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Infectivity Suppression of the Entomopathogenic Fungus, Nomuraea rileyi, by Galleria melonella Hemolymph【Scientific note】

大蠟蛾幼蟲血液對綠殭菌感染寄主之抑制作用【科學短訊】

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Abstract

The 5th instar larvae of Spodoptera litura can be infected with both Nomuraea rileyi and Beauveria bassiana through cuticular contact inoculation with conidia, whereas those of Galleria mellonella can only be infected with B. bassiana but not with N. rileyi. Similarly, the fungal infection in both insect species was obtained through inoculation by injecting their hyphal bodies into a hemocoel. When the N. rileyi hyphal bodies were treated with the hemolymph of G. mellonella without hemocyte, the number of colonies on solid PDB+Y medium formed from the treated hyphal bodies was greatly reduced to approx. 43% compared with that of the control group grown in medium only. However, when the hemolymph was pre-treated with proteinase K or 95oC for 5 min, the number of colonies remained at 67 or 95%, respectively. When assayed with the hemolymph of S. litura without hemocyte, the number of N. rileyi colonies remained the same as the control group, indicating that S. litura hemolymph does not contain a humoral factor against this fungus. Therefore, the hemolymph of G. mellonella contains humoral factor(s), which seems to be proteinaceous based on its inactivation by proteinase and heat, to suppress infection of G. mellonella with N. rileyi.

摘要

以綠殭菌 (Nomuraea rileyi) 與白殭菌 (Beauveria bassiana) 的分生孢子 (conidia) 黏附昆蟲體表方式可感染斜紋夜蛾 (Spodoptera litura) 五齡幼蟲，而大蠟蛾 (Galleria mellonella) 幼蟲可受白殭菌的感染但不受綠殭菌的感染；另以注射綠殭菌與白殭菌的菌絲體 (hyphal body) 方式也得到相同結果。抽取大蠟蛾幼蟲的血液經去除血球後，可抑制綠殭菌菌絲體在PDB + Y固體培養基的生長，產生的菌落數與對照組相比降至僅 43%；但將血液先分別以蛋白質分解酵素 (proteinase K) 或 95°C 加熱 5 min 處理，則產生的菌落數與對照組相比仍各維持在 67 及 95%。若以斜紋夜蛾幼蟲的血液，無論是否予以不同處理，產生的綠殭菌菌落數皆與對照組相當。由以上結果顯示大蠟蛾幼蟲不受綠殭菌感染，乃因其血液中之體液性因子可抑制其菌絲體的生長，而斜紋夜蛾幼蟲受感染，則由於其血液中不含此因子，以抑制菌絲體生長之故。因此，大蠟蛾血液內含有疑似蛋白質成分，能抑制其受綠殭菌之感染。

Key words: entomopathogenic fungus, Nomuraea rileyi, Galleria mellonella, Spodoptera litura, hemolymph
關鍵詞: 蟲生真菌、綠殭菌、大蠟蛾、斜紋夜蛾、血液。

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大蠟蛾幼蟲血液對綠殭菌感染寄主之抑制作用

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摘　要

以綠殭菌 (*Nomuraea rileyi*) 與白殭菌 (*Beauveria bassiana*) 的分生孢子 (conidia) 黏附昆蟲體表方式可感染斜紋夜蛾 (*Spodoptera litura*) 五齡幼蟲，而大蠟蛾 (*Galleria mellonella*) 幼蟲可受白殭菌的感染但不受綠殭菌的感染；另以注射綠殭菌與白殭菌的菌絲體 (hyphal body) 方式也得到相同結果。抽取大蠟蛾幼蟲的血液經去除血球後，可抑制綠殭菌菌絲體在 PDB＋Y 固體培養基的生長，產生的菌落數與對照組相比降至僅 43%；但將血液先分別以蛋白質分解酵素 (proteinase K) 或 95℃ 加熱 5 min 處理，則產生的菌落數與對照組相比仍各維持在 67 及 95%。若以斜紋夜蛾幼蟲的血液，無論是否予以不同處理，產生的綠殭菌菌落數皆與對照組相當。由以上結果顯示大蠟蛾幼蟲不受綠殭菌感染，乃因其血液中之體液性因子可抑制其菌絲體的生長，而斜紋夜蛾幼蟲受感染，則由於其血液中不含此因子，以抑制菌絲體生長之故。因此，大蠟蛾血液內含有疑似蛋白質成分，能抑制其受綠殭菌之感染。

關鍵詞：蟲生真菌、綠殭菌、大蠟蛾、斜紋夜蛾、血液

蟲生真菌如綠殭菌 (*Nomuraea riley*)、白殭菌 (*Beauveria bassiana*) 等廣泛分布於全球，其能引發鱗翅目 (Lepidoptera) 昆蟲的地區性流行病 (Ignoffo, 1981; Tanada and Kaya, 1993)。蟲生真菌感染寄主昆蟲之過程首由分生孢子 (conidia) 黏附在昆蟲體表，其次在孢子發芽時藉由分泌酵素及機械作用穿透體壁進入血腔 (Boucias and Pendland, 1991; Pendland *et al*., 1993; Hayek and St. Leger, 1994)。隨後其菌絲體 (hyphal body) 進入血腔克服昆蟲細胞性及體液性免疫機制，完成感染寄主昆蟲 (Götz, 1991; Mazet *et al*., 1994; Vilcinskas *et al*., 1997; Griesch and Vilcinskas, 1998; Tseng *et al*., 2008)。

斜紋夜蛾 (*Spodoptera litura*) 已被證實可受綠殭菌與白殭菌感染 (Shih, 2005; Uma

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Maheswara Rao *et al.*, 2006)，而大蠟蛾 (*Galleria mellonella*) 則可受白殭菌感染 (Wojda *et al.*, 2009)。我們分別取 1×10^8 conidia/mL 的白殭菌與綠殭菌的分生孢子置入 10 cm 培養皿內，再將各 10 隻的五齡斜紋夜蛾與大蠟蛾幼蟲依序置入培養皿內 1 min，使分生孢子能黏附在昆蟲體表上，進行體表接觸接種，隨後將蟲體置於 25℃ 下 10 天。表一結果顯示斜紋夜蛾可受白殭菌 (86.67%) 與綠殭菌 (80%) 感染形成殭蟲，但大蠟蛾僅受白殭菌感染 (96.7%)，而綠殭菌則無法成功地感染。爲進一步證實綠殭菌無法感染大蠟蛾，是否因蟲體體表具防禦性所致，我們先把白殭菌與綠殭菌的分生孢子置於 SMA＋Y 液體培養基 (Shih, 2005)，在 25℃ 培養 2 天，使其發芽成菌絲體。將各 10 隻的五齡斜紋夜蛾與大蠟蛾幼蟲分別以微量注射器注入 1×10^5 hyphal bodies/larva，隨後將蟲體置於 25℃ 下 10 天。結果顯示斜紋夜蛾均可受白殭菌 (100%) 與綠殭菌 (93.33%) 感染形成殭蟲，但大蠟蛾僅受白殭菌感染 (100%)，而綠殭菌則無法成功地感染 (表二)。所以即使藉人工方式突破體壁障礙，綠殭菌也難以成功地感染大蠟蛾，但白殭菌卻可成功地感染，顯示大蠟蛾血液的免疫反應可成功抑制綠殭菌感染；相對地，斜紋夜蛾的血液之免疫反應則無法抑制白殭菌與綠殭菌的感染。

我們先前試驗已經證實大蠟蛾的血球可對抗綠殭菌的分生孢子及菌絲體，進行吞噬 (phagocytosis) 及瘤結形成 (nodule formation) 等細胞性免疫 (cellular immunity) 反應 (Tseng *et al.*, 2008)。爲了確認體液性免疫 (humoral immunity) 反應是否參與抑制綠殭菌感染，我們抽取五齡大蠟蛾幼蟲血液並予以離心 (1,500 rpm, 5 min)，取除去血球之上清液備用。再取 10 μl 的菌絲體懸浮液 (1×10^7 hyphal bodies/mL) 分別加入 100 μL 上清液或 SMA＋Y 液體培養基，並置於 37℃ 恆溫器 1 h，加入 900 μl 的 SMA＋Y 液體培養基，混合均勻後取 100 μL 塗抹於 PDB+Y 培養基 (含 100 ng/mL 之 ampicillin) (Shih, 2005)，並置於 25℃ 培養箱 72 h 後計算菌落的數目。表二顯示大蠟蛾幼蟲的上清血液處理後之菌落數與 SMA＋Y 液體培養基者相比僅爲 43%，即表示有明顯抑制菌落數的效果。取 100 μL 大蠟蛾幼蟲的上清血液加入 10 μL 蛋白分解酵素 (10 mg/mL, proteinase K)，於 37℃ 下 2 h，重複上述試驗方法，發現菌落數維持爲對照組之 67%。比較未經處理的大蠟蛾幼蟲上清血液，則比值上升約 24%；另取 100 μl 大蠟蛾幼蟲的上清血液於 95℃ 加熱 5 min，重複上述試驗方法，發現綠殭菌的菌落數達 95%，比較未經處理的大蠟蛾幼蟲的上清血液，則比值上升約 52%，顯示大蠟蛾幼蟲的上清血液含有抑制綠殭菌菌絲體生長的成分，其抑制功能對於熱不具耐受性且可受蛋白質分解酵素的破壞，推測可能是屬於抗真菌蛋白成分。

同樣試驗法方法改用斜紋夜蛾幼蟲的上清血液，表二數據顯示綠殭菌的菌落數與 SMA＋Y 液體培養基者相近，表示無任何抑制效果。將其上清血液先經蛋白分解酵素或加熱處理，則比值仍分別維持在 95 及 88%。綜合以上結果顯示大蠟蛾幼蟲不受綠殭菌感染，乃因其血液中含有體液性因子 (humoral factor)，以抑制綠殭菌菌絲體生長；而斜紋夜蛾受綠殭菌的感染，由於其血液則不具此抑制因子之故。因此，昆蟲是否受綠殭菌的感染，其體液性免疫反應扮演極重要的角色。文獻上已經有一些抗真菌蛋白自昆蟲體內分離出來 (Lamberty *et al.*, 1999; Lee *et al.*, 2009; Zhang and Zhu, 2009)。此外，大蠟蛾幼蟲已

表一　斜紋夜蛾與大蠟蛾幼蟲以粘附或注射綠殭菌或白殭菌分生孢子方式之感染率 [1)]

Table 1. The infection (or mummified) rates of *Spodoptera litura* and *Galleria mellonella* larvae infected with *Nomuraea rileyi* or *Beauveria bassiana* through contact or by injecting inoculation[1)]

Fungi	% Mummified larvae[2)]			
	Spodpptera litura		*Galleria mellonella*	
	Contact	Injection	Contact	Injection
Nomuraea rileyi	80.00 ± 10.00	93.33 ± 5.77	0.00 ± 0.00	0.00 ± 0.00
Beauveria bassiana	86.67 ± 5.77	100.00 ± 0.00	96.67 ± 5.77.	100.00 ± 0.00

[1)] All experiments were conducted in triplicate.

[2)] Means ± standard deviation.

表二　斜紋夜蛾或大蠟蛾幼蟲血液處理綠殭菌菌絲體後之菌落數 [1)]

Table 2. Number of colonies of *Nomuraea rileyi* after treating the hyphal bodies with the hemolymph of *Spodoptera litura* or *Galleria mellonella* larvae[1)]

Hemolymph	No. of colonies [2)3)]		
	Without treatment	Proteinase K	Heat (95℃, 5 min)
SMA+Y medium	103.33 ± 18.61 (100%)	None	None
Galleria mellonella	43.67 ± 6.66 (43%)	68.67 ± 5.69 (100%)	98.00 ± 16.64 (100%)
Spodoptera litura	102.67 ± 9.71 (100%)	90.00 ± 10.54 (100%)	97.00 ± 7.00 (100%)

[1)] All experiments were conducted in triplicate.

[2)] Means ± standard deviation.

[3)] The values in parenthesis represent the percentage against the SMA+Y medium group.

證實可受細菌的脂多醣 (lipopolysaccharide) 誘導產生 gallermycin 的抗真菌蛋白，其對黑殭菌 (*Metarhizium anisopliae*) 孢子的生長具有 60% 抑制效果 (Schuhmann *et al.*, 2003)。但黑殭菌仍可感染大蠟蛾 (Götz *et al.*, 1997)，顯示單一的 gallermycin 無法有效抑制黑殭菌感染。由於白殭菌可感染大蠟蛾，故推測 gallermycin 對白殭菌感染也是無抑制之效。但有文獻指出少量注射白殭菌孢子可以在 24 小時後誘發大蠟蛾的 gallermycin 與 galiomicin 基因之表現及提升溶菌酶 (lysozyme) 活性，使其血液具有抗真菌感染的活性 (Wojda *et al.*, 2009)；至於大蠟蛾幼蟲的血液可抑制綠殭菌感染及菌絲體生長，其成分是否為 gallermycin、galiomicin、溶菌酶或其他成分，則有待進一步分析研究。

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ABSTRACT

The 5th instar larvae of *Spodoptera litura* can be infected with both *Nomuraea rileyi* and *Beauveria bassiana* through cuticular contact inoculation with conidia, whereas those of *Galleria mellonella* can only be infected with *B. bassiana* but not with *N. rileyi*. Similarly, the fungal infection in both insect species was obtained through inoculation by injecting their hyphal bodies into a hemocoel. When the *N. rileyi* hyphal bodies were treated with the hemolymph of *G. mellonella* without hemocyte, the number of colonies on solid PDB+Y medium formed from the treated hyphal bodies was greatly reduced to approx. 43% compared with that of the control group grown in medium only. However, when the hemolymph was pre-treated with proteinase K or 95°C for 5 min, the number of colonies remained at 67 or 95%, respectively. When assayed with the hemolymph of *S. litura* without hemocyte, the number of *N. rileyi* colonies remained the same as the control group, indicating that *S. litura* hemolymph does not contain a humoral factor against this fungus. Therefore, the hemolymph of *G. mellonella* contains humoral factor(s), which seems to be proteinaceous based on its inactivation by proteinase and heat, to suppress infection of *G. mellonella* with *N. rileyi*.

Key words: entomopathogenic fungus, *Nomuraea rileyi*, *Galleria mellonella*, *Spodoptera litura*, hemolymph

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