Growth and Cultural Characteristics of *Cordyceps cardinalis* Collected from Korea

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Cordyceps cardinalis was reported in Japan and the USA in 2004, and its fruiting bodies have recently been cultured in Korea. Herbarium specimens preserved at the Cordyceps Research Institute, Mushtech, Korea were revised and identified as *C. cardinalis*, based on morphological characters and conidial structures. Most of the *C. cardinalis* specimens were collected from Mt. Halla in Jeju-do. The effects of various nutritional sources and environmental conditions such as temperature and pH on mycelial growth of *C. cardinalis* were studied. Oatmeal agar, Martin's peptone dextrose agar, and *Schizophyllum* (mushroom) genetics complete medium plus yeast extract resulted in the best mycelial growth. Among carbon sources, cereals, and nitrogen sources, maltose, oatmeal, and peptone resulted in the best mycelial growth respectively. Mineral salts helped to increase growth rate but only resulted in thin mycelial density, similar to water agar. A temperature of 25°C and a pH of 7 resulted in the highest mycelial growth. Based on these results, a *Cordyceps cardinalis* composite medium (CCM) was formulated with 1% maltose, 2% oatmeal, 1% peptone, and 2% agar. Use of the CCM resulted in slightly better mycelial growth than that of other commonly used agar media. Only organic nitrogen sources imparted a reddish pigmentation to the agar media, but this character diminished after several subcultures. A 7 day culture duration resulted in the best mycelial growth.

KEYWORDS : *Cordyceps cardinalis* composite medium, Environmental factors, Mycelial growth, Nutrition sources, Optimum condition

More than 500 reported species of *Cordyceps* are distributed worldwide. The scientific enumeration of *Cordyceps* species started in the early 18th century, and new *Cordyceps* species are still being reported from different parts of the world [1-5]. *Cordyceps* species are regarded as medicinal mushrooms especially in east Asian countries such as Korea, China, and Japan [6-14], and interesting stories of *Cordyceps* collections are frequently cited in the mycological literature [15]. Very recently, the megagenus *Cordyceps* was revised and separated into four genera, viz., *Cordyceps*, *Metacordyceps*, *Ophiocordyceps*, and *Elaphocordyceps*, mainly based on molecular phylogeny [16]. *Cordyceps cardinalis* is a recently reported species with a known distribution in the US and Japan [17]. After the report of Sung and Spatafora [17], herbarium specimens preserved at the Cordyceps Research Institute (CRI), Mushtech, Korea were revised, and it was confirmed that *C. cardinalis* had been regularly collected from different mountains of Korea by CRI personnel.

Recent biochemical studies have shown that *Cordyceps* species contain many bioactive compounds with different effects on the human body, including the immune system

[6-8, 10, 12-14]. The cultural and nutritional requirements of *Cordyceps* species have been studied for many years with the objective of cultivating them under artificial conditions [18-24]. Cultures of *Cordyceps* species are usually established from ascospores, and the germination rates and growth rates differ depending on the species. For example, *C. bassiana*, *C. bifusispora*, *C. militaris*, *C. pruinosa*, *C. scaraeaeicola*, *M. yongmunensis*, *O. pentatoma*, and *Shimizuomyces paradoxa* grow quickly, while *C. nakazawai*, *O. gracilis* and *O. heteropoda* grow more slowly [22-26]. *C. martialis*, *C. ochraceostromata*, *C. ramosopulvinata*, *C. rosea*, *O. longissima*, *O. sphecocephala*, and *O. yakushimensis* grow very slowly [25, 26]. Conidiation differs by species in *Cordyceps* [25, 27, 28], and microcycle conidiation has been reported in several *Cordyceps* species [25, 27]. Colonies of *Cordyceps* species also show different types of pigmentations in culture; *C. militaris* has yellowish white to orange pigmentation, *C. pruinosa* is red with white sectors, *M. yongmunensis* is greenish white, while *C. bassiana* does not develop pigmentation [19, 21, 22, 24]. Fruiting body production under artificial conditions has been reported recently for *C. cardinalis* [29]. The present study was undertaken to describe the morphological characters of *C. cardinalis* collected

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from Korea and to understand its cultural and nutritional characteristics for optimum mycelial growth.

Materials and Methods

Fungal specimens and isolates. *C. cardinalis* specimens preserved at the CRI were used to describe the morphological characters and for use in cultural studies. Multi-ascospore isolates were derived from *C. cardinalis* specimens CRI C-10376 and CRI C-10734. Specimen CRI C-10376 was collected from Mt. Halla on July 12, 2003, while the CRI C-10734 specimen was collected from Mt. Duryun on August 13, 2003. Ascospores were discharged from fresh specimens over 2% water agar (WA) plates, and then the agar blocks containing ascospores were cut and inoculated on Sabouraud dextrose agar plus yeast extract (SDAY; dextrose 20 g, yeast extract 5 g, peptone 5 g, and agar 15 g per 1,000 mL; pH 5.6) agar plates. After inoculation, the SDAY plates were incubated at 25°C under continuous white fluorescent light for 3 wk. The isolates were given the same number as their respective specimen numbers and were designated as original isolates to distinguish them from subcultures. The conidial structures were observed by the slide culture method, as described by Shrestha *et al.* [28].

Effect of medium, temperature, and pH on *C. cardinalis* mycelial growth. To observe the effect of medium type on *C. cardinalis* mycelial growth characteristics, the original isolates, CRI C-10376 and CRI C-10734, were inoculated on 11 different agar media, including potato dextrose agar (PDA), SDAY, yeast-extract malt-extract peptone dextrose agar, Hamada agar, malt-extract agar, ebiose sucrose agar, Martin's peptone dextrose agar (MPDA), *Schizophyllum* (mushroom) genetics complete medium plus yeast extract (MCM), *Schizophyllum* (mushroom) genetics minimal medium, oatmeal agar (OA), and Czapek-dox agar (CDA). WA was used as a control medium. The media compositions followed Shrestha *et al.* [21]. For inoculation, 4-mm diameter mycelial discs were cut from the isolates and inoculated in the center of agar plates containing the various media. The inoculated agar plates were incubated at 25°C under continuous white fluorescent light and were observed for colony diameter, mycelial density, and pigmentation after 3 wk. Mycelial discs were inoculated on SDAY agar plates and incubated at different temperatures ranging from 15°C to 35°C under continuous light to observe the effect of temperature on mycelial growth. To understand the effect of pH on *C. cardinalis* mycelial growth, 100 mL of SDAY broth (SDAY without agar) was prepared in 250 mL Erlenmeyer flasks and adjusted to pHs ranging from 4.0 to 9.0 before sterilization. Five mycelial discs were inoculated in SDAY broth and incubated at 25°C for 7 days on a rotary

shaker at 120 rpm. The broth cultures were filtered through Whatman no. 2 filter paper, and the fungal mass was dried at 60°C for 24 hr to measure dry wt. The original isolates were also subcultured on SDAY agar plates every 3 wk up to the 12th generation. The effect of subculture on colony morphology was recorded after the 3rd, 6th, 9th, and 12th subcultures.

Effect of carbon source, nitrogen source, and mineral salts on *C. cardinalis* mycelial growth. WA was supplemented with eight different carbon sources at a 2% (w/v) concentration and was inoculated with mycelial discs to observe the effect of carbon source on mycelial growth. Of the different carbon sources, maltose and fructose were further tested to observe the effect of different concentrations (1~7%, w/v) on mycelial growth. Different cereals were also used to observe their effect on mycelial growth. Water extracts were prepared by boiling cereals (2%, w/v) in distilled water for 20 min and filtering the cereals through a piece of cotton cloth. Agar was then added to the water extracts at a concentration of 2% (w/v). Mycelial discs were inoculated on WA plates prepared with cereal extracts and incubated at 25°C for 3 wk under white fluorescent light. Based on growth, millet and oatmeal were selected for further tests to investigate the effect of different concentrations of their water extracts (1~4%, w/v) on mycelial growth.

Similarly, isolates supplemented with 13 organic and inorganic nitrogen sources were inoculated on WA. The isolates were further grown on WA supplemented with different concentrations of peptone and KNO₃ (0.5~3.5%, w/v). The isolates were also inoculated on WA supplemented with ten different types of mineral salts at a concentration of 0.2% (w/v). Isolates were tested for growth at different concentrations of MgSO₄·7H₂O and NaCl, ranging from 0.025% to 0.1% (w/v).

Colony diameter was measured in mm, while mycelial density was categorized as thin (+), moderate (++), or compact (+++). Colony color and medium color were described as white, yellowish white, reddish white, light yellow, pale yellow, grayish ruby, and pale red, based on Kornerup and Wanscher [30]. Based on the above results, a *C. cardinalis* composite medium (CCM) was formulated and compared with CM, OA, PDA, and SDAY.

Optimum conditions for *C. cardinalis* liquid culture. To determine the mycelial growth rate in liquid culture, five mycelial discs of each isolate were inoculated in 100 mL of SDAY broth and incubated on a rotary shaker at 120 rpm. Fungal growth was recorded starting from the 3rd to the 12th day of the shaking culture, as described above. Also, the effect of inoculum size on mycelial growth was studied by inoculating different numbers of mycelial discs (3~8 mm) of both isolates in 100 mL of

SDAY broth and incubating them for 7 days at 25°C on a rotary shaker at 120 rpm. The dry wt. of the mycelium was measured, as described above.

Results and Discussion

Morphological characters of *C. cardinalis*. *C. cardinalis* is characterized by gregarious, 1~26 stromata per host, orange reddish to reddish, (4) 10~40 (50) long and 0.5~1.5 mm wide stromata growing on lepidopteran larva (Fig. 1A and 1B). Head is 2~9 × 1~4 mm in size. Perithecia are ovoid and semi-immersed (Fig. 1B, 1C and 1I). Asci have a distinct cap (Fig. 1D and 1H). The ascospores are irregularly septate but do not disarticulate into part-spores (Fig. 1E and 1G). The sizes of perithecia, asci, and ascospores were all within the range of Sung and Spatafora [17]. The conidial structures were intermediate between *Clonostachys* and *Mariannaea* (Fig. 1F and 1J). However, distinct rhizomorphs were not observed, as reported previously [17]. The CRI herbarium specimens were collected in 1993 from Mt. Chiak, Mt. Gujeol, Mt. Duryun, and Mt. Halla in Korea (nearly 90% of the specimens were collected from Mt. Halla). The collection period extended from June to August, and July was the most fertile month.

Effect of medium, temperature, and pH on mycelial growth. The best mycelial growth was observed on OA, MPDA, and MCM (Table 1). Based on mycelial density, OA and MCM showed better growth than the others. OA was the best overall medium (Table 1). The colony pigmentation was mostly white, but a reddish violet color

Table 1. Effect of medium on mycelial growth of the two *Cordyceps cardinalis* isolates

Medium	Isolate No.					
	CRI C-10376			CRI C-10734		
	CD	MD	MC	CD	MD	MC
OA	34.3	+++	GR	36.6	+++	GR
MCM	31.5	+++	GR	36.8	+++	GR
HA	26.8	+++	RW	33.8	+++	RW
MM	25.9	+++	GR	34.9	+++	GR
YMA	25.1	+++	GR	30.6	+++	GR
SDAY	23.4	+++	GR	24.8	+++	GR
MPDA	32.3	++	GR	36.6	++	GR
ES	31.0	++	RW	36.3	++	RW
MEA	30.3	++	RW	35.3	++	RW
PDA	26.6	++	GR	35.6	++	GR
CDA	22.1	+	W	27.6	+	W
WA	12.5	+	W	18.0	+	W

CRI, Cordyceps Research Institute; OA, oatmeal agar; MCM, *Schizophyllum* (mushroom) genetics complete medium plus yeast extract; HA, Hamada agar; MM, *Schizophyllum* (mushroom) genetics minimal medium; YMA, yeast-extract malt-extract peptone dextrose agar; SDAY, Sabouraud dextrose agar plus yeast extract; MPDA, Martin's peptone dextrose agar; ES, ebirose sucrose agar; MEA, malt-extract agar; PDA, potato dextrose agar; CDA, Czapek-dox agar; WA, water agar; CD, colony diameter; MD, mycelial density; MC, medium color; GR, grayish ruby; RW, reddish white; W, white.

developed on the media, except for CDA, as reported earlier [17]. No organic nitrogen source is present in CDA, and this may have been the reason for no change in the medium color. The optimum temperature for mycelial growth was 25°C, and no growth occurred at 35°C (Fig. 2). A pH of 7.0 was optimum for mycelial growth (Fig.

Fig. 1. Morphological characteristics of the *Cordyceps cardinalis* CRI C-10376 specimen. A, Natural specimen; B, Enlarged stroma; C, I, Perithecia; D, H, Ascus; E, G, Ascospores; F, J, Conidial stage (scale bars = 20 µm [G, H], 200 µm [I], 10 µm [J]). CRI, Cordyceps Research Institute.

Fig. 2. Effect of temperature on colony diameter of the two *Cordyceps cardinalis* isolates.

Fig. 3. Effect of pH on mycelial growth of the two *Cordyceps cardinalis* isolates.

3). At the end of culture, the pH of all media had decreased to approximately 5, except in one, which was adjusted to pH 4 at the time of medium preparation (Fig. 3).

The mycelial growth rate increased as the number of subcultures increased. Mycelial density remained compact until the 9th subculture, but changed to moderate after the 12th subculture. However, a significant change in medium color was noticed after subculturing. *C. cardinalis* imparted a red color to the medium, but the color disappeared after several subcultures, indicating a degeneration in the isolates after subculture. SDAY agar plates inoculated with the CRI C-10376 isolate remained grayish ruby until the 6th subculture but turned reddish white after the 9th subculture. SDAY agar plates inoculated with the CRI C-10734 isolate remained grayish ruby but changed to reddish white after the sixth subculture.

Effect of carbon source, nitrogen source, and mineral salts on mycelial growth. All of the carbon sources tested resulted in moderate mycelial density, except lactose, which was only thinly dense, similar to WA (Table 2). Fructose and xylose resulted in a better growth rate of the CRI C-10376 isolate, whereas maltose, starch, dextrose, sucrose, and lactose resulted in better growth of the CRI C-10734 isolate. None of the carbon sources tested induced color on media.

The use of maltose resulted in slightly better growth than fructose for all concentrations (Table 3). The differences in growth were very slight among the different maltose concentrations. Hence, 1% maltose was selected as the minimal optimum concentration. All maltose and fruc-

Table 2. Effect of carbon source on mycelial growth of the two *Cordyceps cardinalis* isolates

Carbon source	Isolate No.			
	CRI C-10376		CRI C-10734	
	CD	MD	CD	MD
Fructose	41.9	++	48.3	++
Maltose	37.5	++	51.2	++
Xylose	41.3	++	45.0	++
Starch	38.0	++	50.5	++
Dextrose	37.5	++	49.8	++
Dextrin	37.0	++	47.2	++
Sucrose	35.8	++	50.7	++
Lactose	38.3	+	49.7	+
WA	15.4	+	18.7	+

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; WA, water agar.

Table 3. Effect of carbon concentration on colony diameter of the two *Cordyceps cardinalis* isolates

Carbon source concentration (w/v)	Isolate No.	
	CRI C-10376	CRI C-10734
Maltose	1%	42.0
	2%	41.3
	3%	41.4
	4%	42.3
	5%	42.4
	6%	42.1
	7%	39.8
Fructose	1%	39.0
	2%	39.6
	3%	40.9
	4%	40.3
	5%	38.8
	6%	37.9
	7%	38.4
WA	16.7	18.4

CRI, Cordyceps Research Institute; WA, water agar.

Table 4. Effect of cereal extracts on mycelial growth of the two *Cordyceps cardinalis* isolates

Cereal extracts 2% (w/v)	Isolate No.			
	CRI C-10376		CRI C-10734	
	CD	MD	CD	MD
Oatmeal	44.8	++	48.3	++
Millet	33.0	++	43.8	++
Barley	34.3	+	37.5	+
Indian millet	30.5	+	37.8	++
German millet	30.4	+	44.4	+
Adlay	29.0	+	46.7	+
Brown rice	24.7	+	33.8	+
Black rice	19.0	+	30.9	+
WA	17.6	+	23.8	+

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; WA, water agar.

tose concentrations resulted in moderate mycelial density.

C. cardinalis isolates showed faster mycelial growth and higher mycelial density in oatmeal than in the other cereals (Table 4). Generally, the use of oatmeal and millet resulted in better growth. Black rice showed the least effect on mycelial growth. Only oatmeal and millet cultures developed moderate mycelial density (except Indian millet for the CRI C-10734 isolate), whereas all other cereals resulted in a thin mycelial density, similar to WA (Table 4). All of the cereals imparted a reddish white to grayish red color to the media.

Using oatmeal in the media resulted in better growth than that of millet for all concentrations tested (Table 5), which supports the results shown in Table 4. Oatmeal at

Table 5. Effect of cereal extract concentration on mycelial growth of the two *Cordyceps cardinalis* isolates

Grain extract concentration (w/v)		Isolate No.			
		CRI C-10376		CRI C-10734	
		CD	MD	CD	MD
Millet	1%	21.3	++	28.4	++
	2%	21.1	++	35.3	++
	3%	23.6	++	36.3	++
	4%	33.3	++	37.9	++
Oatmeal	1%	24.0	++	36.5	++
	2%	40.3	++	45.6	++
	3%	40.7	++	46.1	++
	4%	41.1	++	44.8	++
WA		18.1	+	24.8	+

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; WA, water agar.

Table 6. Effect of nitrogen source on mycelial growth of the two *Cordyceps cardinalis* isolates

Nitrogen source	Isolate No.							
	CRI C-10376				CRI C-10734			
	CD	MD	CC	MC	CD	MD	CC	MC
Peptone	44.3	+++	W	GR	47.8	+++	W	GR
Typtone	39.4	+++	W	GR	41.3	+++	W	GR
Yeast extract	34.6	+++	W	PR	32.1	+++	W	GR
Urea	8.90	+++	W	W	12.1	+++	W	W
KNO ₃	54.1	++	YW	W	56.5	++	W	W
NaNO ₃	53.0	++	YW	W	55.4	++	W	W
Alanine	44.9	++	W	PR	45.0	++	W	RW
NH ₄ NO ₃	41.9	++	YW	RW	45.6	++	W	RW
(NH ₄) ₂ HPO ₄	41.5	++	W	W	42.9	++	PY	W
Asparagine	39.4	++	W	PR	44.9	++	W	PR
(NH ₄) ₂ SO ₄	39.1	++	W	W	38.3	++	YW	W
(NH ₄) ₂ C ₄ H ₄ O ₆	27.5	++	LY	W	31.1	++	W	YW
WA	16.8	+	W	W	19.9	+	W	W

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; CC, colony color; MC, mycelial color; W, white; GR, grayish ruby; YW, yellowish white; RW, reddish white; PR, pale red; LY, light yellow; WA, water agar.

2~4% showed higher mycelial growth than 1%. Hence, 2% oatmeal was selected as the minimal optimum concentration. Both millet and oatmeal resulted in moderate mycelial density, irrespective of the concentration (Table 5). This study showed that chemically refined carbon sources did not impart any pigmentation to the medium; however, cereals induced a reddish color, probably due to the presence of organic nitrogen.

The use of peptone in the media resulted in the best mycelial growth among the organic nitrogen sources, whereas KNO₃ was the best among inorganic nitrogen sources (Table 6). All organic nitrogen source cultures developed compact mycelial density, whereas the inorganic nitrogen source cultures developed only moderate density. Interestingly, organic nitrogen source cultures developed only white colonies, while some of the inorganic nitrogen source cultures developed yellowish white to light yellow colonies (Table 6). The medium color change was very pronounced in the presence of the organic nitrogen sources (except urea), whereas only a few inorganic nitrogen sources induced a faint reddish color to the medium (Table 6). All media containing organic nitrogen sources developed a reddish color (Table 1), indicating that nitrogen sources, especially organic, were responsible for the development of a reddish color in the medium. This was also demonstrated by the observation no change in color occurred on the CDA plates that did not contain any organic nitrogen source.

KNO₃ promoted faster mycelial growth than peptone at all concentrations tested (Table 7). However, peptone resulted in a compact mycelial density (except at 0.5%),

Table 7. Effect of nitrogen source concentration on mycelial growth of the two *Cordyceps cardinalis* isolates

Nitrogen source concentration (w/v)		Isolate No.			
		CRI C-10376		CRI C-10734	
		CD	MD	CD	MD
Peptone	0.5%	42.4	++	44.9	++
	1%	38.0	+++	39.4	+++
	1.5%	37.3	+++	41.9	+++
	2%	37.9	+++	41.0	+++
	2.5%	37.5	+++	39.1	+++
	3%	36.3	+++	36.4	+++
	3.5%	33.3	+++	35.4	+++
KNO ₃	0.5%	45.5	++	49.3	++
	1%	45.8	++	50.6	++
	1.5%	43.9	++	47.8	++
	2%	41.6	++	44.6	++
	2.5%	41.0	++	42.6	++
	3%	39.6	++	39.8	++
	3.5%	38.1	++	38.4	++
WA		16.5	+	18.9	+

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; WA, water agar.

whereas KNO_3 produced only moderate density (Table 7). Mycelial density and growth rate usually have an opposing relationship on agar plates, as shown by Shrestha *et al.* [21]; the higher the colony density, the smaller the colony diameter. Developing a high mycelial density probably consumes more time, and, consequently, results in a smaller colony diameter. In other words, a poor medium contain less nutrition; hence, prompting the colony to grow rapidly in a radial direction in search of nutrition. Media with less nutrition cannot sustain a thicker mycelial density [21]. Peptone (1%) resulted in both a compact mycelial density and a longer colony diameter (Table 7).

Adding K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and NaCl to the media resulted in a better growth rate (Table 8). However, all of the mineral salts resulted in only a thin mycelial density, similar to WA. None of the mineral salts induced color to the medium. Mineral salts have diverse effects on fungal growth. They probably change the pH of the medium, and may even be toxic to fungal growth. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and NaCl did not result in any differences in colony diameter at all concentrations tested (Table 9), and mycelial den-

sity remained thin. Although mineral salts resulted in a faster growth rate than WA, no mineral salt was selected to formulate the CCM.

Using the CCM resulted in the same growth as OA but was better than PDA and SDAY (Table 10). However, all media induced an almost compact mycelial density. Organic nitrogen sources are important nutritional factors for mycelial growth of entomopathogenic fungi [21, 22, 24], which could be due to their insect hosts.

Optimum conditions for liquid culture. Mycelial dry wt. increased until the 7th day of liquid culture, after which wt. remained almost constant (Fig. 4). During the early stage of growth in a liquid culture, hyphae disperse in the medium and have direct contact with oxygen in the medium. As the culture gets older, mycelial pellets form, and the growth rate slows. Also, less oxygen availability and a depletion of nutrition, accompanied by metabolic by-products, retards growth. Mycelial growth can be quantified in a liquid culture under any environmental or nutritional condition, which is an advantage over agar culture. Furthermore, a liquid culture is much more preferable to an agar culture for industrial production. Growth is limited in agar culture, and it is difficult to separate the mycelia from the agar. Also, a liquid culture can be efficiently used as an inoculum for fruiting body production [18].

Not much variation in the dry wt of the inoculum was

Table 8. Effect of mineral salts on colony diameter of the two *Cordyceps cardinalis* isolates

Mineral salt	Isolate No.	
	CRI C-10376	CRI C-10734
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	46.4	44.8
NaCl	45.5	46.6
NaSO_4	45.5	42.9
K_2HPO_4	46.4	42.5
CaCO_3	45.0	42.4
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	44.3	44.2
$\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$	43.3	42.4
KH_2PO_4	43.3	40.1
CaCl_2	43.0	42.4
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	38.1	38.9
WA	14.0	19.5

CRI, Cordyceps Research Institute; WA, water agar.

Table 9. Effect of mineral salt concentration on colony diameter of the two *Cordyceps cardinalis* isolates

Mineral salt concentration (w/v)	Isolate No.	
	CRI C-10376	CRI C-10734
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.025%	48.7
	0.05%	48.8
	0.075%	48.9
	0.1%	49.1
NaCl	0.025%	47.6
	0.05%	48.3
	0.075%	48.5
	0.1%	49.0
NaCl	0.025%	48.3
	0.05%	48.5
	0.075%	48.5
	0.1%	48.5
WA	18.4	18.7

CRI, Cordyceps Research Institute; WA, water agar.

Table 10. Mycelial growth of the two *Cordyceps cardinalis* isolates on different media

Medium	Isolate No.			
	CRI C-10376		CRI C-10734	
	CD	MD	CD	MD
CCM	48.0	+++	48.1	+++
OA	47.0	+++	49.5	+++
PDA	42.0	+++	43.5	++
SDAY	35.6	+++	40.4	+++

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; CCM, *Cordyceps cardinalis* composite medium; OA, oatmeal agar; PDA, potato dextrose agar; SDAY, Sabouraud dextrose agar plus yeast extract.

Fig. 4. Effect of culture period on mycelial growth of the two *Cordyceps cardinalis* isolates.

Fig. 5. Effect of the number of mycelial discs on mycelial growth of the two *Cordyceps cardinalis* isolates.

observed in the liquid cultures (Fig. 5). Using five to seven mycelial discs resulted in better growth of isolate CRI C-10734, whereas growth of the CRI C-10376 isolate was unstable. Usually, mycelial growth increases as the amount of inoculum increases, but when the inoculum increases excessively, the nutritional sources become depleted quickly, retarding mycelial growth. The optimum number of mycelial discs was found to be five (Fig. 5).

C. cardinalis imparted color to the medium. In the present study, no carbon sources or mineral salts imparted color to the medium, whereas only organic nitrogen sources induced a reddish color to the medium. But, this medium color characteristic changed after several subculture generations. The mycelial growth rate increased slightly during later subcultures, but mycelial density decreased.

In summary, the use of OA, MPDA, and MCM resulted in good growth of *C. cardinalis*. The optimum temperature and pH were 25°C and 7, respectively. Nutritionally, 2% oatmeal, 1% maltose, and 1% peptone resulted in better growth, whereas mineral salts had a minimal effect. Hence, a CCM was formulated with 1% maltose, 2% oatmeal, 1% peptone and 2% agar. For liquid growth, inoculation of five mycelial discs in 100 mL of broth medium and an incubation period of 7 days showed the best results.

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