survey of Mental Health and Functional Foods

To gain insight into the modern customers, including psychiatric users' attitudes toward psychobiotics and their incorporation in foods of the new generation, an online questionnaire was administered (Google form). The link to the open survey was shared by the private psychiatric practice Psihocentrala (Belgrade, Serbia) with patients who applied to the internal mailing list. One response per participant was allowed. The data collection ran for one month. The questionnaire contained 23 closed questions about the survey participants' personal data (age, sex, and health status), their preferences regarding probiotic consumption, and their efforts allocated for staying educated about functional foods and mental health, with a focus on how informed they were about psychobiotics. The survey participation was anonymous and on a voluntary basis. The survey is available in translated form as Supplementary Material S1.

2.8. Statistical Analysis

Statistical analysis was performed using the software GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). The analysis of variance (one-way ANOVA) was used to determine the significant differences ($p < 0.05$) among treatments. The significant differences between the mean value of data obtained from the bacterial counts were analyzed by Tukey's multiple comparison test, while data from the MTT assay were analyzed by Dunnett's test. The results were expressed as mean value ± standard deviation (SD). All experiments were performed at least in duplicate.

3. Results and Discussion

3.1. The impact of L. reuteri on the Bioactivity of Acid-Coagulated Cheese

There is an increasingly prevalent trend of the investigations for a link between *L. reuteri* healing properties on gastrointestinal ailments, mainly inflammatory bowel disease (IBD), as well as colorectal cancer, and mental disorders [14]. Constant exposure to stressors, such as social conflict, social isolation, and pathogen infection, stimulate the release of the adrenal hormones noradrenaline, adrenaline, and glucocorticoids through activation of the hypothalamic–pituitary–adrenal axis and the secretion of pro-inflammatory cytokines IL-6 and TNF. This could lead to the occurrence of psychiatric disorders, including anxiety and depression, as well as colitis and gut dysbiosis [28]. Patients with IBD have approximately a 20% prevalence rate of anxiety and 15% prevalence rate of depression [14]. A vast amount of research has demonstrated that a variety of *L. reuteri* strains have the ability to reduce depression. Particularly, *L. reuteri* DSM 17938 was found to exert an anti-depressive impact [16] and the ability to reduce the detrimental effect of pathogenic *Escherichia coli*, which is a leading cause of infectious diarrhea in humans and animals [14]. Also, Han et al. [28] demonstrated in vivo that infection with *E. coli* causes colitis with anxiety and depression through the activation of the MGB axis. In the same study, it was shown that *L. reuteri* NK33 may alleviate depression and colitis by ameliorating gut dysbiosis.

Considering the established link between depression and intestinal disorders, our study investigated the potential benefits of cheese fermented with the psychobiotic strain *L. reuteri*. Specifically, we examined whether such cheese samples support the growth of beneficial LAB and modulate the growth of beneficial and pathogenic *E. coli* strains. Additionally, we tested whether extracts of the cheese could inhibit the growth of colorectal cancer cells in vitro. Our findings suggest that cheese fermented with *L. reuteri* may have potential health benefits for individuals suffering from depression and intestinal disorders.

High viability of LAB was observed in all tested samples (>log 7.64) (Table 1).

Although cheese is recognized as one of the most suitable dairy matrices for the survival of LAB, it also provides favorable conditions for the viability of certain foodborne pathogens [29]. While some strains of *E. coli* can cause illness, non-pathogenic strains lacking genomic genes for virulence factors may confer benefits to the host, such as the production of vitamins K and B12. Furthermore, *E. coli* is among the first members to colonize the intestinal tract of infants and establishes itself as a life-long resident of the normal microbiota [30]. Thus, within the genus *Escherichia*, both beneficial strains, such as the probiotic strain *E. coli* Nissle 1917 [31], and pathogenic strains, such as *E. coli* O157:H7, can be found.

Table 1. LAB viability of cheese samples with and without *Limosilactobacillus reuteri* DSM 17938 and mushroom powders.

Sample	LAB Viability (log CFU/mL)
control cheese	8.10 ± 0.28
cheese + *L. reuteri*	7.67 ± 0.25
cheese + *A. bisporus*	8.06 ± 0.20
cheese + *L. reuteri* + *A. bisporus*	7.80 ± 0.34
cheese + *P. ostreatus*	7.64 ± 0.23
cheese + *L. reuteri* + *P. ostreatus*	7.67 ± 0.07

Considering the above-mentioned, it was necessary to assess the *in situ* viability of *E. coli* strains. The viability of tested pathogenic *E. coli* strains (0157:H7, ATCC 35218) remained high in all tested cheese samples (up from 4.91 ± 0.37) (Table 2). Pathogenic strains of *E. coli* are significant from a public health perspective, as they have been associated with outbreaks and severe food poisoning. Therefore, the increased viability of pathogenic *E. coli* strains in cheeses suggests that additional caution is required in cheese manufacturing [15,30]. On the other hand, Darwish et al. [31] demonstrated that the addition of fruit peel powder to yogurt had a beneficial effect ($p < 0.05$) on counts of probiotic *E. coli* Nissle 1917. Additionally, edible mushrooms may stimulate the growth of gut microbiota and act as prebiotics [32], potentially leading to increased viability of *E. coli* Nissle 1917. In another study, it was shown that an isolated probiotic strain of *E. coli* was compatible with commercial probiotics and standard LAB strains and could be used in conjunction with strains available in the food and pharmaceutical industries [33]. In our investigation, it was demonstrated that cheese with and without *L. reuteri* and gourmet mushroom powder supported the high viability of *E. coli* Nissle 1917 (>7.11 log CFU/mL). The potential to enrich cheese samples with both gram-positive (e.g., LAB strains) and gram-negative health-promoting strains (e.g., *E. coli* Nissle 1917) would render the product particularly desirable from a health-benefit perspective due to the fact that gram-positive and gram-negative probiotics have different mechanisms of action [34].

Table 2. *In situ Escherichia* viability of cheese samples with and without *Limosilactobacillus reuteri* DSM 17938 and mushroom powders.

Sample	*E. coli* Strain (log CFU/mL)		
	0157:H7	**ATCC 35218**	**Nissle 1917**
control cheese	4.91 ± 0.37 ^{a}	5.20 ± 0.28	7.11 ± 0.26
cheese + *L. reuteri*	4.64 ± 0.39 ^{bc}	5.06 ± 0.13	7.47 ± 0.13
cheese + *A. bisporus*	5.26 ± 0.64 ^{d}	5.50 ± 0.46	7.24 ± 0.10
cheese + *L. reuteri* + *A. bisporus*	5.17 ± 0.33 ^{e}	5.51 ± 0.37	7.23 ± 0.07
cheese + *P. ostreatus*	6.03 ± 0.04 ^{b}	5.81 ± 0.06	7.25 ± 0.05
cheese + *L. reuteri* + *P. ostreatus*	6.70 ± 0.39 ^{acde}	5.81 ± 0.04	7.40 ± 0.13

^{abcde} Values with the same letter are significantly different in comparison to each other according to Tukey's multiple comparison test ($p < 0.05$).

The presence of high LAB viability in fresh cheese samples, in addition to supporting health-promoting properties, such as modulating the viability of commensal and pathogenic microbes, may also affect cancer cell growth. It is particularly noteworthy that a large initial count of *L. reuteri* BCRC14652 (>10^8 CFU/mL) induced cell membrane damage to human colon carcinoma cells HT29, while a mixture of *Lactobacillus acidophilus* and *Lacticaseibacillus casei* (>10^7 CFU/mL) could dose-dependently increase the apoptosis-induction activity of 5-fluorouracil, a conventional chemotherapeutic [35]. Furthermore, the anti-tumor potential of LAB-cultured supernatants and inactivated cells, i.e., paraprobiotics, has also been reported [35]. Our previous findings confirm that yogurt-extracted whey [36] and extract obtained from yogurt supplemented with *Ganoderma lucidum* residues [15] decreased colon cancer cell line HCT116 viability to some extent. Consistent with these results, a statistically significant decrease in HCT116 viability was observed in this study, with cells being most susceptible to extract obtained from cheese samples without mushroom powder supplementation (up to 30.96% inhibition of cell viability, Figure 1).

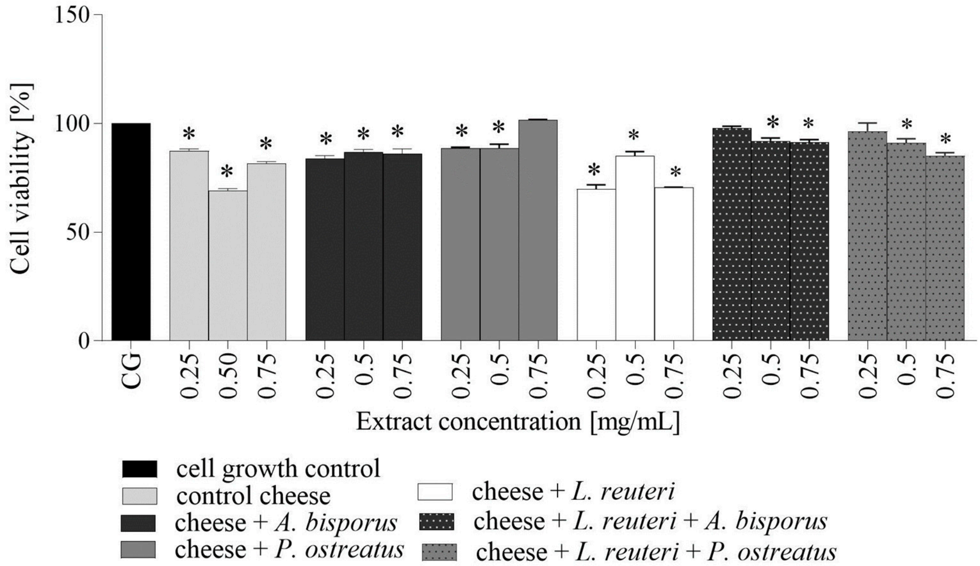

Figure 1. Viability of HCT116 cells treated with extracts of cheeses with and without *L. reuteri* and mushrooms powders. The asterisk indicates a significant difference of means between samples and cell growth control (CG) according to Dunnett's test ($p < 0.05$).

3.2. Sensory and Organoleptic Evaluation of Cheese Supplemented with Psychobiotics and Gourmet Mushroom Powders

The incorporation of a potential psychobiotic strain during cheese preparation may influence both the fermentation process and the organoleptic properties of the final product. Furthermore, the fortification of cheese with powdered mushrooms served to increase the glutamate content, which is a substrate required for the expression of psychobiotic activity of *L. reuteri* and also contributed to the formation of an acceptable final taste of the cheese. Mushrooms are highly valued for their health-enhancing potential as well as their desirable flavor and aroma. Button mushrooms are among the four most popular types of mushrooms globally [37]. As psychobiotic products represent a new generation of food, the addition of a familiar and desirable taste provides an additional incentive for the acceptance of such an innovative product. The results of this study indicate that cheeses fortified with *A. bisporus* mushroom powder received the highest ratings (96.11% and 97.21% for cheese with and without *L. reuteri*, respectively, Table 3). These findings are consistent with those of Khider et al. [38], who reported that the addition of dried mushrooms to cheese resulted in a product with good sensory characteristics and improved microbiological quality. In contrast, cheese prepared with oyster-mushroom powder scored slightly lower than other samples due to its acidity (77.46% and 84.68% for cheese without and with *L. reuteri*, respectively). Similarly, Tupamahu et al. [39] supplemented yogurt with oyster-mushroom powder and found that a higher concentration of 1% led to increased acidity. The preliminary sensory data obtained in this study may inform future efforts to formulate cheese containing gourmet mushroom powders and psychobiotics.

Table 3. Sensory evaluation of cheese samples with and without *Limosilactobacillus reuteri* DSM 17938 and mushroom powders.

Sensory Attribute * / Sample	Apparency	Odor	Taste	Texture	% Max. Overall Quality
control cheese	13.82 ± 0.45	18.43 ± 0.54 [af]	33.00 ± 0.37 [ai]	29.14 ± 0.28 [ae]	94.39
cheese + *P. ostreatus*	13.93 ± 0.22	16.14 ± 0.49 [abcd]	21.25 ± 0.74 [abcde]	26.36 ± 0.54 [abcd]	77.46
cheese + *A. bisporus*	14.04 ± 0.35	19.57 ± 0.20 [bg]	34.25 ± 0.20 [bgj]	29.36 ± 0.13 [bf]	97.21
cheese + *L. reuteri*	14.13 ± 0.42	18.71 ± 0.38 [ce]	32.50 ± 0.57 [cfg]	28.71 ± 0.37 [c]	94.05
cheese + *L. reuteri* + *P. ostreatus*	14.14 ± 0.40	17.00 ± 0.39 [efgh]	26.75 ± 0.57 [dfij]	26.79 ± 0.47 [efg]	84.68
cheese + *L. reuteri* + *A. bisporus*	14.25 ± 0.40	19.00 ± 0.19 [dh]	33.50 ± 0.27 [e]	29.36 ± 0.20 [dg]	96.11

* For each sensory attribute, appearance (3), smell (4), taste (7), and texture (6), a coefficient of importance (CI) was assigned. [abcdefghij] Values with the same letter in the same column are significantly different in comparison to each other according to Tukey's multiple comparison test ($p < 0.05$).

3.3. Exploring Consumer Awareness and Preferences for Psychobiotic Foods

Many probiotic species, including *L. reuteri*, can be found in commercially available foods. However, these products do not provide information regarding the psychobiotic capacity of the probiotic strains they contain. In the subsequent phase of this study, a survey was conducted to address the following questions: How frequently do modern consumers and patients suffering from depression and anxiety choose products containing probiotics? Would they like to know if these probiotic strains qualify as psychobiotics? Additionally, would they prefer to select foods with psychobiotic activity that have the potential to improve mood and cognition, or would they rather continue using familiar probiotic-based products? A total of 105 respondents participated in the survey, with approximately equal representation of both genders (45.2%; n = 47 female and 54.8%; n = 57 male). Confirmed gastrointestinal disorders, depression, and/or anxiety were reported by 5.8% (n = 6), 3.8% (n = 4), and 16.5% (n = 11) of subjects, respectively. A significant percentage of survey respondents were aware of what psychobiotics are (77.9%; n = 81) and expressed interest in trying dairy products fermented with a psychobiotic strain. Of the participants, 63.5% (n = 66) and 57.3% (n = 59) stated that they would try yogurt and cheese containing psychobiotics, respectively. The majority of respondents (92.3%; n = 96) agreed that mental health care is very important and that there is a link between mental health and food choices. When asked if they would try a psychobiotic product if recommended by a health professional, 86.5% (n = 90) responded affirmatively. However, most respondents (87.5%; n = 91) felt that they required additional education regarding probiotics, psychobiotics, prebiotics, synbiotics, paraprobiotics, and postbiotics. Furthermore, only 32% (n = 33) indicated that they would be willing to take both the latest probiotic and psychobiotic products, while just 6.8% (n = 7) expressed interest in trying a psychobiotic product alone.

Therefore, it is important that information about health-promoting microbes, in viable or inactivated form, especially beneficial psychobiotic bacteria, become widely available to modern consumers, including psychiatric users. Above all, it is crucial that a fermented product containing probiotics with psychobiotic capacity be adequately labeled. A total of 88.5% (n = 92) of consumers, including psychiatric users, pointed out that it is very important for them that fermented products containing psychobiotic strains are adequately marked. When addressing the need to make probiotics and probiotic-containing products more accessible to the general public, Stanczak and Heuberger [40] stated, "Because the results of scientific studies related to probiotics may be complicated, it is important that this information be translated into simple and understandable messages that the consumer can relate to". This message remains relevant today with respect to the acceptance of products based on psychobiotics.

4. Conclusions

A fermentation technique incorporating a potential psychobiotic strain and natural glutamate-rich supplements has yielded dual benefits, promoting both biological effects and desirable sensory characteristics. High LAB viability was maintained in all cheese samples, and cheese extracts exhibited significant cytotoxicity against colon cancer cells in vitro. Sensory evaluation revealed high scores for all tested samples. The designed cheese may have value in dietary therapy. The cheeses that contain psychobiotics appeal to modern consumers, including those who suffer from depression and anxiety. Considering the unambiguous confirmation of the bioactivities and sensory acceptability of cheeses fermented with the selected strain presented in this study, we recommend further research to confirm the psychobiotic activity of the newly developed cheeses.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fermentation9080745/s1, File S1: Questionnaire on awareness of psychobiotics.

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Institutional Review Board Statement: Testing of product sensory characteristics and conducting a survey with psychiatric users was approved by the local Ethical Committee at Ćuprija General Hospital, Serbia (No. 868/1/3, decision made on 31 January 2022).

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Data Availability Statement: The data of this study are available upon request from the corresponding author.

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