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Isolation, identification, and fermentation characteristics of endogenous lactic acid bacteria derived from edible mushrooms

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Abstract

A total of 83 acid-producing strains were isolated from fresh *Lentinus edodes*, *Pleurotus eryngii*, *Flammulina velutipes*, *Agaricus bisporus*, *Pleurotus ostreatus*, *Pleurotus djamor*, *Pleurotus abalones*, and *Pleurotus citrinopileatus* by $CaCO_3$-MRS plate medium. These 83 strains were divided into 9 species, including 52 strains of *Lactococcus lactis*, 13 strains of *Pediococcus pentosaceus*, 8 strains of *Enterococcus faecium*, 3 strains of *Lactococcus garvieae*, 2 strains of *Enterococcus casseliflavus*, 2 strains of *Lactobacillus plantarum*, 1 strain of *Enterococcus lactis*, 1 strain of *Pediococcus acidilactici*, and 1 strain of *Lactobacillus pentosus* based on the catalase test, Gram staining, and 16S rDNA molecular identification. Among these *P. acidilactici*, *P. pentosus*, and *L. plantarum* could be used in food. *P. acidilactici* had a strong acid-producing capacity and fast growth rate, thus showing preferable fermentation characteristics in MRS broth and edible mushroom medium than *P. pentosus* and *L. plantarum*. In the medium of *L. edodes*, *P. eryngii*, and *F. velutipes*, the best sensory state can be reached within 12-24 h, among them *P. eryngii* had the best fermentation effect, which was characterized by uniform and bright color, moderate acidity, good flavor, and tight tissue state. This study investigated the types and fermentation characteristics of lactic acid bacteria (LAB) derived from edible mushrooms, enriched the resources of LAB suitable for the fermentation, and provided a theoretical reference for the application of LAB fermentation technology of edible mushrooms.

Keywords: edible mushrooms; lactic acid bacteria; separation and identification; 16S rDNA; fermentation characteristics.

Practical Application: Enriching the microbial resources suitable for LAB fermentation of edible mushrooms.

1 Introduction

Lactic acid bacteria (LAB) are a general term for microorganisms that can utilize fermentable sugars such as glucose and lactose and convert them into lactic acid (Wang et al., 2021). LAB not only maintain the balance of intestinal flora (Dempsey & Corr, 2022), but also enhance human immunity (Rastogi & Singh, 2022), hypoglycemic (Archer et al., 2021), hypolipidemic (Wiciński et al., 2020), anti-oxidation (Bryukhanov et al., 2022), and so on. In recent years, LAB have been widely used in the production and processing of fermented foods because of their universally recognized safety, excellent acid-producing properties, and probiotic effects (Alan & Yildiz, 2022), such as the use of LAB fermentation technology to make fruit and vegetable products, thus giving them better taste and nutritional value (Choi et al., 2019; Gumus & Demirci, 2022). In addition, LAB are used in the food industry as a natural antibacterial agent to play a role in antiseptic and fresh preservation (Kousha et al., 2022).

Edible mushrooms are large fungi that can be eaten by people, which have high nutritional value and are rich in nutrients and active ingredients such as polysaccharides, proteins, amino acids, minerals, vitamins, polyphenols, and terpenoids (Kumar et al., 2021; Mleczek et al., 2021; Podkowa et al., 2021). Moreover, it has functions such as immunomodulatory (Pathak et al., 2022), anti-tumor (Xu et al., 2022), anti-inflammatory (Kushairi et al., 2020), antioxidant (Muñoz-Castiblanco et al., 2022), anti-bacterial (Moussa et al., 2022), hypolipidemic activity (Sheng et al., 2019), and various other effects. In recent years, edible mushroom industry has developed rapidly in China, with an annual output of more than 40 million tons, accounting for more than 75% of global output (China Edible Fungi Association, 2022), thus making important contributions to poverty alleviation and rural revitalization. The contradiction between production and sales has become increasingly prominent with the substantial increase in the output of edible mushroom, thereby affecting the income of mushroom farmers and the healthy development of the industry. Therefore, vigorously developing the intensive processing of edible mushroom will help extend the industrial chain, increase added value, and increase the income of mushroom farmers. However, at present, there are relatively few fine and deep processing technologies applicable to edible mushroom in China, and the processing forms are relatively single.

The use of LAB fermentation technology to process *Lentinus edodes* (shiitake mushrooms) can improve the flavor, taste, and nutritional and health value of shiitake mushrooms, which is a processing approach with development potential (Nie et al., 2022). The selection of strains is very important for the fermentation of LAB in fruits and vegetables. *L. edodes* is a type of edible mushroom with the largest output in China, and *Flammulina velutipes* and *Pleurotus eryngii* are two kinds of edible mushrooms with the highest output in factory cultivation. In this study, self-derived LAB were isolated and screened

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from *L. edodes*, *F. velutipes*, *P. eryngii*, and other five kinds of mushrooms. Furthermore, the fermentation characteristics were determined to enrich the resource bank of LAB suitable for edible mushroom fermentation to provide a useful reference for the application of LAB fermentation technology in edible mushroom processing.

2 Materials and methods

2.1 Materials and reagents

Fresh *L. edodes*, *F. velutipes*, and *P. eryngii* were purchased from three local supermarkets in Xinxiang City, Henan Province, China. In addition, fresh *Agaricus bisporus*, *Pleurotus ostreatus*, *Pleurotus djamor*, *Pleurotus abalones,* and *Pleurotus citrinopileatus* were picked on-site from the edible mushroom greenhouse in Henan Institute of Science and Technology, Xinxiang City, Henan Province, China. Lyophilized strain of *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) was purchased from Guangdong Microbial Culture Preservation Center (Guangzhou, China). Lyophilized strain of *Limosilactobacillus fermentum* (Lf) was provided by Zhengzhou Hehe Bioengineering Technology Co., Ltd., (Zhengzhou, China). Moreover, MRS culture medium were purchased from Guangdong Huankai Microbial Technology Co., Ltd., (Guangzhou, China). Bacterial Genomic DNA Extraction Kit, lysozyme, proteinase K, λDNA HindIII Marker, D2000 DNA Marker, ddH_2O, and 2×Taq PCR MasterMix II were purchased from Tiangen Biochemical Technology (Beijing, China) Co., Ltd. Agarose, Goldview Nucleic Acid Stain, Gram staining kit, 50×TAE electrophoresis buffer, and 6×DNA loading buffer were purchased from Beijing Solarbio Science & Technology Co., Ltd. Agarose Gel DNA Mini Recovery Kit was purchased from Guangzhou Meiji Biotechnology Co., Ltd. 16SrRNA universal primers (27F and 1492R) were synthesized by Sangon Bioengineering (Shanghai, China) Co., Ltd. Furthermore, calcium carbonate, absolute ethanol, glycerol, and sodium chloride were purchased from Tianjin Deen Chemical Reagent Co., Ltd.

2.2 Medium

The primary screening medium ($CaCO_3$-MRS) is the MRS medium containing 1.5% calcium carbonate. The edible mushroom culture medium were prepared as follows: fresh *L. edodes*, *F. velutipes*, and *P. eryngii* were washed and dried, and then cut into small pieces of uniform sizes, the ratio of edible mushroom to distilled water was 1:1 (W:W). Subsequently, 2% of glucose and salt were added, and sterilized at 115 °C for 15 min, then finally cooled to room temperature.

2.3 Isolation and screening of acid-producing strains

Fresh *L. edodes*, *F. velutipes*, and *P. eryngii* were cut into 3 mm × 3 mm × 3 mm uniform pieces by sterile operation, added 10 times the mass of sterile water (containing 2% glucose and 2% NaCl). Then, mixed and incubated at 37 °C for 2 h, and the culture medium was diluted to 10^{-7}. A total of 200 μL diluent of each gradient was spread to $CaCO_3$-MRS medium plate and cultured at 37 °C for 48 h. Subsequently, single colonies with transparent zone and different diameter, shape, and color was selected from each plate (Zhao et al., 2022). The colonies were purified and cultured at 37 °C for 24-48 h each time. Afterward, the colonies were continuously purified at least thrice until pure colonies were obtained. The color, smoothness, morphological characteristics, and transparency of the colonies were observed and recorded. Then, the purified strains were inoculated into MRS liquid medium, cultured at 37 °C for 48 h, and the bacterial liquid was collected. Finally, the bacterial liquid was evenly mixed with 20% sterile glycerol and frozen at -20 °C based on the proportion of 1:1.

2.4 Preliminary identification of acid-producing strains

The purified strains were tested by hydrogen peroxide reaction and Gram staining. The bacteria with positive Gram staining and negative catalase could be preliminarily identified as LAB (Kanak & Yilmaz, 2021). In brief, the inoculation ring was used to drop a small amount of bacterial solution on the slope plate, 3% of hydrogen peroxide solution was dropped and mixed. It is considered catalase positive if bubbles are produced within 5-10 s, otherwise it is negative. After Gram staining, the morphological characteristics of the purified strains were observed under inverted microscope, and were simply classified based on the staining results and cell morphology.

2.5 16s rDNA molecular identification

The DNA was extracted based on the instructions of the bacterial genomic DNA extraction kit, and the extraction results were detected by 1% agarose gel electrophoresis and gel imaging system, and then amplified by 16S rDNA PCR using the universal primers 27F (5'AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTACGACTT-3') (Pato et al., 2022). PCR reactant (50 μL): template DNA 4 μL, primer 27F 2 μL, primer 1492R 2 μL, 2 × Taq PCR MasterMix 25 μL, and ddH_2O 17 μL. PCR amplification condition: pre-denatured at 94 °C for 5 min, 30 cycles (denatured at 94 °C for 30 s, annealed at 52 °C for 45 s, extended at 72 °C for 90 s), last extended at 72 °C for 7 min. In addition, the PCR amplification products were detected by 1.5% agarose gel electrophoresis, and the target bands about the length of 1500 bp were cut off. Moreover, the PCR products were purified using agarose gel DNA small recovery kit, and the purified product was detected by 1.5% agarose gel electrophoresis and sent to Sangon Bioengineering (Shanghai, China) Co., Ltd., for sequencing. Then, the obtained sequences were searched for the closest sequences of known species and genera by BLAST on NCBI, and the homology analysis was carried out.

2.6 Study on the fermentation characteristics of LAB in MRS broth

Based on the "list of bacteria that can be used in food" released by the National Health Commission of China, LAB that can be used in food were selected to determine pH and growth rate, and compared with the commercial strains *L. delbrueckii* subsp. *bulgaricus* (Lb) and *L. fermentum* (Lf) preserved in laboratory. The activated LAB were inoculated into MRS broth with 3% inoculum, cultured at 37 °C for 48 h, and the pH value was determined every 8 h. The control group and experimental group were diluted with water to 30 times

with un-inoculated MRS broth as colorimetric control, and OD_{600nm} was determined every 8 h.

2.7 Study on the fermentation characteristics of LAB in edible mushroom medium

The activated LAB were inoculated into the edible mushroom culture medium with 3% inoculum, cultured at 37 °C for 48 h, and the pH value was determined every 12 h. The fermentation broth of edible mushroom was diluted 10 times using clear water as colorimetric control, and OD_{600nm} was determined every 12 h. The sensory evaluation of fermented edible mushroom was carried out every 12 h, and the changes of color, taste, smell, and tissue state were subsequently evaluated and recorded.

2.8 Statistical analysis

All experiments were performed at least in triplicate. Statistical evaluations were performed using SPSS version 17.0 software for Windows (SPSS Inc, Chicago, IL, USA). In addition, one-way analysis of variance (ANOVA) was used to test the significant differences between means, and a post-hoc test (Dunnett's T3) was used to perform multiple comparisons between means at a $P < 0.05$ significance level.

3 Results and discussion

3.1 Isolation and purification results of acid-producing strains

The bacteria with evident calcium-dissolving circle were selected for isolation and purification, and a total of 83 strains were screened (Table 1), which were recorded as L1-L83. More than 70% of the acid-producing strains were isolated from *L. edodes*, *F. velutipes*, and *P. eryngii*, which were sourced from local supermarkets. Less than 30% of the acid-producing strains were isolated from the five species of edible mushrooms from the greenhouse. Based on the colony morphology characteristics of acid-producing strains, 39 strains had milky white, smooth edges, large, and raised colonies (Figure 1a); 27 strains had milky white, smooth edges, small, and raised colonies (Figure 1b); 11 strains had light white, smooth edges, small, and raised colonies (Figure 1c); and 6 strains had light yellow, smooth edges, small, and raised colonies (Figure 1d). The difference of colony morphology of 83 strains were primarily in color and colony size, however, no significant difference was observed in other aspects.

Table 1. Screening results of acid-producing strains.

Edible mushroom	Source	Number of acid-producing strains	Proportion
Lentinus edodes	Local supermarket	25	30.12%
Flammulina velutipes		20	24.10%
Pleurotus eryngii		16	19.28%
Agaricus bisporus	Edible mushroom greenhouse	7	8.43%
Pleurotus ostreatus		4	4.82%
Pleurotus djamor		4	4.82%
Pleurotus abalones		4	4.82%
Pleurotus citrinopileatus		3	3.61%

3.2 Preliminary identification results of acid-producing strains

Based on the results of the catalase tests, a total of 83 acid-producing strains did not produce air bubbles, hence, they were all catalase negative. The cell morphology and color purified strains stained with Gram were observed by oil microscope (1000× magnification), and the staining results of some bacteria were shown in Figure 1e and 1f. All the 83 primary screening strains were Gram-positive bacteria, most of which were spherical (80 strains) and a few were rod-shaped (3 strains). Thus, 83 acid-producing strains could be preliminarily judged for further experiments based on the results of catalase test and Gram staining test.

3.3 16s rDNA molecular identification results

The purified 83 strains were identified by 16S rDNA, and the results were shown in Table 2. Among the 83 strains,

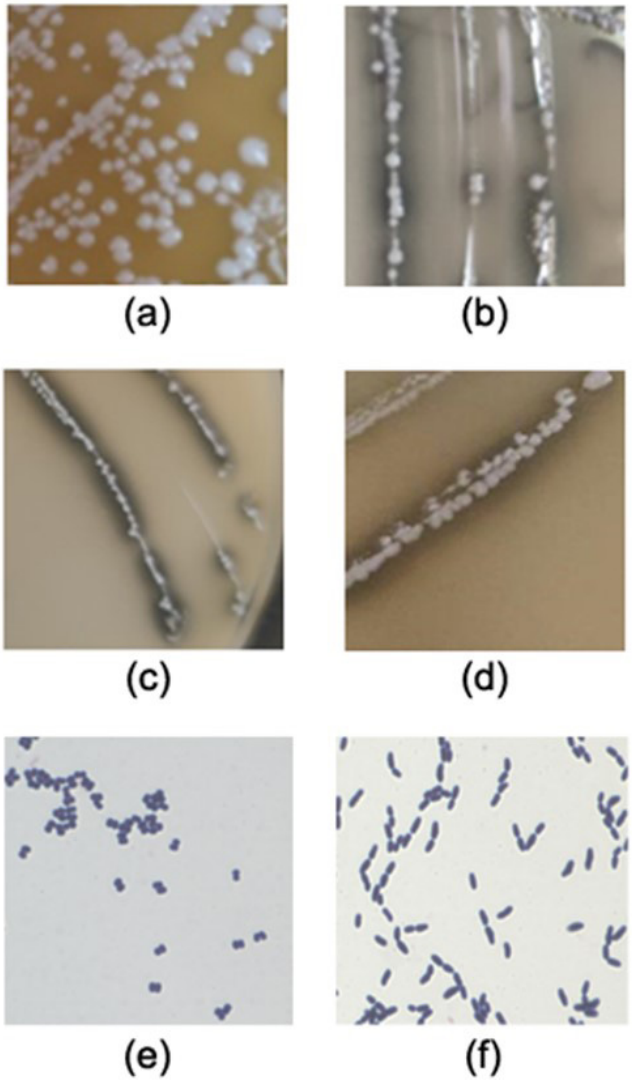

Figure 1. Colony morphology (a, b, c, and d) and cell morphology (e and f) of some acid-producing strains.

Table 2. 16S rDNA sequence alignment results of the acid-producing strains.

Serial number of the acid-producing strains	Amount	The most similar strain	Accession number in GenBank	Homology
L1, L3, L9, L11, L15, L16, L21, L22, L24, L25, L26, L28, L29, L32-L35, L37-L40, L42-L47, L55, L56, L59, L61-L63, L65-L83	52	*Lactococcus lactis*	MT573832.1	100.00%
L41, L48	2	*Enterococcus casseliflavus*	MN166296.1	100.00%
L2, L27, L36	3	*Lactococcus garvieae*	KX671996.1	99.90%
L4-L8, L10, L13, L17, L18, L20, L23, L30, L31	13	*Pediococcus pentosaceus*	MT515988.1	99.84%
L49, L50, L51, L53、L54, L57, L58, L60	8	*Enterococcus faecium*	MW256493.1	99.46%
L12, L19	2	*Lactobacillus plantarum*	GQ337867.1	99.33%
L14	1	*Lactobacillus pentosus*	KX057548.1	99.30%
L64	1	*Pediococcus acidilactici*	MT463550.1	99.07%
L52	1	*Enterococcus lactis*	OM570329.1	99.03%

there were 9 different types of strains, including 52 strains of *Lactococcus lactis*, 13 strains of *Pediococcus pentosaceus*, 8 strains of *Enterococcus faecium*, 3 strains of *Lactococcus garvieae*, 2 strains of *Enterococcus casseliflavus*, 2 strains of *Lactobacillus plantarum*, 1 strain of *Enterococcus lactis*, 1 strain of *Pediococcus acidilactici*, and 1 strain of *Lactobacillus pentosus*. *L. lactis* was the dominant wild strain of edible mushroom, accounting for 62.65% of the total. However, more *L. lactis* were isolated from edible mushroom from different purchase locations, thereby indicating that the surface of edible mushroom was the most suitable for the growth of *L. lactis* in the local environment, and its source may be edible mushroom sticks or attached in the process of growth, transportation, and storage. In addition, *L. lactis* is widely used in Western cheese production, however, it has not been included in the "list of bacteria that can be used in food" released by the National Health Commission of China. Among the strains isolated and purified in this experiment, only *P. acidilactici*, *P. pentosaceus* and *L. plantarum* were included in the "list of strains that can be used in food".

P. acidilactici is widely used in animal husbandry and added to animal feed, which can antagonize pathogenic microorganisms in animals, enhance immunity, and prevent the production of harmful substances (Merati et al., 2022). In addition, *P. acidilactici* is often used as a starter and bacteriostatic in the food industry. If used in fermented meat products, it can significantly improve the texture and flavor of meat products (Jiang et al., 2023). It can also regulate intestinal flora and improve the immunity (Olajugbagbe et al., 2020).

Moreover, *P. pentosaceus* can control foodborne pathogens, regulate intestinal microflora, reduce cholesterol, and so on. It widely exists in fermented plants, such as pickled pickles, sugar beets, and other foods. Moreover, it is often used as a starter for soy sauce fermentation and fish pickling, as well as a preservative to prolong the preservation time of natural fruit (Jiang et al., 2022).

Furthermore, *L. plantarum* is often found in fermented vegetables and fruit juices. It can reduce serum cholesterol, prevent cardiovascular disease, alleviate lactose intolerance, regulate intestinal flora, immune regulation, and so on. *L. plantarum* is also widely used in food industry, such as fermented meat products, kimchi and other fermented condiments, fermented fruit, vegetable juice, and so on (Yilmaz et al., 2022).

3.4 Fermentation characteristics of LAB in MRS broth

The acid-producing capacity of LAB is an important index to evaluate its fermentation characteristics. Strong acid-producing capacity reduces fermentation time and cost in the actual industrial production (Alan & Yildiz, 2022). Pa and Pp had the same change trend of pH as compared with Lb and Lf. The pH decreased rapidly before 8 h and stabilized after 16 h, however, the acid-producing capacity of Pp was slightly weaker than Pa. The acid-producing ability of Lp was evidently weaker than that of other strains, and the acid-producing rate was the slowest (Figure 2a).

The growth curve can directly reflect the adaptability of LAB to the fermentation environment in the fermentation process, and can show the differences in growth rules among different strains. The faster-growing strains have greater advantages in the actual industrial production (Seo et al., 2021). As shown in Figure 2(b), the growth trend of Pa was consistent with that of Lb and Lf. In addition, the logarithmic growth period of Pa was in the range of 0-8 h. After 16 h, the growth tended to be stable, and the growth ability of Pa was even slightly stronger before 8 h. Pp rapidly grew before 8 h, and still slowly grew from 8 h to 40 h, and became stable after 40 h. However, the growth ability of Lp was the worst and entered the logarithmic growth phase after 8 h, which showed a poor strain activity. Combined with acid-producing curve and growth curve in MRS broth, Pa has good fermentation characteristics, Pp was slightly weaker than Pa, and Lp was the worst.

3.5 Fermentation characteristics of LAB in edible mushroom

Acidogenic capacity

Based on Figure 3a, the pH change trend of five strains was roughly the same, and the pH value of Pa was the lowest at the end of fermentation for 48 h. The acid-producing ability

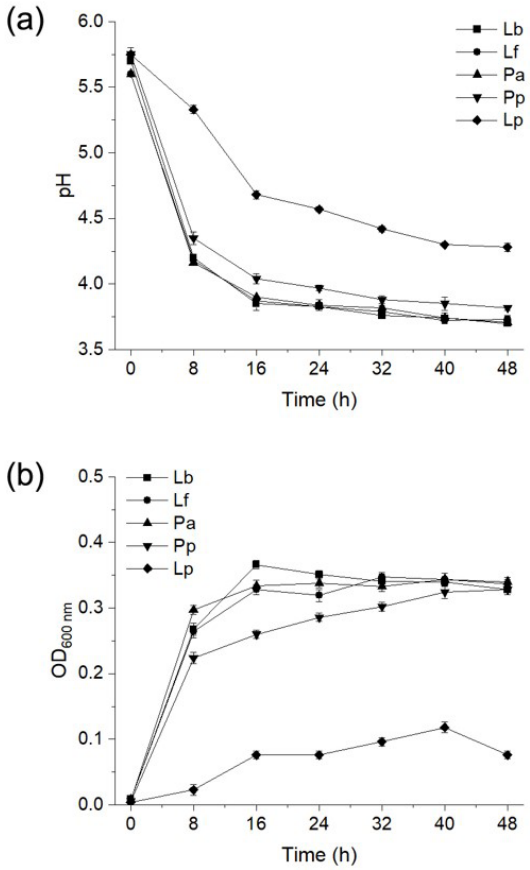

Figure 2. Acid-producing curve (a) and growth curve (b) of different LAB in MRS broth.

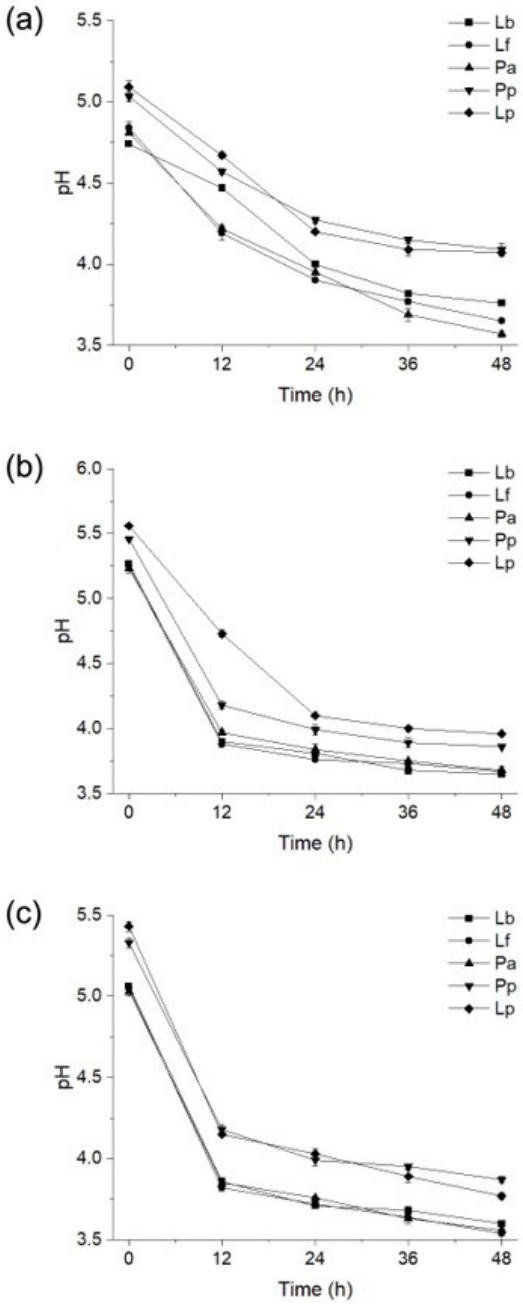

Figure 3. Acid-producing curve of different LAB fermented in *L. edodes* (a), *F. velutipes* (b), and *P. eryngii* (c).

of Pp in *L. edodes* fermentation environment was weak, which was consistent with the Lp. In the fermented *F. velutipes* environment, the other four strains produced acid rapidly before 12 h, except for the rapid decrease of pH value of Lp before 24 h (Figure 3b). The acid-producing ability of Pa was not significantly ($P > 0.05$) different from that of commercial strains. In addition, the acid-producing ability of Pp was weak, meanwhile, that of Lp was the worst. In the environment of fermented *P. eryngii*, the acid-producing curve of the three LAB was the same as that of commercial strains. The pH rapidly decreased before 12 h and slowly decreased after 12 h (Figure 3c). Among these, no significant ($P > 0.05$) differences were observed in acid-producing between Pa and commercial strains, and between Pp and Lp, however, the overall acid-producing ability was poor.

Growth curve

Pa has stronger growth ability under *L. edodes* fermentation conditions than commercial strains, of which $OD_{600\ nm}$ was larger when it reached the fermentation end point (Figure 4a). The growth curve of Pp was consistent with that of Lp, and a big gap between *L. edodes* and commercial strains was observed. As shown in Figure 4b, the overall growth trend was consistent in the fermented *F. velutipes* environment. Under these conditions, the growth ability of Pa was slightly weaker than that of commercial strains, however, no significant difference was found. Meanwhile, the growth ability of Pp and Lp was the same, which was significantly ($P < 0.05$) weaker than that of commercial strains. In the environment of fermented *P. eryngii*, the growth trend of the three LAB was not significantly ($P > 0.05$) different from that of commercial strains, and the logarithmic growth period was in the range of 0-12 h (Figure 4c). Under these conditions, the growth ability of Pa was slightly ($P > 0.05$) lower than that of commercial strains, and the growth ability of Pp and Lp was significantly ($P < 0.05$) weaker than that of commercial strains.

Sensory evaluation

The sensory evaluation was carried out every 12 h, and the changes of color, taste, smell, and tissue state of fermented *L. edodes* were recorded, as shown in Table 3. The sensory evaluation of Pa was consistent with that of commercial strains Lb and Lf. Combined with Figures 4, the color, taste, and tissue state all deteriorated in 36 h because of the rapid growth rate and strong acid-producing ability of Pa. Given that *L. edodes* had its own rich flavor, it does not have evident fermentation flavor until the taste was sour. The taste of Pp and Lp was always light, and a strange smell was observed after 36 h. Overall, the sensory evaluation of *L. edodes* fermented by Pa was the best among the three strains of LAB, and the sensory evaluation reached the best in 24 h, which can greatly save time and reduce cost in the actual fermentation industry.

When fermenting *F. velutipes*, the soup became sticky after 36 h, even though the fermentation characteristics of the five strains were significantly different (Table 3). This indicated that the stickiness of fermented *F. velutipes* soup was not directly related to the fermentation characteristics of LAB, but may be caused by the characteristics of *F. velutipes*. For example, the specific reasons for the stickiness of *F. velutipes* polysaccharides need to be further studied. No significant difference was found in Pa as compared with Lb and Lf, however, the difference between Pp and Lp was primarily reflected in the taste. It took a long time for Pp to taste moderately, and Lp took the longest time, and the sweet and sour taste was lighter. Generally, Pa was the most suitable for *F. velutipes* fermentation, which can be completed in a short time.

P. eryngii was more suitable for LAB fermentation than *L. edodes*, and was evidently better than *L. edodes* in color and tissue state (Table 3). Although the growth rate of Pa in the fermentation of *P. eryngii* was lower than that of the two commercial strains Lb and Lf, it was highly consistent with the commercial strain in all aspects of sensory evaluation,

Table 3. Sensory evaluation of fermented edible mushroom by different LAB.

Edible mushroom	LAB	Color	Taste	Smell	Tissue state
L. edodes	Lb	The color is uniform and bright, and the color becomes dark after 36 h.	The taste is mild at 12 h, moderate at 24~36 h, and sour at 48 h.	The smell has a strong flavor of *L. edodes* but no obvious fermented flavor, fermented flavor appears after 36 h.	The tissue state is tight, and the soup is dark yellow, which becomes cloudy after 36 h.
	Lf	The color is uniform and bright, and the color becomes dark after 36 h.	The taste is mild at 12 h, moderate at 24~36 h, and sour at 48 h.	The smell has a strong flavor of *L. edodes* but no obvious fermented flavor, fermented flavor appears after 36 h.	The tissue state is tight, and the soup is dark yellow, which becomes cloudy after 36 h.
	Pa	The color is uniform and bright, and the color becomes dark after 36 h.	The taste is mild at 12 h, moderate at 24 h, and sour after 36 h.	The smell has a strong flavor of *L. edodes* but no obvious fermented flavor, fermented flavor appears after 36 h.	The tissue state is tight, and the soup is dark yellow, which becomes cloudy after 36 h.
	Pp	The color is uniform and bright, and the color becomes dark after 36 h.	The taste is always mild.	The smell has a strong flavor of *L. edodes* but no obvious fermented flavor, bad flavor appears after 36 h.	The tissue state is tight, and the soup is dark yellow, which becomes slightly cloudy after 36 h.
	Lp	The color is uniform and bright, and the color becomes dark after 36 h.	The taste is always mild.	The smell has a strong flavor of *L. edodes* but no obvious fermented flavor, bad flavor appears after 36 h.	The tissue state is tight, and the soup is dark yellow, which becomes slightly cloudy after 36 h.
F. velutipes	Lb	The color is uniform and bright.	The taste is moderate at 12~24 h, and sour after 36 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white and yellowish, which becomes sticky after 36 h.
	Lf	The color is uniform and bright.	The taste is moderate at 12~24 h, and sour after 36 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white and yellowish, which becomes sticky after 36 h.
	Pa	The color is uniform and bright.	The taste is moderate at 12~24 h, and sour after 36 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white and yellowish, which becomes sticky after 36 h.
	Pp	The color is uniform and bright.	The taste is mild at 12~24 h, and moderate at 36~48 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white and yellowish, which becomes sticky after 36 h.
	Lp	The color is uniform and bright.	The taste is mild at 12~36 h, and moderate at 48 h.	Good smell, mild sweet and sour	The tissue state is tight, and the soup is white and yellowish, which becomes sticky after 36 h.
P. eryngii	Lb	The color is uniform and bright.	The taste is moderate at 12~24 h, and sour after 36 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white.
	Lf	The color is uniform and bright.	The taste is moderate at 12~24 h, and sour after 36 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white.
	Pa	The color is uniform and bright.	The taste is moderate at 12~24 h, and sour after 36 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white.
	Pp	The color is uniform and bright.	The taste is mild at 12~24 h, and moderate at 36~48 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white.
	Lp	The color is uniform and bright.	The taste is mild at 12~24 h, and moderate at 36~48 h.	Good smell, sweet and sour	The tissue state is tight, and the soup is white.

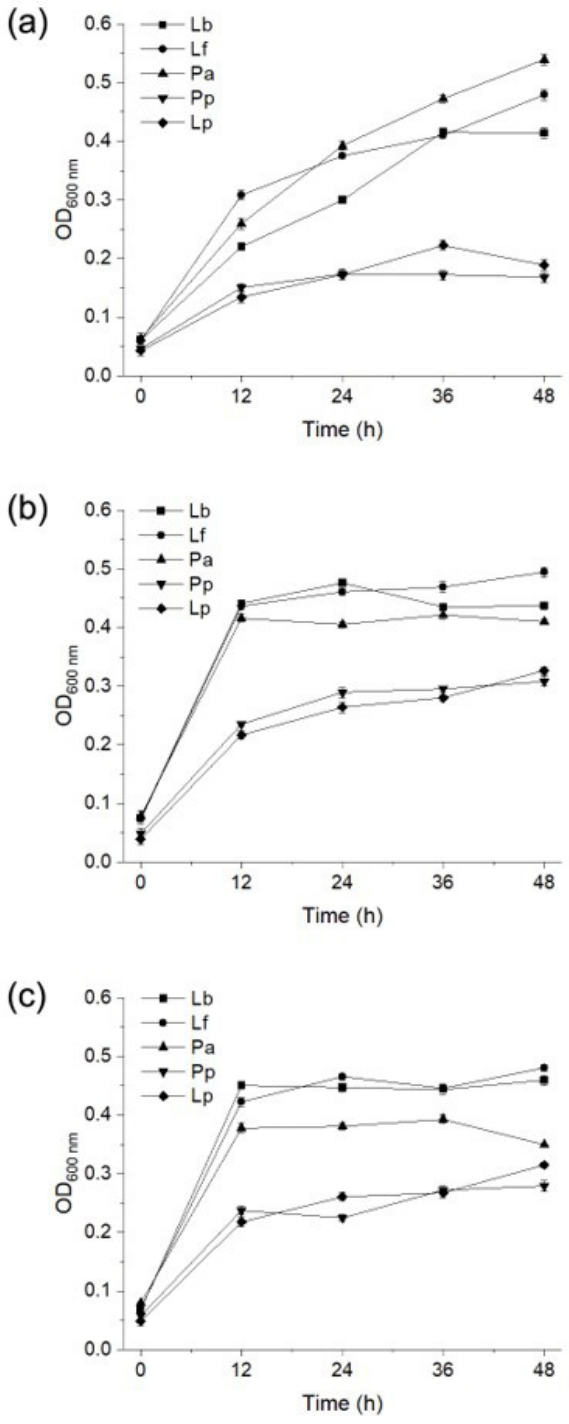

Figure 4. Growth curve of different LAB fermented in *L. edodes* (a), *F. velutipes* (b), and *P. eryngii* (c).

thus reached the best sensory state at 12 h and tasted sour after 36 h. Although the fermentation characteristics of Pp and Lp are poor, the best sensory state can be achieved in *P. eryngii* fermentation for a long time. In summary, Pa was the most suitable for *P. eryngii* fermentation, which could be completed in a short time, whereas Pp and Lp were suitable for long-term fermentation.

4 Conclusions

In this study, a total of 83 acid-producing strains were isolated and purified from fresh *L. edodes*, *P. eryngii*, *F. velutipes*, *A. bisporus*, *P. ostreatus*, *P. djamor*, *P. abalones*, and *P. citrinopileatus*. These 83 strains were preliminarily identified as LAB by catalase test and Gram staining and were divided into 9 types of species based on the 16S rDNA molecular identification.Based on the list of strains that can be used in food released by the National Health Commission of China, three kinds of bacteria can be used in fermentation characteristics test, which are *L. lactis*, *P. pentosus*, and *L. plantarum*. The strain with the highest homology was selected to study its fermentation characteristics. In MRS broth, Pa had the strongest acid-producing ability and the fastest growth rate, and had the same good fermentation characteristics as commercial strains. However, Pp and Lp were the worst. In the fermentation system of *L. edodes*, *F. velutipes*, and *P. eryngii*, Pa could complete fermentation within 12 h and reached the best sensory state, whereas Pp and Lp taken longer fermentation time, 36 h and 48 h, respectively. Furthermore, *P. eryngii* was the most suitable for LAB fermentation under the same conditions, which was better than fermented *L. edodes* and fermented *F. velutipes* in color, taste, and smell and tissue state as a whole. This study not only enriches the resource bank of natural LAB in edible mushroom and provides a strain source for the development of new food of fermented edible mushroom, but also provides a new reference way for deep processing of edible mushroom.

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