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Characteristics of *Metacordyceps yongmunensis*, a New Species from Korea

Gi-Ho Sung[1], Bhushan Shrestha[2] and Jae-Mo Sung[3]*

[1]*Mushroom Research Division, National Institute of Horticultural and Herbal Science, Rural Development Administration, Suwon 441-707, Korea*
[2]*Green Energy Mission/Nepal, Anam Nagar, Kathmandu, P.O. Box 10647, Nepal*
[3]*Cordyceps Institute of Mushtech, Chuncheon 200-936, Korea*

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***Metacordyceps yongmunensis* is a newly reported species from Korea, which is very similar to *Cordyceps* species in morphological characters. It grows on large lepidopteran pupa, and numerous white stromata grow on a single host. Mycelial growth characteristics of *M. yongmunensis* isolates were studied in different media and at different temperatures. Also, different carbon sources, nitrogen sources, and mineral salts were tested for mycelial growth of *M. yongmunensis*. *Schizophyllum* (mushroom) genetics complete medium plus yeast extract, *Schizophyllum* (mushroom) genetics minimal medium, and Martin's peptone dextrose agar produced longer colony diameters and more compact mycelial density than other media. The optimum temperature for mycelial growth was 25°C. Carbon sources such as sucrose, soluble starch, dextrose, glucose, dextrin, maltose, and fructose showed better mycelial growth, whereas peptone, yeast extract and tryptone resulted in the best mycelial growth of all of the nitrogen sources tested. All of the mineral salts tested showed similar growth as the control, except K_2HPO_4 which showed longer colony diameter and more compact mycelial density. The compact colonies were white and cottony with a greenish margin. The results showed that *M. yongmunensis* is an easy fungus to growas it grew from 30 to more than 50 mm in 2 wk.**

KEYWORDS : Carbon source, *Metacordyceps yongmunensis*, Nitrogen source, Optimum medium, Optimum temperature

Metacordyceps is a newly erected genus in the family Clavicipitaceae (Hypocreales: Ascomycota) [1]. Its morphological characters are very similar to those of *Cordyceps* species. *M. yongmunensis* G.H. Sung, J.M. Sung, and Spatafora is a newly reported *Metacordyceps* species from Korea (Fig. 1) [1]. Cultural characteristics of different *Cordyceps* species have been studied recently to produce them in artificial culture conditions [2-5]. In Korea, many studies have reported on the culture of different *Cordyceps* species [6-10]. In this regard, cultural characteristics of *M. yongmunensis* isolates were studied to understand the optimum medium and environmental conditions for its growth.

Materials and Methods

Fungal specimens and isolates. *M. yongmunensis* specimens EFCC C-2134 and EFCC C-2396 were collected in July and August 1998, respectively, from Mt. Yongmun in Gyunggi Province, Korea. The other *M. yongmunensis* EFCC C-8807 specimen was collected in July 2002 from Bukbang-myeon in Kangwon Province, Korea. All specimens were growing on large lepidopteran pupa. The ascospores were isolated from fresh stromata by the spore discharge method on 2% water agar (WA). WA blocks with numerous ascospores were transferred to potato dextrose agar (PDA) plates and incubated at 25°C for 3 wk. The specimens and isolates were preserved in the Entomopathogenic Fungal Culture Collection (EFCC) of Kangwon National University, Korea.

Effect of medium and temperature on mycelial growth of *M. yongmunensis*. Mycelial discs (5 mm) of *M. yongmunensis* isolates EFCC C-2134, EFCC C-2396, and EFCC C-8807 were grown on PDA agar plates and inoculated on 12 different agar media including WA (Tables 1 and 2). The medium compositions of Shrestha *et al*. [8] were followed. Agar was added at a concentration of 20 g/L for all media. The inoculated agar plates were incubated at 25°C under continuous white light conditions and observed for colony diameter (CD) and mycelial density (MD) after 2 wk of incubation. WA was used as the control. The effect of temperature on mycelial growth was also observed by inoculating mycelial discs (5 mm) on *Schizophyllum* (mushroom) genetics complete medium plus yeast extract (MCM) agar plates and incubating them at different temperatures ranging from 15°C to 35°C, with regular intervals of 5°C for 2 wk, after which CD and MD were observed. CD was measured in mm, while MD was categorized as thin (+), moderate (++), or compact (+++).

Effect of carbon source, nitrogen source, and mineral salts on mycelial growth of *M. munensis*. Martin's peptone dextrose agar (MPDA) was used to study the

*Corresponding author <E-mail : cordyceps@hanmail.net>

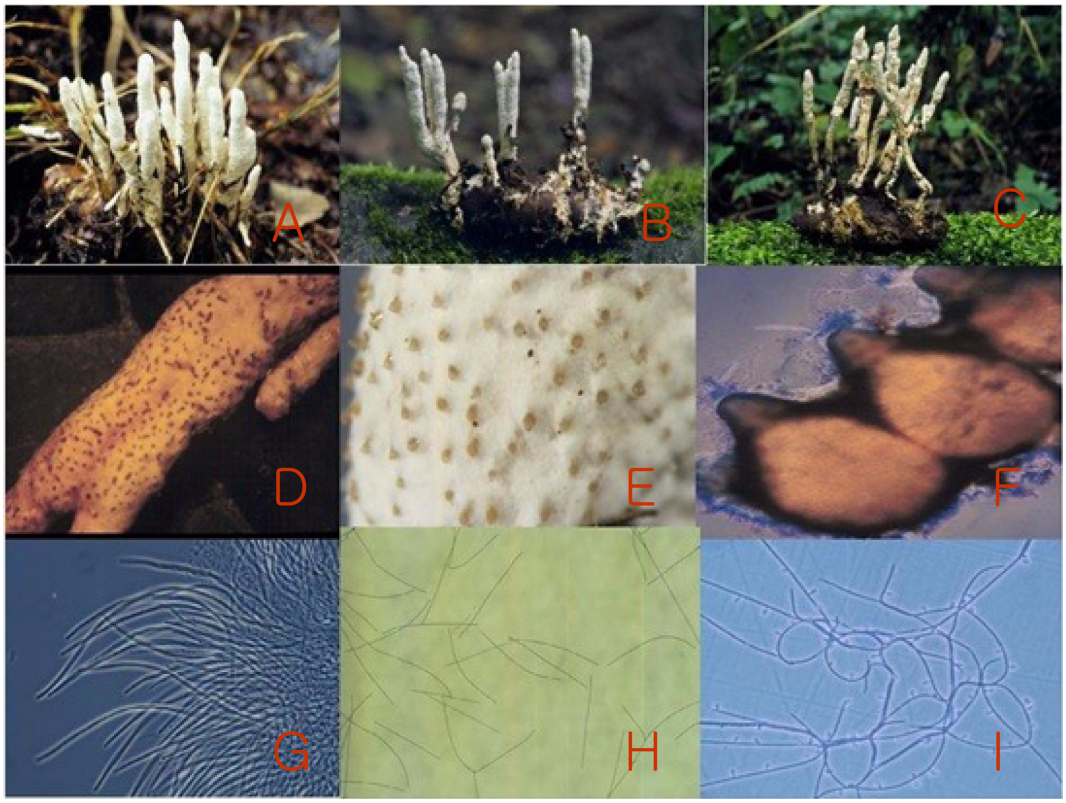

Fig. 1. Morphological characteristics of *Metacordyceps yongmunensis*. A~C, Natural specimens; D, Magnification of head; E, Immersed perithecia; F, Perithecia; G, Asci; H, Threadlike ascospore; I, Direct conidia formation from ascospores.

Table 1. Composition of culture media

Components (g/L)	Medium										
	PDA	MEA	SDA	MYA	SDAY	YMA	HA	MPDA	CDA	MM	MCM
Dextrose	20	20	40	4	40	10	20	10		20	20
Malt extract		20		10		3					
Sucrose									30		
Potato	200										
Peptone		1	10		10	5		5			2
Yeast extract				4	10	3	3				2
DL-Asparagine										2	
$NaNO_3$									3		
Ebiose							5				
$MgSO_4 \cdot 7H_2O$								0.5	0.5	0.5	0.5
KCl									0.5		
$FeSO_4 \cdot 7H_2O$									0.01		
KH_2PO_4								1		0.46	0.46
K_2HPO_4									1	1.0	1
Thiamine-HCl										120 μg	
Hyponex							3				

PDA, potato dextrose agar; MEA, malt-extract agar; SDA, Sabourand dextrose agar; MYA, malt-yeast agar; SDAY, Sabourand dextrose agar plus yeast extract; YMA, yeast-extract malt-extract peptone dextrose agar; HA, Hamada agar; MPDA, Martin's peptone dextrose agar; CDA, Czapek-dox agar; MM, *Schizophyllum* (mushroom) genetics minimal medium; MCM, *Schizophyllum* (mushroom) genetics complete medium plus yeast extract.

effect of carbon source, nitrogen source, and mineral salts on *M. yongmunensis* growth characteristics. Eleven different types of carbon sources were used in the MPDA agar medium at a concentration of 1% (w/v) to study the effect of carbon source on mycelial growth (Table 3). MPDA without dextrose was used as the control. Similarly, 12

Table 2. Effect of medium on growth characteristics of *Metacordyceps yongmunensis* isolates

Medium	Isolates					
	EFCC C-2134		EFCC C-2396		EFCC C-8807	
	CD	MD	CD	MD	CD	MD
MCM	54	+++	51	+++	49	+++
CDA	52	++	47	++	45	++
MM	50	+++	47	+++	33	+++
SDA	50	++	40	+++	40	+++
MPDA	48	+++	42	+++	43	+++
MEA	47	++	38	++	33	+++
PDA	44	+++	44	+++	45	+++
MYA	41	+++	38	+++	37	+++
YMA	40	+++	35	+++	40	+++
SDAY	32	+++	29	+++	35	+++
HA	27	+++	31	+++	32	+++
WA	15	+	25	+	18	T

CD, colony diameter; MD, mycelial density; MCM, *Schizophyllum* (mushroom) genetics complete medium plus yeast extract; CDA, Czapek-dox agar; MM, *Schizophyllum* (mushroom) genetics minimal medium; SDA, Sabourand dextrose agar; MPDA, Martin's peptone dextrose agar; MEA, malt-extract agar; PDA, potato dextrose agar; MYA, malt-yeast agar; YMA, yeast-extract malt-extract peptone dextrose agar; SDAY, Sabourand dextrose agar plus yeast extract; HA, Hamada agar; WA, water agar.

Table 3. Effect of carbon source on growth characteristics of *Metacordyceps yongmunensis* isolates

Carbon source	Isolates					
	EFCC C-2134		EFCC C-2396		EFCC C-8807	
	CD	MD	CD	MD	CD	MD
Sucrose	46	+++	39	+++	31	+++
Lactose	46	+	34	+	29	+
Soluble starch	45	+++	37	++	32	++
Dextrose	45	+++	41	+++	36	+++
Maltose	44	++	36	+++	28	+++
Glucose	44	+++	38	+++	30	+++
Dextrin	44	+++	38	++	36	++
Fructose	43	++	41	+++	33	+++
Arabinose	36	+	31	++	26	+++
Galactose	8	+	8	+	8	+
Xylose	8	++	9	++	8	++
Control	38	+	35	+	26	+

CD, colony diameter; MD, mycelial density.

different types of organic and inorganic nitrogen sources were added to MPDA at a concentration of 0.5% (w/v) to study the effect of nitrogen source on mycelial growth (Table 4). MPDA without peptone was used as control. Ten different types of mineral salts were also added to MPDA at a concentration of 0.05% (w/v) to study the effect of mineral salts on mycelial growth (Table 5). MPDA without $MgSO_4 \cdot 7H_2O$ and KH_2PO_4 was used as the control. Growth characteristics were recorded after 2 wk of incubation under continuous white light condition at 25°C.

Table 4. Effect of nitrogen source on growth characteristics of *Metacordyceps yongmunensis* isolates

Nitrogen source	Isolates					
	EFCC C-2134		EFCC C-2396		EFCC C-8807	
	CD	MD	CD	MD	CD	MD
Peptone	44	+++	39	+++	38	+++
Yeast extract	44	+++	40	+++	42	+++
Tryptone	41	+++	41	+++	41	+++
KNO_3	38	++	35	++	31	++
$NaNO_3$	38	++	41	++	33	++
dl-Alanine	35	++	29	+++	21	+++
Ammonium tartrate	32	++	23	+++	21	+++
Glycine	31	+++	27	+++	21	+++
NH_4NO_3	28	+++	23	+++	19	+++
L-Asparagine	18	+++	24	+++	20	+++
$(NH_4)_2SO_4$	17	+++	18	+++	17	+++
$(NH_4)_3PO_4$	14	+++	15	+++	18	+++
Control	26	+	30	+	26	+

CD, colony diameter; MD, mycelial density.

Table 5. Effect of mineral salts on growth characteristics of *Metacordyceps yongmunensis* isolates

Mineral salt	Isolates					
	EFCC C-2134		EFCC C-2396		EFCC C-8807	
	CD	MD	CD	MD	CD	MD
K_2HPO_4	53	+++	50	+++	50	+++
$CaCO_3$	41	+++	44	+++	45	++
$MgSO_4 \cdot 7H_2O$	41	++	41	++	32	++
$CaCl_2 \cdot 2H_2O$	40	++	36	++	36	++
NaCl	40	++	33	++	36	++
$MnSO_4 \cdot 7H_2O$	37	++	39	++	32	++
Na_2SO_4	37	++	37	++	27	++
$ZnSO_4 \cdot 7H_2O$	36	++	34	++	32	++
KH_2PO_4	35	++	32	+++	28	+++
KCl	35	+++	36	++	40	+++
Control	38	++	37	++	31	++

CD, colony diameter; MD, mycelial density.

CD and MD were measured as described above.

Results and Discussion

Effect of medium and temperature on mycelial growth of *M. yongmunensis*. MCM produced the widest CD, followed by Czapek-dox agar (CDA), *Schizophyllum* (mushroom) genetics minimal medium (MM) and PDA (Table 2). All media produced compact MD, except CDA, Sabourand dextrose agar (SDA), and malt-extract agar (MEA). CDA produced moderate MD in all isolates, whereas SDA produced moderate MD only in the EFCC C-2134 isolate and MEA in the EFCC C-2134 and C-2396 isolates (Table 2, Fig. 2). In total, MCM, MM, and MPDA produced better mycelial growth than the other

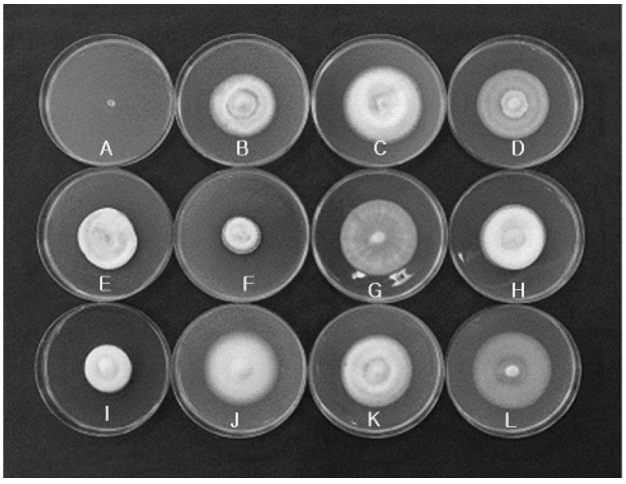

Fig. 2. Effect of medium on mycelial growth of *Metacordyceps yongmunensis* isolate EFCC C-2134. A, Water agar; B, Potato dextrose agar; C, *Schizophyllum* (mushroom) genetics complete medium plus yeast extract; D, Malt-extract agar; E, Yeast-extract malt-extract peptone dextrose agar; F, Hamada agar; G, Sabourand dextrose agar; H, Malt-yeast agar; I, Sabourand dextrose agar plus yeast extract; J, *Schizophyllum* (mushroom) genetics minimal medium; K, Martin's peptone dextrose agar; L, Czapek-dox agar.

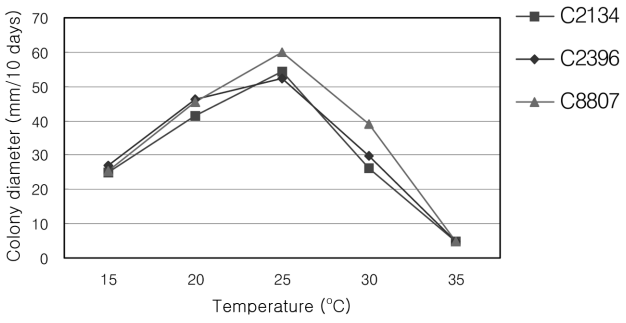

Fig. 3. Effect of temperature on mycelial growth of three *Metacordyceps yongmunensis* isolates.

media. Hence, MCM was selected for the experiment to observe the effect of temperature on mycelial growth. Compact isolates produced white, cottony colonies with greenish margins. WA always produced thin MD, almost invisible; however, WA sustained mycelial growth by showing continuous radial growth. Mycelial growth was highest at 25°C, followed by 20°C (Fig. 3). No mycelial growth occurred at 35°C, however no loss of viability was observed. The mycelium started growing again after a transfer from 35°C to 25°C.

Effect of carbon source, nitrogen source, and mineral salts on mycelial growth of *M. yongmunensis*. All the carbon sources produced larger CDs than the control, except arabinose, galactose, and xylose (Table 3). Galactose and xylose produced almost no mycelial growth in any of the isolates, whereas arabinose produced colonies slightly smaller than the control (Table 3). Lactose and galactose produced only thin MD, which was similar to the control. In the C-2134 isolate CDs on different carbon sources were similar; however, only sucrose, soluble starch, dextrose, glucose, and dextrin produced compact MD. In the C-2396 isolate, dextrose and fructose produced the highest CD followed by sucrose and glucose. Sucrose, dextrose, maltose, glucose, and fructose produced compact MD. In the third isolate, dextrose and dextrin produced the largest CDs, followed by fructose and soluble starch. However, sucrose, dextrose, maltose, glucose, fructose, and arabinose produced compact MD. Thus, of 11 different carbon sources, sucrose, soluble starch, dextrose, glucose, dextrin, maltose, and fructose resulted in better colony growth and MD than the others. It could not be determined whether galactose or xylose inhibited mycelial growth of *M. yongmunensis*.

In all three isolates, peptone, yeast extract, and tryptone produced large CDs and compact MD (Table 4). All nitrogen sources resulted in compact MD except KNO_3 and $NaNO_3$, which produced moderate MD. Only thin MD was produced in the control. dl-Alanine, ammonium tartrate, glycine, NH_4NO_3, L-asparagine, $(NH_4)_2SO_4$, and $(NH_4)_3PO_4$ produced shorter CDs than the control, but all of them produced compact MD. Complex organic nitrogen sources such as peptone, yeast extract, and tryptone resulted in higher mycelial growth than the others. The higher growth of mycelia might be due to the presence of different types of amino acids and inorganic nitrogen sources present in the peptone, yeast extract, and tryptone.

Most of the mineral salts produced moderate MD, whereas K_2HPO_4, $CaCO_3$, KH_2PO_4, and KCl produced either moderate or compact MD (Table 5). Some of the salts such as $MnSO_4 \cdot 7H_2O$, Na_2SO_4, $ZnSO_4 \cdot 7H_2O$, KH_2PO_4, and KCl produced smaller CDs than the control. Thus, mineral salts, except K_2HPO_4, $CaCO_3$, KH_2PO_4, and KCl, had no visible effect on the mycelial growth of *M. yongmunensis*.

Shrestha *et al*. [8] showed that *Cordyceps militaris* produces various types of pigmentation on different agar media, but *M. yongmunensis* produced no pigmentation except a greenish margin on the colonies. In this study, it was clearly shown that MD of *M. yongmunensis* was thin in the absence of both carbon and nitrogen sources. Further studies are necessary to determine the optimum culture conditions to produce fruiting bodies.

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