Instability in *in vitro* fruiting of *Cordyceps militaris* (L.) Link

Bhushan Shrestha, Seok-Un Hong, Sang-Kuk Han, Ho-Kyung Kim* and Jae-Mo Sung,
Dept. of Environmental Biology and Entomopathogenic Fungi Culture Collection,
Kangwon National University, Chunchon, Kangwon-Do, *Mushtech Co., Chunchon, Kangwon-Do
Tel.: 033-250-6435, Fax: 033-241-6435, email: jmsung@cc.kangwon.ac.kr

Abstract

Traditionally, *Cordyceps* species have been used as a part of herbal medicine in Oriental countries, including Korea for internal health, vigorosity and to cure different diseases related to heart, lung etc. In the recent years, research on artificial fruiting of different *Cordyceps* species including *C. militaris* has been carried out in the world because of their medicinal value. Variation as well as instability occur during artificial fruiting. Instability observed in the *in vitro* fruiting of *C. militaris* is reported in the present study.

1. Introduction

Cordyceps belongs to Div. Ascomycota, Class Pyrenomycetes, Order Hypocreales and Family Clavicipitaceae. There are over 300 species of *Cordyceps* in the world (6,7). More than 30 species of *Cordyceps* have been reported from Korea (13). *Cordyceps* species are highly valued in oriental medicine (3). *C. militaris* is specially well known for the production of cordycepin, which is effective in inhibiting the growth of tumor cells (7). Artificial fruiting of *C. militaris* has been reported in rice medium (5,9,10) as well as in defined medium (1). Similarly, its cultural characteristics and *in vitro* fruiting have also been reported from Korean isolates (12,14,2). But, continuous fruiting has been found difficult (13).

2. Materials and Methods

Isolates of *C. militaris* preserved at Entomopathogenic Fungi Culture Collection, Kangwon National University were used for the present experiment. Liquid spawn was inoculated in 1000cc pp bottles containing 80gm of brown rice and 10gm of pupa. The number of stromata per bottle was counted after two months of cultivation. The fruiting was graded as high, medium and low when the numbers of stromata per bottle were 100 or more, 50-99, or less than 50 respectively. Similarly, three media were used to compare their effect on fruiting.

3. Results and Discussion

The number of *in vitro* produced stromata varied from few to over 200 per bottle. Out of total 105 isolates, 49 isolates produced low fruiting, 30 produced medium fruiting, while 21 produced high fruiting. 5 isolates produced only white mycelium. Out of 17 high fruiting isolates, only 7 isolates produced same quality of fruiting for the second time, whereas 5 isolates

produced medium fruiting and the rests produced low fruiting (Table 1).

Table 1. Difference between Ist and 2nd fruiting from original isolates

S. No.	Isolate No.	Original isolate	
		First Fruiting	Second fruiting
1	3551	High	High
2	3559	〃	Medium
3	3561	〃	High
4	3577	〃	Low
5	3578	〃	High
6	3710	〃	Low
7	3713	〃	Medium
8	3741	〃	Low
9	3746	〃	Low
10	3747	〃	Medium
11	3751	〃	High
12	3927	〃	High
13	3931	〃	Medium
14	3945	〃	High
15	3966	〃	High
16	3970	〃	Medium
17	3973	〃	Low

Seven isolates showed high fruiting, when their subcultures were used. But, subcultures of 4 isolates produced medium fruiting, while those of 2 isolates produced low. Four isolates produced only white or yellow mycelium, when their subcultures were used (Table 2).

Table 2. Difference between fruitings from original and subculture isolates

S. No.	Isolate No.	Original isolate	Subculture
1	3264	High	Medium
2	3533	〃	High
3	3551	〃	Medium
4	3553	〃	Low
5	3561	〃	Only white mycelium
6	3563	〃	High
7	3568	〃	High
8	3578	〃	High
9	3746	〃	High
10	3747	〃	Yellow cotton like mycelium
11	3921	〃	Light yellow mycelium
12	3937	〃	High
13	3954	〃	Yellow mycelium
14	3956	〃	Medium
15	3970	〃	Low
16	3973	〃	Medium
17	4013	〃	High

Out of 7 high fruiting isolates, only 2 isolates showed consistency in fruiting from original as well as their subcultures. Subcultures of 2 isolates showed improved fruiting than the second time fruiting of their original cultures. One isolate showed medium fruiting for the second time from its original isolate, but produced low when its subculture was used (Table 3). Two isolates produced only white or yellow mycelium when their subcultures were used.

Table 3. Stability of fruiting from good isolates

S. No.	Isolate No.	Original isolate		First subculture
		First fruiting	Second fruiting	
1	3551	High	High	High
2	3561	〃	High	Only white mycelium
3	3578	〃	High	High
4	3746	〃	Low	High
5	3747	〃	Medium	Yellow cotton like mycelium
6	3970	〃	Medium	Low
7	3973	〃	Low	Medium

Fruiting was more unstable when further subcultures were used (Table 4). Fruiting was high when the original isolates were used, but their first subcultures produced low fruit bodies. But, two of them produced medium type of fruiting when their second subcultures were used.

Table 4. Effect of subculture on artificial fruiting

S. No.	Strain no.	Original isolate	First subculture	Second subculture
1	5252	High	Low	Low
2	5253	〃	〃	Medium
3	5254	〃	〃	Medium

Original as well as its first and second subcultures of isolate no. 3551 produced high fruiting, but its third subculture produced only few stromata. When three different types of liquid media were used for liquid culture, the quality of fruiting varied from isolate to isolate, irrespective of media used (Table 5).

Table 5. Effect of medium on artificial fruiting

S. No.	Isolate no.	Medium	Fruiting	S. No.	Isolate no.	Medium	Fruiting
1	5014	SDAY	High	3	5253	SDAY	High
		PDA	High			PDA	High
		SS	Medium			SS	High
2	5252	SDAY	Medium	4	5254	SDAY	High
		PDA	Low			PDA	High
		SS	Low			SS	High

4. Conclusion

Nothing is yet known about mating system in *Cordyceps* species (4). Mating type of filamentous ascomycetous is governed by bipolar system (11), whereas there is multiple mating system in filamentous basidiomycetes (8). Unidirectional Mating type switching has been observed in filamentous fungi (11). In the present experiment, only one fifth of the total number of tested isolates produced high fruiting. Even high fruiting isolates did not produce same quality of fruiting when they were used for the second time or their subcultures were used. Subculture is one of the low cost methods for long-term preservation of microbiol isolates, although phenotypic as well as genotypic changes occur. *In vitro* fruiting of *C. militaris* generally degraded when their subcultures were used or there was no consistency among subcultures. All the three types of liquid media used in the present experiment did not show any significant effect on fruiting. Further genetic studies on fruiting as well as suitable methods of

preservation are necessary for continuous successful cultivation of *C. militaris*.

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