Study on the Technological Conditions for Submerged Fermenter Culture of Eight Strains of *Flammulina velutipes*

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Abstract: The growth of eight strains of *Flammulina velutipes* under liquid fermentation conditions was examined. The most promising results were obtained with strains Fv-19 and Fv-K.

Key words: *Flammulina velutipes*, liquid culture, plant, application

1 Introduction

Flammulina velutipes has many desirable characteristics including having a delicious taste, a highly nutritious composition, a capacity to prevent illness, and an ability to promote the growth of the young. In recent years, consumption has increased rapidly, and some countries have established factories for producing this mushroom. In China, we have also developed a set of conditions for cultivating *F. velutipes* that are suitable for the conditions in this country. However, many production facilities still use solid spawn to inoculate the growth substrate. There are many shortcomings associated with this approach such as the growth cycle is too long and the procedure is not user efficient. On the other hand, there are many benefits of using liquid spawn including uniformity of growth and relatively rapid spawn run. Hence, this is the future development. The aim of this study was to examine strains of *F. velutipes* with a view to selecting strains suitable for use in the preparation of liquid spawn on a large scale. Of eight strains examined, two (Fv-19 and Fv-K) were the best in terms of growth under liquid fermentation conditions, and showed the highest application potential.

2 Materials and Methods

2.1 Name and source of strains

The strains used in this study and their origin are shown in Table 1.

Table 1. Strains used in this study and their source

Strain	Source	Strain	Source
Fv-8801	Fujian Sanming Epiphyte Graduate School	Fv-K	Shanghai Agriculture Science School, Fungi Graduate School
Fv-19	Fujian Sanming Epiphyte Graduate School	Fv-01	Zhenjiang Fungi Graduate School
Fv-yuxre	Chinese Agricultural College	Fv-02	Zhenjiang Fungi Graduate School
Fv-baixue	Chinese Agricultural College	Fv-gold	Zhenjiang Fungi Graduate School

2.2 Culture media

Solid media consisted of potato 200g, cottonseed hulls 50g, corn flour 20g, bran 50g , grape sugar 20g , KH_2PO_4 1g, $MgSO_4$ 1g, vitamin B1 100mg, agar 20g , water 1000ml.
Liquid seed culture medium consisted of peptone 0.6%, grape sugar 2%, KH_2PO_4 0.1%, $MgSO_4$ 0.05%, vitamin B_1 0.01%, corn flour 3%, bran 2% , water 6400ml, pH 6.82 (adjusted using NaOH).

2.3 Stirred flask liquid seed culture

Flasks (500ml) were loaded with 400ml culture medium, sterilised by autoclaving (1.2kg/cm^2, 35 min) and, after cooling inoculated with the test fungus. Each flask was aerated with sterile air (60L/min) and incubated at 25 ±1°C for 7 days.

2.4 Two-step 10L fermenter seed culture

Two flasks of stirred flask liquid seed culture (800 ml) were used to inoculate a 10L fermenter containing 8L of sterile liquid seed culture medium. The fermenter was operated for 5 days at 25 ±1°C and an aeration rate of 85L/min.

2.5 Cultivation bag inoculation

Fifty growth bags (15 x28 cm) were inoculated with fermenter seed culture of each of the test strains. The growth substrate consisted of cottonseed hulls 65% corn flour 6%, bran 23%, gypsum 1%, CaO 1% sugar 2% and water 60%. Each bag was inoculated aseptically under pressure with 10-15 ml of liquid spawn and then placed in the cultivation room at 20±1°C. Parameters recorded were the rate of mushroom development, the level of contamination, and the manner in which the crop grew.
Bags producing mushrooms were first placed in a refrigerator and then opened for 'scratching' prior to management of fruit body development under conditions of 12±1°C and 85% humidity.

2.6 Measurements

Mycelium wet weight was measured by taking aliquots of fermenter culture and filtering through filter paper prior to weighing. Mycelial growth was determined by measuring the radial growth at several points on solid media and taking an average value. Dry weight determinations were made by taking 5 ml aliquots of culture, filtering under vacuum, washing with distilled water and then drying at 60°C to constant weight. The amount of life form was determined according to the formulation: Quantity of life-form (kg/m^3) = (DCB/V) × 10^6 where DCB = cell dry weight (kg) and V = sampling volume (ml). Growth curves were prepared by taking daily dry weight measurements (5 ml culture) throughout the experimental period. The pH of the culture medium was measured directly using a pH meter.

3 Results and Analysis

3.1 Analysis of stirred flask culture fluid

The physical parameters of the fermentation liquid taken from stirred flask cultures are shown in Table 2. It can be seen that the smallest reduction in pH (0.96 units) occurred with Fv-K, while the greatest decrease (1.95 units) was seen with Fv-8801. Changes in pH associated with the other strains ranged from 1.11 to 1.70 units.

These reductions in pH value are probably associated with the production of organic acids during fungal growth. Table 2 also shows that, of the eight strains, Fv-K produced the highest concentration of mycelial pellets (343/ml) which varied in diameter from 0.4 mm to 0.8 mm. By all appearances, this strain was the best fitted for the production of liquid spawn, followed closely by strain Fv-19. Culture fluids were red and brown in colour, and very clear indicating that the fungus had absorbed all the nutrients. The cultures had a fragrant odour indicating the absence of any bacterial contamination.

3.2 Two-step cultivation in 10L fermenter

Since the 10L fermenter was made of stainless steel, the mycelium could not be observed. Therefore, the end-point of the fermentation was confirmed by determining the end point of mycelial biomass production (Table 3). In all cases, this occurred between 5 and 6 days after inoculation.

Table 2. Physical parameters of samples of fermentation liquid taken from stirred flask cultures

Parameters	Fungal strains							
	Fv-K	Fv-yuxue	Fv-baixue	Fv-01	Fv-19	Fv-02	Fv-gold	Fv-8801
Original pH	6.82	6.82	6.82	6.82	6.82	6.82	6.82	6.82
Final pH	5.86	5.49	5.65	5.27	5.16	4.92	5.14	4.87
pH change	0.96	1.33	1.17	1.55	1.66	1.90	1.68	1.95
Mycelial pellets (per ml)	343	289	249	297	305	301	267	251
Diameter of pellet (mm)	0.4-1.0	0.3-1.1	0.4-1.4	0.6-1.2	0.6-1.1	0.8-1.5	1.0-2.3	0.7-1.6
Mycelial dry weight (g/100ml)	1.36	1.21	0.89	1.26	1.30	1.28	0.95	0.92
Odour of fermentation liquid	Fungi spicy	Fungi spicy	Fungi spicy	Fungi spicy	Fungi spicy	Fungi spicy	Fungi spicy	Fungi spicy
Colour	Red brown	Red brown	Red brown	Red brown	Red brown	Dust yellow	Red brown	Dust yellow
Clear ratio	highest	higher	higher	higher	higher	low	high	lowest

Table 3. Number of mycelial pellets produced by each strain over a time-course

Time(days) \ Strain	Fv-K	Fv-yuxue	Fv-baixue	Fv-01	Fv-19	Fv-02	Fv-gold	Fv-8801
1	31	26	25	22	28	29	25	22
2	56	44	51	39	52	52	49	33
3	150	132	166	132	165	156	172	129
4	370	293	282	257	344	324	267	212
5	782	592	584	522	706	672	602	522
6	666	458	496	476	585	633	487	403

3.3 Growth in bags after inoculation with liquid spawn

Fifty bags of soid substrate was inoculated with the liquid spawn of each strain and various characteristics including the rate of growth and fruiting body development, the quality of the fruiting bodies, and the degree of contamination, were monitored (Table 4).

Table 4. Fungal growth and fruit body development in cultivation bags

Items / Strains	No. of bags contaminated	Pollution ratio (%)	Mycelial growth rate (cm/day)	Days before fruit bodies appeared	Mycelial growth tendency
Fv-K	0	0	1.3	10	++++
Fv-yuxue	1	2	1.1	12	+++
Fv-baixue	2	4	0.95	14	++
Fv-01	4	8	1.1	12	++
Fv-19	0	0	1.2	11	++++
Fv-02	2	4	1.1	12	++
Fv-gold	2	4	1.1	12	+++
Fv-8801	5	10	0.87	15	++

Values are for 50 bags inoculated with liquid spawn of a single strain.

From Table 4, it can be seen that bags inoculated with strains Fv-K and Fv-19 produced thick, white, dense and strong mycelia, were least likely to become contaminated, and produced fruit bodies in the shortest time.

3.4 Yields and economic properties of fruiting bodies produced by different strains of *F. velutipes*

When all eight strains of *F. velutipes* were grown under the same cultivation conditions, the longest stipes and smallest pilei were produced by strains Fv-19 and Fv-K. The properties of the fruiting bodies were in accord with the high quality standards for this fungal commodity (see Table 5).

Table 5. Economic properties of hymenia produced by the different strains

Strains	Yield (gm)	Height (cm)	Pileus diameter (mm)	Stipe diameter (mm)	Number (/container)	Color
Fv-K	104	13-19	4-8	2-4	260	white
Fv-yuxue	96	13-17	3-6	2-4	245	white
Fv-baixue	92	13-17	3-6	2-5	233	white
Fv-01	102	14-18	3-7	3-5	240	white
Fv-19	115	13-19	4-8	3-5	253	yellow
Fv-02	99	13-17	3-6	2-4	242	white
Fv-gold	99	14-18	4-9	3-5	226	yellow
Fv-8801	88	13-17	3-6	2-4	180	white

4 Summary

Based on analysis of data obtained from submerged cultivation of eight strains of *F. velutipes*, strains Fv-K and Fv-19 are the more suitable for submerged fermentation among the white and yellow breeds, respectively. The most suitable medium formulation is: peptone 0.6%, glucose 2%, KH_2PO_4 0.1%, $MgSO_4$0.05%, vitamin B_1 0.01%, corn flour 3%, and bran 2%. The pH should be adjusted to 6-6.5. The optimum temperature is 25°C. Cultures should be aerated for 5 to 6 days at a rate of 60L/min. In solid-state cultures, strains Fv-K and Fv-19 grew most rapidly, were subject to the lowest levels of contamination, and produced the

highest fruiting body yields as well as hymenia with the best economic properties. Therefore, this strain was adjudged to be the most suitable for production purposes.