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韓應昆誌 27(1) : 41—46 (1988)
Korean J. Appl. Entomol.

Effect of Nutrients and pH on the Growth and Sporulation of Four Entomogenous Hypomycetes Fungi (Deuteromycotina)

培地의 營養源 및 pH가 數種 昆虫寄生菌의 菌糸生長 및 胞子生產에 미치는 影響

Dae Joon Im[1], Moon Hong Lee[1], R.M. Aguda[2] and M.C. Rombach[2]
任大準[1] · 李文弘[1] · R.M. Aguda[2] · M.C. Rombach[2]

ABSTRACT Growth of *Metarrhizium flavoviride* var. *minus* and *Hirsutella strigosa* showed good yield in the carbon source media adding dextrose, starch and saccharose, but *Hirsutella* sp. from Korea grew well in the other media except in the dextrose media. Yeast extract was necessary for the mecelial growth of the fungi, but the fungi tested in this experiment showed a difference in the amount of required yeast extract. Growth of *Nomurea rileyi* was fastidious in the carbon and nitrogen sources media and the optimum pH of the media for growth was at 6.7. Sporulation of *M. flavoviride* var. *minus* was high on media, containing 1%～2% of yeast extract as nitrogen and carbon source media, but *N. rileyi* sporulated abundantly on the media with nitrogen and dextrose.

KEY WORDS entomogenous hypomycetes, nutrient, pH, mycelial growth, sporulation.

抄 錄 數種 昆虫寄生菌의 菌糸生長 및 胞子形成에 미치는 炭素源 (dextrose, starch, sucrose, sorbitol)과 窒素源(Yeast extract 濃度別) 培地 및 pH의 影響을 調査하였다.
Metarrhizium flavoviride var. *minus*와 *Hirsutella strigosa*는 dextrose, starch, sucrose 源培地에서 菌糸生長이 많은 反面 韓國에서 發見된 *Hirsutella* sp.(Korea)는 dextrose를 除外한 培地에서 좋은 生長을 보였다. 使用한 窒素源은 菌糸의 生長에 必須的이나 寄生菌의 種類에 따라 生長의 差異가 있었다. 그러나 *Nomurea rileyi*는 培地源에 關係없이 까다로운 菌糸生長을 나타냈으며 供試菌 모두 pH 部近의 培地에서 菌糸生產量이 많았다.
한편 2種 寄生菌을 供試하여 胞子形成을 調査한 結果, *M. flavoviride* var. *minus*는 供試窒素源과 dextrose源 培地에서 胞子生產이 많아 寄生菌別 培地 選擇性의 差異를 나타냈다.

檢 索 語 昆虫寄生菌, 培地營養, pH, 菌糸生長, 胞子形成

Microbial control of insect pests using entomogenous fungi is increasing in importance. Mass culture and sporulation with good growth are important considerations in accordance with maintaining high virulence. Mass production methods of entomogenous hypomycetes fungi were successfully developed by using solid nutrient agar and liquid media (Agudelo & Falcon 1983, McCoy et al. 1972). However, the sporulation is often determined by the type of medium used for culturing the fungi (Schaeffenberg 1964).

Several authors reported studies on nutrition of entomogenous fungi which were concerned with the effect of the medium on the growth and sporulation of the fungi rather than on virulence (Barnes et al. 1975, Campbell et al. 1983). However, other reports showed that virulence of the fungi is dependent on the character of the culture substrate and on the number of transfers

1 Department of Entomology, Agricultural Sciences Institute, RDA, Suwon. Korea. 440～707(農業技術研究所 生物部 昆虫科)
2 Department of Entomology, International Rice Research Institute, P.O. Box 933, Manila, Philippines. (國際米作研究所 昆虫科)

in artificial culture. The relative amounts of carbon and nitrogen available to the fungus determine whether they produce mainly conidia or mycelia.

Further, Smith & Grula(1981) reported that the carbon-energy source compounds having great chemical variability (from glucose to complex wax) allow extensive growth of *Beauveria bassiana*. Boucias & Pendland (1984) reported the nutritional substrates including cuticular extract as promising for the conidial germination of *Nomurea rileyi*. Campbell et al.(1978) showed the growth and sporulation of two hypomycetes fungi, *B. bassiana* and *Metarrhizium anisopliae*, on the liquid media containing 24 amino acids. The latter fungus produced greater mycelial growth in neopeptone (Difco) than in sodium nitrate (Roberts 1966).

Information on nutrient utilization of these fungi is imoprtant for the development of efficient mass prodution media. Hence, this study was conducted to determine the growth and sporulation of the four fungi species in different liquid and solid culture media. Also, effect of pH of the culture medium on the growth and sporulation was tested.

MATERIALS AND METHODS

Submerged growth, different carbon and nitrogen source

Four species of fungi, *M. flavoviride* var. *minus*, *N. rileyi*, *Hirsutella* sp. (Korean isolate) and *H. strigosa* were tested. The origin of the fungi is given in Table 1.

Different carbon sources such as dextrose, starch, saccharose, and sorbitol were tested. The different carbon sources 2% were mixed with equal volume of 1% yeast extract. In another test, different concentrations of yeast extract such as 5, 1, 2, 4 and 8% were used to determined their effects on the growth of the fungi. Basal salt solution was used as basic liquid for all the media (Macleod 1959a). The media were placed in small bottles (dia 3.5cm×9cm long) and were autoclaved at 110°C for 15 minutes. Then the media were cooled and inoculated with 2.5ml of the fungi suspension respectively. The fungi suspension or inoculum was grown using liquid media of Emerson's YpSs Sabouraud dextrose in the shaker. The inoculated bottles mere grown in rotary shaker for 3-5days, until the best medium has achieved optimum growth. Growth of the fungi was measured by filtering the mycelium in a dried pre-weighed filter paper using a buchner funnel attached to a vacuum flask. The filtered mycelia were dried up in the oven at 70°C for 24 hours and after which they were weighed. Three replicates per treatment per fungus species were prepared.

Table 1. Origin of fungal isolates

ARSEF[a]	Fungus	Host
2044	*Hirsutella strigosa*	Brown planthopper, *Nilaparvata lugens*(Stal), (Philippines)
2187	*Hirsutella* sp.	*N. lugens*, (Korea)
1547	*Metarrhizium flavoviride*var. *minus*	*N. lugens* (Philippines)
2174	*Nomurea rileyi*	Rice leaf folder, *Cnaphalocrosis medinalis* Guenee, (Philippines)

[a] ARSEF numbers refer to the USDA collection of entomopathogenic fungal culture. Boyce Thompson Institute for Plant Research, Ithaca, NY14853, U.S.A.

Submerged growth, influence of pH

The same fungi as the above test were used. A mixture 1% dextrose those 1% yeast extract was used as culture medium in determining the effect of pH on the growth of the four species of fungi. The pH of the culture medium was adjusted into 3.5, 5.0, 6.0, 6.7, 7.0, 8.0 and 9.5 respectively. These media were placed in small bottles and autoclaved at 110°C for 15 minutes. They were cooled for 24 hours. The pH was again readjusted using sterile acid and basic buffer. The bottles were inoculated individually with 2.5ml of their respective fungal suspenion. Three replicates per treatment per fungus were done. The rest is the same as the above experiments.

Aerial growth, sporulation on different media

Sporulation of *M. flavoviride* var. *minus* and *N. rileyi* were determined by using different solid media comprising different sources of carbon as well as nitrogen. The sources of carbon such as dextrose, saccharose, starch and sorbitol added 1% yeast extract were used. Also, 1, 2 and 4% of yeast extract with 1% dextrose were tested. To all media 1.5% agar was added. They autoclaved and poured in sterile plates. The plates were inoculated with 0.3ml of the conidia suspension. Conidia suspension were prepared by suspending dry conidia in sterile water containing 0.15% Tween 80. The inoculated plates were incubated for about 10 days at room temperature or until conidiation was completed. Since conidia developed above a tough mycelial mat that covered the medium, conidia were easily harvested with a rubber policeman without disturbing the mat or the medium. The conidia produced were measured by standard haemacytometer techniques (conidia/ml) and were compared to the measurement taken by turbidity with the spectrometer.

RESULTS AND DISCUSSION

Submerged growth, different carbon and nitrogen sources

The growth of the different fungi species on four carbon sources as well as on six nitrogen sources is shown in Table 2. *M. flavoviride* var. *minus* grew very well in all the media tested except sorbitol, probably because sorbitol is a sugar alcohol and was already oxidized thus weaker source of carbon for the fungus. The growth of *H. strigosa* was remarkably high in all the media used and yielded highest growth in dextrose. *Hirsutella* sp. (Korea) did not grow in this medium. This fungus grow well in saccharose as did *N. rileyi* and *M. flavoviride* var. *minus*. However, the different fungi showed significantly different reactions in growth in all carbon sources used except in saccharose (Campbell et al. 1983). As reported by Schaerffenberg(1964), pure carbon substrates such as glucose, maltose, and dextrose agar can be successfully used in the culture of some pathogenic fungi only if albumin is added. Further, Benham & Miranda(1953) has shown that fungi can not thrive if the substrate contains no vitamin B_1 or B_2. In this experiment, these vitamin requirements are supplied by the addition of 1% yeast extract to the medium.

The mycelial growth of *M. flavoviride* var. *minus*, *N. rileyi*, *H. strigosa* and *Hirsutella* sp. (Korea) were also determined using different concentrations of yeast extract as nitrogen source. It was shown in Table 2 that the *M. flavoviride* var. *minus* and *H. strigosa* grew very well in 1% dextrose with 4% yeast extract. However, the two fungi,

Table 2. Growth in grams of the different fungi species on different media

Media	*Metarrhizium flavoviride*	*Nomuraea rileyi*	*Hirstella* sp.(Korea)	*Hirsutella strigosa*
Carbon Source				
DYE	256.64 a (a)	93.38 ab(b)	6.69 c (c)	297.79 a (a)
StYe	249.15 a (a)	35.35 b (c)	117.00 b (b)	159.58 b (b)
SaYe	280.34 a (a)	145.23 a (a)	223.22 a (a)	208.33 b (a)
SoYe	89.53 b (b)	88.34 ab(b)	186.6 ab(a)	189.36 b (a)
Nitrogen Source				
Ye 0D	59.19 ab(a)	22.70 bc(a)	4.15 b (a)	43.12 d (a)
Ye 5D	201.97 ab(a)	51.44 b (a)	146.41 a (a)	133.79 ad(a)
Ye 1D	200.91 ab(a)	97.76 a (b)	111.51 a (b)	233.18 bc(a)
Ye 2D	190.63 ab(a)	0.00 c (b)	225.37 a (a)	269.92 b (a)
Ye 4D	239.32 a (b)	0.00 c (c)	0.00 b (c)	518.00 a (a)
Ye 8D	15.96 b (c)	123.97 a (b)	210.62 a (a)	204.38 bc(a)

In a column, means followed by a common letter are not significantly different($p = 0.05$) by DMRT.
In a row, means followed by a common letter in parenthesis are not significantly different($p = 0.05$) by DMRT.

DYe-Dextrose 2% + Yeast extract 1%
StYe-Starch 2% + Yeast extract 1%
SaYe-Saccharose 2% + Yeast extract 1%
SoYe-Sorbitol 2% + Yeast extract 1%
Ye 0D-0% Yeast extract + Dextrose 1%
Ye 5D-.5% Yeast extract + Dextrose 1%
Ye 1D-1% Yeast extract + Dextrose 1%
Ye 2D-2% Yeast extract + Dextrose 1%
Ye 4D-4% Yeast extract + Dextrose 1%
Ye 8D-8% Yeast extract + Dextrose 1%

Table 3. Growth in grams of the different fungi species on the different pH of medium

pH	*Metarrhizium flavoviride*	*Nomuraea rileyi*	*Hirsutella* sp. (Korea)	*Hirsutella strigosa*
3.5	3.42 c (b)	108.33 b (a)	91.28 bc (ab)	0.00 c (b)
5.0	142.97 b (b)	245.50 a (a)	156.56 a (b)	53.472 ab(b)
6.0	175.58 ab(a)	229.32 a (a)	146.92 ab (ab)	81.950 a (b)
6.7	197.63 a (b)	251.70 a (a)	138.83 ab (c)	45.46 b (d)
7.0	200.00 a (ab)	245.50 a (a)	129.88 ab (bc)	52.287 ab(c)
8.0	171.96 ab(a)	198.35 a (a)	117.07 abc(b)	33.050 b (c)
9.5	27.43 c (bc)	81.15 b (a)	62.33 c (ab)	0.00 c (c)

In a column, means followed by a common letter are not significantly different ($p = 0.05$) by DMRT.
In a row, means followed by a common letter in parenthesis are not significantly different ($p = 0.05$) by DMRT.

N. rileyi and *Hirsutella* sp. (Korea), showed no growth in this medium. *Hirsutella* sp. (Korea) grew well in the medium with 2% yeast extract while *N. rileyi* in 8% yeast extract.

In general, the nutritional requirements of *N. rileyi* appear to be more fastidious than that reported for other entomogenous fungi(Boucias & Pendland, 1984). It was also noted that all the fungi achieved the optimal mycelial growth from 0.5% to 8% yeast extract except for *N. rileyi* and *Hirsutella* sp. (Korea) in 2% and 4% yeast extract (Table 2). Yeast extract is known to be an excellant source of B-compex vitamins and also the best peptone source of both mycelial growth and sporulation. This was proved by Barnes et al.(1975) on his studies of growth and sporulation of *M. anisopliae* on media containing various peptone sources. Highly informative in this respect is the culture experiments which MacLeod (1959a,

Table 4. Sporulation of *Metarrhizium flavoviride* and *Nomuraea rileyi* on different solid media

Media	Spore count (spores/ml)	
Carbon source	*Metarrhizium flavoviride*	*Nomuraea rileyi*
DYE	1.042×10^8	6.09×10^8
StYE	2.432×10^8	1.26×10^6
SaYE	2.524×10^8	3.80×10^6
SoYE	1.733×10^8	6.30×10^5
Nitrogen Source		
YE 1 D	2.888×10^8	6.74×10^8
YE 2 D	4.240×10^7	1.13×10^9
YE 4 D	4.450×10^6	3.49×10^8

DYE-Dextrose 1% + Yeast Extract 1% StYE-Starch 1% + Yeast Extract 1%
SaYE-Saccharose 1% + Yeat Extract 1% SoYE-Sorbitol 1% + Yeas Extract 1%
YE 1D-1% Yeast Extracs + Dextrose(1%) YE 2D-2% Yeast Extrtact + Dextrose(1%)
YE 4D-4% Yeast Etxtract + Dextrose(1%)

b) performed using *H. gigantea* Petch in which he obtained optimum growth and the highest yield of mycelium in yeast extract-dextrose medium.

In this experiment, it showed that the four fungi could be grown in yeast extract-detrose medium. This is probably because of the vitamins profusely present in yeast and the carbon in dextrose which are very important as growth factors for the fungi.

Submerged growth, influence of pH

Mycelial growth of *M. flavoviride* var. *minus*, *N. rileyi*, *H. strigosa* and *Hirsutella* sp. (Korea) in different pH of the media were illustrated in Table 3. The results showed that all the fungi tested grew well from the medium pH 5 to 8. The same result of growth obtained from *Paecilomyces farinosus* at pH 5.5 (Agudelo & Falcon 1983) and from *H. thompsonii* at pH 6.0 (McCoy et al. 1972). They yielded highest growth on the media pH which were near to the range or to the level equal to or greater than the initial pH of the medium which is 6.7. However, the growth of these fungi was suppressed from the very acidic and basic medium. This suggests that these fungi could be successfully grown in the media with pH which is slightly basic or acidic near to neutral.

Aerial growth, sporulation on different media

Sporulation of *M. flavoviride* var. *minus* and *N. rileyi* on different media with different carbon sources and nitrogen sources is shown in Table 4. The best carbon source for sporulation of *N. rileyi* was dextrose while sorbitol yielded the lowest. *M. flavoride* var. *minus* showed high conidia production in all the carbon sources used. This fungus also sporulated well on 1% yeast extract.

It showed that the higher the percentage of the yeast extract in the medium the lower the sporulation. *N. rileyi*, on the other hand, produced the highest conidia on the medium with 2% yeast extract. Further, the results showed that all the yeast extract concentrations tested showed remarkably high sporulation.

Thus, the relative amounts of carbon and nitrogen available to the fungi determine whether they produce mainly conidia or mycelia (Schaerffenberg 1964). Roberts (1966) investigated the effect of various inorganic and organic nitrogen sources on the growth and toxin production of *M. anisopliae*. In this experiment it is evident that yeast extract is a good source of nitrogen and vitamins utilized both fungi for sporulation.

ACKNOWLEDGEMENTS

We thank the International Rice Research Institute and the Rural Development Administration of Korea for funding this research through the IRRI-RDA Cooperative Agreement.

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(Received May 7, 1988)