

# 真菌内共生细菌研究进展

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**摘要:** 自真菌内共生细菌在 1970 年被首次发现以来, 各个时期的学者都采用当时流行的研究方法关注宿主真菌及其内共生细菌之间的关联现象。近年来科技手段日益多样, 对二者相互作用的探索逐渐成为新的研究热点, 随着研究的不断拓展和深入, 越来越多的生物学现象和原理被揭示。本文在真菌内共生细菌的研究方法、定殖位置、形态、分类、宿主类群、共生关联的建立、生物学功能、宿主治愈、分离和重新植入等方面进行综述, 并在此基础上进行展望, 以期对真菌内共生细菌的广泛深入研究提供借鉴。  
**关键词:** 植物病原菌, 菌根菌, 内生菌, 蓝细菌, 小孢根霉素

## Research progress on endofungal bacteria

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**Abstract:** Since the discovery of endofungal bacteria (EFB) for the first time in 1970, scholars have applied techniques popular at their times to pay attention to this biological correlation phenomenon between host fungi and their endobiont bacteria. In recent years, investigations on their interactions have gradually become a new hotspot using diverse research methods. With the continuous expansion and deepening of research on EFB, more and more biological phenomena and theories have been revealed. The research progress concerning investigation methods, colonization location, morphology, classification, host groups, development of the symbiosis, biological function, host healing, isolation, and re-introduction of EFB to fungi are reviewed. The main trend of further studies of EFB is also predicted.

**Key words:** plant pathogens, AM fungi, endophytic fungi, cyanobacteria, rhizoxin

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真菌内共生细菌 (endofungal bacterium, EFB) 是生活在真菌营养菌丝或繁殖结构内的细菌。在特定环境中, 真菌宿主与其内共生细菌在生长和繁殖过程中互惠互利, 作为一个整体发挥各种生物学和生态学功能, 影响着农业和林业的诸多方面。

这种现象首先在内囊霉属 *Endogone* Link 真菌中发现。1970年, Barbara Mosse 研究 *Endogone* 孢子精细结构时, 发现了一种不同寻常的细胞器, 推测为能自我复制的类细菌生物 (bacteria-like organisms)。这些类细菌生物自由地生活于宿主真菌孢子细胞质中, 没有任何外膜, 亦未引起宿主的任何异常。当真菌宿主孢子进入休眠状态时, 类细菌生物急剧增生, 形成大量聚群 (Mosse 1970)。1980–1990年, 研究人员在丛枝菌根真菌巨孢囊霉 *Gigaspora* spp. 和球囊霉 *Glomus* spp. 中发现类似的细胞结构, 为这种现象补充了证据, 但称为类细菌细胞器 (bacteria-like organelles) (MacDonald & Chandler 1981; MacDonald *et al.* 1982; Walley & Germida 1996; Maia & Kimbrough 1998)。由于当时技术条件有限, 无法对这些类细菌生物或类细菌细胞器进行分离、培养和鉴定。之后的 10 余年, 随着研究方法的突飞猛进, 这些类细菌生物或者类细菌细胞器最终明确为真菌内共生细菌 (EFB) (Lackner *et al.* 2011a)。此后, 真菌和内共生细菌间的关系和相互作用逐渐成为新的研究热点。本文综述了目前对真菌内共生细菌的研究结果和进展, 主要包括研究方法、形态、定殖位置、分类、宿主类群、生物学功能、宿主治愈、分离和重新植入等, 并在此基础上对其研究领域进行展望。

## 1 真菌内共生细菌的研究方法

早期主要通过扫描电子显微镜和透射电子显微镜直接观察真菌菌丝或孢子中的 EFB (Mosse 1970; MacDonald *et al.* 1982; Buscot 1994; Walley & Germida 1996; Filippi *et al.*

1998; Maia & Kimbrough 1998)。后来, 用细菌特异性染料进行染色或者使用细菌特异性探针进行荧光原位杂交 (fluorescent *in situ* hybridization, FISH), 用实时荧光定量 PCR (real-time quantitative PCR, qPCR) 检验 EFB 的垂直传播, 用变性梯度凝胶电泳 (denaturing gradient gel electrophoresis, DGGE) 进行 EFB 聚群鉴定, 用焦磷酸测序 (pyrosequencing) 快速鉴定 EFB, 用脂肪酸甲酯谱 (fatty acid methyl ester, FAME) 对 EFB 细胞进行脂肪酸分析, 用高效液相色谱分析 (high performance liquid chromatography, HPLC) 进行毒素检测 (Barbieri *et al.* 2000; Bertaux *et al.* 2003; Xavier & Germida 2003; Izumi *et al.* 2008; Hoffman & Arnold 2010; Kai *et al.* 2012; Desirò *et al.* 2015; Guo *et al.* 2017; Naito *et al.* 2017)。最近的主流方向是用细菌特异性引物进行 PCR 扩增和测序研究 EFB 的分类群 (Bianciotto *et al.* 2004; Partida-Martinez & Hertweck 2005; Sato *et al.* 2010; Hoffman *et al.* 2013; Desirò *et al.* 2015, 2018), 用基因组学和蛋白组学解析 EFB 控制宿主真菌产孢能力、配制 EFB 生长特殊培养基和解读 EFB 从真菌宿主水平转移获得的基因功能等 (Moebius *et al.* 2014; Mondo *et al.* 2017; Naito *et al.* 2017; Sun *et al.* 2019)。除以上原位研究方法之外, 还有少数纯培养的方法, 将 EFB 自宿主中分离出来进行离体培养, 然后通过传统形态结合现代分子的方法进行鉴定 (Partida-Martinez *et al.* 2007b; Arendt *et al.* 2016; Ohshima *et al.* 2016), 采用激光束转化技术和混合培养方法将该 EFB 重新植入真菌中 (Partida-Martinez *et al.* 2007c; Arendt *et al.* 2016; Baltrus *et al.* 2018)。

## 2 真菌内共生细菌的定殖位置和形态

EFB 主要定殖于真菌宿主的菌丝细胞质 (Partida-Martinez & Hertweck 2005; Sato *et al.*

2010; Desirò *et al.* 2013; Ohshima *et al.* 2016; Guo *et al.* 2017)或孢子细胞质内(Bianciotto *et al.* 2000; Lumini *et al.* 2007; Naumann *et al.* 2010; Desirò *et al.* 2018), 仅有 *Nostoc punctiforme* 定殖于 *Geosiphon pyriformis* 囊状体中(Schüßler 2002)。EFB 在真菌宿主细胞壁中也时有发现(Mayo *et al.* 1986; Filippi *et al.* 1998; Long *et al.* 2008), 但此时应该属于还未完成定殖的侵入阶段。外生菌根真菌所形成的特殊菌丝体结构哈蒂氏网(Hartig net)中也发现过 EFB 的定殖(Buscot 1994)。

EFB 的形态主要呈球状和杆状, 个别为卵形和椭圆形。不可培养的支原体近缘内共生细菌(Mycoplasma-related endobacteria, MRE)基本为球状(Naumann *et al.* 2010; Desirò *et al.* 2013, 2015, 2018), 仅有 *Nostoc punctiforme* 呈卵形(Schüßler 2002), 另一种不可培养的内共生细菌暂定类群“*Candidatus Glomeribacter gigasporarum* (CaGg)”则为杆状(Bianciotto *et al.* 2000, 2003, 2004; Jargeat *et al.* 2004; Salvioli *et al.* 2008)。可培养的 EFB 除 *Paenibacillus* spp. 呈椭圆形之外(Bertaux *et al.* 2003, 2005), 其余皆同时具有球状和杆状两种形态(Partida-Martinez & Hertweck 2005; Lumini *et al.* 2007; Pakvaz & Soltani 2016; Naito *et al.* 2017)。菌体细胞体积大小方面, 位于宿主体内的 EFB 可以达到自由生活时的 6 倍(Schüßler *et al.* 1996)。

### 3 真菌内共生细菌的分类和宿主类群

大量研究表明在多种真菌谱系中存在多样的 EFB, 这些真菌谱系包括毛霉菌门 Mucoromycota、球囊菌门 Glomeromycota、子囊菌门 Ascomycota 和担子菌门 Basidiomycota。其 EFB 多数属于  $\alpha$ -变形菌门 Alphaproteobacteria、 $\beta$ -变形菌门 Betaproteobacteria 和厚壁菌门 Firmicutes, 少数属于放线菌门 Actinobacteria 和  $\gamma$ -变形菌门 Gammaproteobacteria (Barbieri *et al.* 2000; Partida-Martinez & Hertweck 2005; Izumi *et al.*

2006; Sharma *et al.* 2008; Agnolucci *et al.* 2015; Ohshima *et al.* 2016; Naito *et al.* 2017; Desirò *et al.* 2018)。

#### 3.1 与毛霉菌门关联的内共生细菌

根据目前报道, 宿生有 EFB 的 Mucoromycota 真菌类群包括 4 个属, 即内囊霉属 *Endogone*、裂孢囊霉属 *Lobosporangium*、被孢霉属 *Mortierella* 和根霉属 *Rhizopus*。其可培养 EFB 属于拟伯克氏菌属 *Paraburkholderia*、卡斯特兰尼氏菌属 *Castellaniella*、*Cryobacterium* 属和 *Mycoavidus* 属; 其不可培养 EFB 暂定为支原体近缘内共生细菌(MRE)。Mucoromycota 真菌宿主及其 EFB 的统计见表 1。

#### 3.2 与球囊菌门关联的内共生细菌

宿生有 EFB 的 Glomeromycota 真菌类群包括 7 个属, 即绕孢囊霉属 *Ambispora*、索形球囊霉属 *Funneliformis*、地管霉属 *Geosiphon*、球囊霉属 *Glomus*、巨孢囊霉属 *Gigaspora*、噬根球囊霉属 *Rhizophagus* 和盾孢囊霉属 *Scutellospora*。其可培养 EFB 属于食酸菌属 *Acidovorax*、无色杆菌属 *Achromobacter* 和土壤杆菌属 *Agrobacterium* 等 31 个属; 其不可培养 EFB 暂定为“*Candidatus Moeniiplasma*”、CaGg 和 MRE。Glomeromycota 真菌宿主及其 EFB 的统计见表 2。

#### 3.3 与子囊菌门关联的内共生细菌

目前报道宿生有 EFB 的 Ascomycota 真菌类群包括 13 个属, 即囊丝核菌属 *Ascorhizoctonia*、枝孢属 *Cladosporium*、*Esteya* 属、镰刀菌属 *Fusarium*、*Gliocladiopsis* 属、小球腔菌属 *Leptosphaeria*、微壳色单隔孢属 *Microdiplodia*、羊肚菌属 *Morchella*、丛赤壳属 *Nectria*、拟盘多毛孢属 *Pestalotiopsis*、棘壳孢属 *Pyrenochaeta*、炭角菌属 *Xylaria* 和块菌属 *Tuber*。其 EFB 皆可培养, 属于芽孢杆菌属 *Bacillus*、慢生根瘤菌属 *Bradyrhizobium* 和 *Cytophaga-Flexibacter-Bacteroides* (CFB) 分子系统发育群等 22 个分类群。Ascomycota 真菌宿主及其 EFB 的统计见表 3。

表 1 Mucoromycota 真菌宿主及其 EFB

Table 1 Mucoromycota hosts and their eudofungal bacteria (EFB)

Host		EFB		Notes	References
Genera	Species	Genera	Species		
Endogone	<i>E. lactiflua</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2015
	<i>E. flammicoron</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2015
	<i>Endogone</i> sp.	Undefined	MRE	-	Desirò <i>et al.</i> 2015
Lobosporangium	<i>L. transversale</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2018
Rhizopus	<i>R. microsporus</i>	Paraburkholderia	<i>P. rhizoxinica</i>	Plant pathogens	Partida-Martinez <i>et al.</i> 2005, 2007a, 2007b; Santos <i>et al.</i> 2018
			<i>P. endofungorum</i>		
Mortierella	<i>R. oryzae</i>	Burkholderia	Burkholderia spp.	Human pathogens	Ibrahim <i>et al.</i> 2008
	<i>M. alpina</i>	Castellaniella	<i>C. defragrans</i>	-	Kai <i>et al.</i> 2012
		Cryobacterium	<i>Cryobacterium</i> sp.	-	
	<i>M. capitata</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2018
	<i>M. elongata</i>	Mycoavidus	<i>M. cysteinexigens</i>	-	Sato <i>et al.</i> 2010; Ohshima <i>et al.</i> 2016; Desirò <i>et al.</i> 2018
		Undefined	MRE	-	Desirò <i>et al.</i> 2018
	<i>M. gamsii</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2018
	<i>M. humilis</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2018
	<i>M. minutissima</i>	Mycoavidus	<i>M. cysteinexigens</i>	-	Desirò <i>et al.</i> 2018
		Undefined	MRE	-	
	<i>M. rostafinskii</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2018
	<i>M. selenospora</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2018
	<i>M. thaxteri</i>	Undefined	MRE	-	Desirò <i>et al.</i> 2018

表 2 Glomeromycota 真菌宿主及其 EFB

Table 2 Glomeromycota hosts and their EFB

Host		EFB		Notes	References
Genera	Species	Genera	Species		
Ambispora	<i>A. appendicula</i>	Undefined	MRE	Arbuscular mycorrhizal fungi	Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013
	<i>A. fennica</i>	Undefined	MRE	Arbuscular mycorrhizal fungi	
Funneliformis	<i>F. coronatum</i>	Agrobacterium	Agrobacterium sp.	Arbuscular mycorrhizal fungi	Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013; Agnolucci <i>et al.</i> 2015
		Arthrobacter	Arthrobacter sp.		
		Undefined	MRE		
		Mycoavidus	<i>M. cysteinexigens</i>		
	<i>F. mosseae</i>	Sinorhizobium	Sinorhizobium sp.	Arbuscular mycorrhizal fungi	Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013; Agnolucci <i>et al.</i> 2015
		Agrobacterium	Agrobacterium sp.		
		Arthrobacter	Arthrobacter sp.		
		Bacillus	Bacillus sp.		
		Cupriavidus	Cupriavidus sp.		
		Geobacillus	Geobacillus sp.		
		Ideonella	Ideonella sp.		

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续表 2

		<i>Lysobacter</i>	<i>Lysobacter</i> sp.		
		<i>Methylibium</i>	<i>Methylibium</i> sp.		
		Undefined	MRE		
		<i>Mycoavidus</i>	<i>M. cysteinexigens</i>		
		<i>Paenibacillus</i>	<i>Paenibacillus</i> sp.		
		<i>Propionibacterium</i>	<i>Propionibacterium</i> sp.		
		<i>Rhizobium</i>	<i>Rhizobium</i> sp.		
		<i>Streptomyces</i>	<i>Streptomyces</i> sp.		
<i>Geosiphon</i>	<i>G. pyriformis</i>	<i>Nostoc</i>	<i>N. punctiforme</i>	Cyanobacteria	Kluge 2002; Schüßler 2002
		Undefined	MRE		
<i>Glomus</i>	<i>G. caledonium</i>	Undefined	MRE	Arbuscular mycorrhizal fungi	MacDonald & Chandler 1981; Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013
	<i>G. claroideum</i>	Undefined	MRE	Arbuscular mycorrhizal fungi	Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013
	<i>G. clarum</i>	<i>Alcaligenes</i>	<i>A. ilicis</i>	Arbuscular mycorrhizal fungi	Walley <i>et al.</i> 1996; Xavier <i>et al.</i> 2003
		<i>Bacillus</i>	<i>B. alvei</i>		
		<i>Bacillus</i>	<i>B. brevis</i>		
		<i>Bacillus</i>	<i>B. chitosporus</i>		
		<i>Bacillus</i>	<i>B. firum</i>		
		<i>Bacillus</i>	<i>B. megaterium</i>		
	<i>G. etunicatum</i>	Undefined	MRE	Arbuscular mycorrhizal fungi	Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013
	<i>G. mosseae</i>	<i>Acidovorax</i>	<i>A. delafieldii</i>	Arbuscular mycorrhizal fungi	MacDonald <i>et al.</i> 1982; Filippi <i>et al.</i> 1998; Bharadwaj <i>et al.</i> 2008
		<i>Arthrobacter</i>	<i>A. atrocyneus</i>		
		<i>Aureobacterium</i>	<i>A. saperdae</i>		
		<i>Bacillus</i>	<i>B. megaterium</i>		
		<i>Burkholderia</i>	<i>B. picketti</i>		
		<i>Curtobacterium</i>	<i>C. citreum</i>		
		<i>Hydrogenophaga</i>	<i>H. pseudoflava</i>		
		<i>Micrococcus</i>	<i>M. halobius</i>		
		<i>Pseudomonas</i>	<i>P. chlororaphis</i>		
		<i>Pseudomonas</i>	<i>P. stutzeri</i>		
	<i>G. intraradices</i>	<i>Achromobacter</i>	<i>A. xylosoxydans</i>	Arbuscular mycorrhizal fungi	Bharadwaj <i>et al.</i> 2008
		<i>Achromobacter</i>	<i>A. piechaudii</i>		
		<i>Bacillus</i>	<i>B. chitosporus</i>		
		<i>Bacillus</i>	<i>B. subtilis</i>		
		<i>Clavibacter</i>	<i>C. michiganense insidiosum</i>		
		<i>Corynebacterium</i>	<i>C. bovis</i>		
		<i>Janthinobacterium</i>	<i>J. lividum</i>		
		<i>Micrococcus</i>	<i>M. luteus</i>		
		<i>Pseudomonas</i>	<i>P. fluorescens</i>		
		<i>Pseudomonas</i>	<i>P. syringae tagetes</i>		
	<i>G. versiforme</i>	Undefined	MRE	Arbuscular mycorrhizal fungi	Mayo <i>et al.</i> 1986; Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013

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续表 2

	<i>Glomus</i> spp.	Undefined	MRE	Arbuscular mycorrhizal fungi	Maia <i>et al.</i> 1998; Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013
<i>Gigaspora</i>	<i>G. heterogama</i>	Undefined	Undefined	Arbuscular mycorrhizal fungi	MacDonald & Chandler 1981; MacDonald <i>et al.</i> 1982
	<i>G. margarita</i>	<i>Burkholderia</i>	BRE	Arbuscular mycorrhizal fungi	MacDonald & Chandler 1981; MacDonald <i>et al.</i> 1982; Bianciotto <i>et al.</i> 1996, 2000, 2003, 2004; Cruz <i>et al.</i> 2008; Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2014
	<i>G. margarita</i>	<i>Janthinobacterium J. lividum</i>		Arbuscular mycorrhizal fungi	MacDonald & Chandler 1981; MacDonald <i>et al.</i> 1982; Bianciotto <i>et al.</i> 1996, 2000, 2003, 2004; Cruz <i>et al.</i> 2008; Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2014
	<i>G. margarita</i>	<i>Paenibacillus</i>	<i>P. polymyxa</i>	Arbuscular mycorrhizal fungi	MacDonald & Chandler 1981; MacDonald <i>et al.</i> 1982; Bianciotto <i>et al.</i> 1996, 2000, 2003, 2004; Cruz <i>et al.</i> 2008; Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2014
	<i>G. margarita</i>	Undefined	MRE	Arbuscular mycorrhizal fungi	MacDonald & Chandler 1981; MacDonald <i>et al.</i> 1982; Bianciotto <i>et al.</i> 1996, 2000, 2003, 2004; Cruz <i>et al.</i> 2008; Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2014
	<i>G. decipiens</i>	<i>Candidatus Glomeribacter</i>	<i>Candidatus G. gigasporarum</i>	Arbuscular mycorrhizal fungi	Bianciotto <i>et al.</i> 2000, 2003, 2004; Jargeat <i>et al.</i> 2004; Salvioli <i>et al.</i> 2008
	<i>G. gigantea</i>	<i>Candidatus Glomeribacter</i>	<i>Candidatus G. gigasporarum</i>	Arbuscular mycorrhizal fungi	Bianciotto <i>et al.</i> 2000, 2003, 2004; Jargeat <i>et al.</i> 2004; Salvioli <i>et al.</i> 2008
<i>Rhizophagus</i>	<i>R. intraradices</i>	<i>Acidobacterium</i>	<i>Acidobacterium</i> sp.	Arbuscular	Agnolucci <i>et al.</i> 2015
		<i>Arthrobacter</i>	<i>Arthrobacter</i> sp.	mycorrhizal fungi	
		<i>Bacillus</i>	<i>Bacillus</i> sp.		
		Undefined	MRE		
		<i>Paenibacillus</i>	<i>Paenibacillus</i> sp.		
		<i>Pseudomonas</i>	<i>Pseudomonas</i> sp.		
		<i>Pseudomonas</i>	<i>Pseudomonas</i> sp.		
		<i>Rhizobium</i>	<i>Rhizobium</i> sp.		
		<i>Sinorhizobium</i>	<i>Sinorhizobium</i> sp.		
		<i>Streptomyces</i>	<i>Streptomyces</i> sp.		
	<i>R. clarus</i>	<i>Candidatus Moeniiplasma</i>	<i>Candidatus M. glomeromycotorum</i>	Arbuscular mycorrhizal fungi	Naito <i>et al.</i> 2017
<i>Scutellospora</i>	<i>S. castanea</i>	<i>Candidatus Glomeribacter</i>	<i>Candidatus G. gigasporarum</i>	Arbuscular mycorrhizal fungi	Bianciotto <i>et al.</i> 2000, 2003
		Undefined	MRE	Arbuscular mycorrhizal fungi	
	<i>S. gilmorei</i>	Undefined	MRE	Arbuscular mycorrhizal fungi	Naumann <i>et al.</i> 2010; Desirò <i>et al.</i> 2013
	<i>S. persica</i>	<i>Candidatus Glomeribacter</i>	<i>Candidatus G. gigasporarum</i>	Arbuscular mycorrhizal fungi	Bianciotto <i>et al.</i> 2000, 2003

表 3 Ascomycota 真菌宿主及其 EFB

Table 3 Ascomycota hosts and their EFB

Host		EFB		Notes	References
Genera	Species	Genera	Species		
<i>Ascorhizoctonia</i>	<i>Ascorhizoctonia</i>	<i>Bacillus</i>	<i>B. pumilus</i>	Endophytic fungi	Pakvaz <i>et al.</i> 2016
	sp.	<i>Sphingomonas</i>	<i>S. paucimobilis</i>		
<i>Cladosporium</i>	<i>Cladosporium</i> sp.	<i>Curtobacterium</i>	<i>Curtobacterium</i> sp.	Endophytic fungi	Shaffer <i>et al.</i> 2016
<i>Esteya</i>	<i>E. vermicola</i>	<i>Pseudomonas</i>	<i>Pseudomonas</i> sp.	<i>Esteya vermicola</i>	Wang 2017
<i>Fusarium</i>	<i>F. concolor</i>	<i>Streptococcus</i>	<i>Streptococcus</i> sp.	Endophytic fungi	Shaffer <i>et al.</i> 2018
		<i>Curtobacterium</i>	<i>Curtobacterium</i> sp.		
		<i>Rothia</i>	<i>Rothia</i> sp.		
	<i>F. keratoplasticum</i>	<i>Chitinophaga</i>	<i>Chitinophaga</i> sp.	Endophytic fungi	Shaffer <i>et al.</i> 2017, 2018
<i>Fusarium</i>	<i>Fusarium solani</i>	<i>Bradyrhizobium</i>	<i>Bradyrhizobium</i> sp.	Oxidized elemental sulfur	Li <i>et al.</i> 2010
<i>Gliocladiopsis</i>	<i>Gliocladiopsis</i> sp.	<i>Enterobacter</i>	<i>Enterobacter</i> sp.	Endophytic fungi	Shaffer <i>et al.</i> 2018
		<i>Variovorax</i>	<i>Variovorax</i> sp.		
<i>Leptosphaeria</i>	<i>Leptosphaeria</i> sp.	<i>Bacillus</i>	<i>B. pumilus</i>	Endophytic fungi	Pakvaz <i>et al.</i> 2016
		<i>Bacillus</i>	<i>B. subtilis</i>		
<i>Microdiplodia</i>	<i>Microdiplodia</i> sp.	<i>Luteibacter</i>	<i>Luteibacter</i> sp.	Endophytic fungi	Shaffer <i>et al.</i> 2016
		<i>Erwinia</i>	<i>Erwinia</i> sp.		
		<i>Rhizobium</i>	<i>Rhizobium</i> sp.		
		<i>Pantoea</i>	<i>Pantoea</i> sp.		
<i>Morchella</i>	<i>Morchella</i> sp.	Undefined	Unnamed	Arbuscular mycorrhizal fungi	Buscot 1994
<i>Nectria</i>	<i>Nectria</i> sp.	<i>Curvibacter</i>	<i>Curvibacter</i> sp.	Endophytic fungi	Shaffer <i>et al.</i> 2018
		<i>Bradyrhizobium</i>	<i>Bradyrhizobium</i> sp.		
		<i>Stenotrophomonas</i>	<i>Stenotrophomonas</i> sp.		
		<i>Pelomonas</i>	<i>Pelomonas</i> sp.		
		<i>Tatumella</i>	<i>Tatumella</i> sp.		
<i>Pestalotiopsis</i>	<i>Pestalotiopsis</i> sp.	<i>Luteibacter</i>	<i>Luteibacter</i> sp.	Endophytic fungi	Arendt <i>et al.</i> 2016; Shaffer <i>et al.</i> 2016
<i>Pyrenochaeta</i>	<i>Pyrenochaeta</i> sp.	<i>Bacillus</i>	<i>B. pumilus</i>	Endophytic fungi	Pakvaz <i>et al.</i> 2016
<i>Xylaria</i>	<i>X. cubensis</i>	<i>Ralstonia</i>	<i>Ralstonia</i> sp.	Endophytic fungi	Shaffer <i>et al.</i> 2018
<i>Tuber</i>	<i>T. borchii</i>	<i>Bacillus</i>	<i>Bacillus</i> spp.	Arbuscular mycorrhizal fungi	Gazzanelli <i>et al.</i> 1999; Barbieri <i>et al.</i> 2000
		Undefined	CFB		
		<i>Pseudomonas</i>	<i>Pseudomonas</i> spp.		
<i>Alternaria</i>	<i>A. mali</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Aureobasidium</i>	<i>A. pullulans</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Biscogniauxia</i>	<i>B. mediterranea</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Botryosphaeria</i>	<i>B. dothidea</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Cladosporium</i>	<i>C. oxysporum</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Dothidea</i>	<i>D. sambuci</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010

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<i>Hormonema</i>	<i>Hormonema</i> sp.	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Lecythophora</i>	<i>Lecythophora</i> sp.	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Monodictys</i>	<i>Monodictys</i> sp.	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Pestalotiopsis</i>	<i>P. besseyi</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
	<i>P. caudata</i>	Undefined	Undefined		
<i>Phaeomoniella</i>	<i>P. chlamydospora</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Phoma</i>	<i>P. glomerata</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Phyllosticta</i>	<i>P. spinarum</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Pithya</i>	<i>P. cupressina</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010
<i>Preussia</i>	<i>P. africana</i>	Undefined	Undefined	Endophytic fungi	Hoffman <i>et al.</i> 2010

3.4 与担子菌门关联的内共生细菌

宿生有 EFB 的 Basidiomycota 真菌类群包括 12 个属，即伞菌属 *Agaricus*、田头菇属 *Agrocybe*、糙缘线革菌属 *Amphinema*、鬼伞属 *Coprinellus*、层孔菌属 *Fomes*、蜡蘑属 *Laccaria*、梨形孢属 *Piriformospora*、侧耳属 *Pleurotus*、红菇属 *Russula*、蜡壳耳属 *Sebacina*、乳牛肝菌属 *Suillus* 和黑粉菌属 *Ustilago*。其 EFB 皆可培养，属于不动杆菌属 *Acinetobacter*、根瘤菌属 *Rhizobium* 和红球菌属 *Rhodococcus* 等 11 个属。Basidiomycota 真菌宿主及其 EFB 的统计见表 4。

4 内共生关系的建立和维持

当前关于 EFB 与其宿主真菌共生关系的建立过程与维持机制的认识仅局限于少数几个例子，主要来自对 *Rhizopus*-EFB 共生关联以及 *Geosiphon*-EFB 共生关联的研究。与其他革兰氏阴性菌一样，EFB *Paraburkholderia rhizoxinica* 的表面由脂多糖覆盖，但其特殊之处在于这些 LPSs 携带有大量的呋喃半乳糖（galactofuranose）同聚物，而呋喃半乳糖是丝状真菌中常见的细胞成分。通过这种相似成分形成分子层面的拟态，消除宿主真菌对 EFB 的排斥（Leone *et al.* 2010）。此时，EFB 通过 II 型分泌系统（type II secretion system, T2SS）分泌壳聚糖酶和几丁质酶，前者直接作用于宿主真菌细胞壁的壳聚糖，后者的酶

活性可通过 EFB 分泌的几丁质结合蛋白与宿主真菌细胞壁几丁质结合得到加强。这些酶对宿主细胞壁进行酶解，随后 EFB 可顺利穿透细胞壁（Lackner *et al.* 2011b, 2011c; Moebius *et al.* 2014）。EFB 的非核糖体多肽合成酶（nonribosomal peptide synthetase, NRPS）基因簇编码线性脂肽“Holrhizin A”，该脂肽作为生物表面活性剂降低表面张力，阻滞真菌宿主细胞膜的成熟，协助 EFB 穿透细胞膜从而侵入宿主真菌细胞内（Niehs *et al.* 2018）。侵入后的 EFB 开始释放有害物质小孢根霉素，而真菌宿主则通过改变  $\beta$ -tubulin 第 100 位氨基酸的方式对小孢根霉素产生抗性（Schmitt *et al.* 2008）。当 EFB 在宿主内繁殖到一定量之后，EFB *P. rhizoxinica* 通过其 III 型分泌系统（type III secretion system, T3SS）引发宿主产孢（Lackner *et al.* 2011a, 2011b, 2011c），将 EFB 垂直传播给宿主后代（Mondo *et al.* 2017）。

此外，EFB 青枯菌 *Ralstonia solanacearum* 也很可能是通过渗透的方式进入宿主真菌黄曲霉 *Aspergillus flavus*。研究发现 *R. solanacearum* 产生一种脂肽罗索霉素（ralsomycin）改变宿主真菌的细胞膜结构，助其自身进入宿主菌丝。但这并非 *R. solanacearum* 侵入宿主真菌的唯一因素，几丁质水解酶和 III 型细菌分泌系统也可能发挥作用（Spraker *et al.* 2016）。



表 4 Basidiomycota 真菌宿主及其 EFB

Table 4 Basidiomycota hosts and their EFB

Host		EFB		Notes	References
Genera	Species	Genera	Species		
<i>Agaricus</i>	<i>A. bisporus</i>	<i>Pseudomonas</i>	<i>P. brassicacearum</i>	-	Aslani <i>et al.</i> 2018
<i>Agrocybe</i>	<i>Agrocybe</i> sp.	<i>Pseudomonas</i>	<i>P. taiwanensis</i>	-	Aslani <i>et al.</i> 2018
<i>Amphinema</i>	<i>A. byssoides</i>	<i>Acinetobacter</i>	<i>A. Johnsonii</i>	Arbuscular	Izumi <i>et al.</i> 2008
		<i>Bacillus</i>	<i>B. andropogonis</i>	mycorrhizal fungi	
		<i>Pantoea</i>	<i>P. ananatis</i>		
<i>Coprinellus</i>	<i>Coprinellus</i> sp.	<i>Brochothrix</i>	<i>B. thermosphacta</i>	-	Aslani <i>et al.</i> 2018
<i>Fomes</i>	<i>Fomes</i> sp.	<i>Pseudomonas</i>	<i>P. chlororaphis</i>	-	Aslani <i>et al.</i> 2018
		<i>Pseudomonas</i>	<i>P. koreensis</i>	-	
		<i>Serratia</i>	<i>S. marcescens</i>	-	
<i>Laccaria</i>	<i>L. bicolor</i>	<i>Paenibacillus</i>	<i>Paenibacillus</i> spp.	Arbuscular	Bertaux <i>et al.</i> 2003,
				mycorrhizal fungi	2005
<i>Piriformospora</i>	<i>P. indica</i>	<i>Rhizobium</i>	<i>R. radiobacter</i>	Arbuscular	Sharma <i>et al.</i> 2008;
				mycorrhizal fungi	Guo <i>et al.</i> 2017
<i>Pleurotus</i>	<i>Pleurotus</i> sp.	<i>Bacillus</i>	<i>B. maycoides</i>	-	Aslani <i>et al.</i> 2018
		<i>Bacillus</i>	<i>B. thuringiensis</i>	-	
		<i>Stenotrophomonas</i>	<i>S. chelatiphaga</i>	-	
<i>Russula</i>	<i>R. paludosa</i>	<i>Bacillus</i>	<i>B. psychrosaccharolyticus</i>	Arbuscular	Izumi <i>et al.</i> 2006
		<i>Paenibacillus</i>	<i>P. graminis</i>	mycorrhizal fungi	
		<i>Paenibacillus</i>	<i>Paenibacillus</i> spp.		
		<i>Pseudomonas</i>	<i>Pseudomonas</i> spp.		
	<i>Russula</i> sp.	<i>Bacillus</i>	<i>B. arvi</i>	Arbuscular	Izumi <i>et al.</i> 2006
		<i>Bacillus</i>	<i>B. psychrosaccharolyticus</i>	mycorrhizal fungi	
		<i>Burkholderia</i>	<i>B. glathei</i>		
		<i>Paenibacillus</i>	<i>P. phyllosphaerae</i>		
		<i>Paenibacillus</i>	<i>P. polymyxa</i>		
		<i>Pseudomonas</i>	<i>P. fluorescens</i>		
<i>Sebacina</i>	<i>S. vermifera</i>	<i>Rhodococcus</i>	<i>R. marinonascens</i>		
		<i>Rhodococcus</i>	<i>R. opacus</i>		
		<i>Acinetobacter</i>	<i>Acinetobacter</i> sp.	Endophytic fungi	Sharma <i>et al.</i> 2008
		<i>Paenibacillus</i>	<i>Paenibacillus</i> sp.		
		<i>Rhodococcus</i>	<i>Rhodococcus</i> sp.		
<i>Suillus</i>	<i>S. conthurnatus</i>	<i>Burkholderia</i>	<i>B. phenazinum</i>	Arbuscular	Izumi <i>et al.</i> 2008
				mycorrhizal fungi	
	<i>S. revillei</i>	<i>Pseudomonas</i>	<i>P. fluorescens</i>	Arbuscular	Varese <i>et al.</i> 1996
		<i>Pseudomonas</i>	<i>P. putida</i>	mycorrhizal fungi	
	<i>S. flavidus</i>	<i>Burkholderia</i>	<i>B. glathei</i>	Arbuscular	Izumi <i>et al.</i> 2006
		<i>Janthinobacterium</i>	<i>J. agaricidamnorum</i>	mycorrhizal fungi	

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续表 4

		<i>Paenibacillus</i>	<i>P. odorifer</i>		
		<i>Pseudomonas</i>	<i>P. fluorescens</i>		
		<i>Pseudomonas</i>	<i>P. tolaasii</i>		
	<i>S. variegatus</i>	<i>Burkholderia</i>	<i>B. glathei</i>	Arbuscular	Izumi <i>et al.</i> 2006
		<i>Pseudomonas</i>	<i>P. brassicacearum</i>	mycorrhizal fungi	
		<i>Pseudomonas</i>	<i>P. spp.</i>		
		<i>Rahnella</i>	<i>R. aquatilis</i>		
<i>Ustilago</i>	<i>U. maydis</i>	<i>Bacillus</i>	<i>B. pumilu</i>	Plant pathogens	Ruiz-Herrera <i>et al.</i> 2015

与上述 EFB 渗透进入宿主真菌从而建立和维持共生关联的机制截然不同，*Geosiphon*-EFB 共生关联的建立主要是通过吞噬的方式形成，其过程大致如下：作为 *Geosiphon* 属真菌的 EFB，*Nostoc punctiforme* 早期原基（primordia）细胞分泌含有甘露糖的粘液至细胞膜表面，被真菌宿主 *G. pyriformis* 特异性识别。宿主菌丝顶端下方细胞质连续多次凸出膨胀，形成不规则“斗篷”（mantle）结构，吞噬 5–15 个识别出的 *N. punctiforme* 早期原基，形成长达 2mm 梨形囊状体（bladder）（Mollenhauer *et al.* 1996；Schüßler 2012）。EFB 位于宿主质膜形成的隔室中（Schüßler *et al.* 1996），共生初始的 12h 内，*N. punctiforme* 遭受严重的胁迫而变形，光合色素减弱，甚至凋亡（Mollenhauer *et al.* 1996；Schüßler & Wolf 2005）。2–3d 后，EFB 细胞开始恢复，色素沉淀，在隔室中生长并呈单列丝状繁殖（Schüßler *et al.* 1996），有规律地间隔形成异形细胞（Mollenhauer *et al.* 1996；Schüßler 2012）。

5 真菌内共生细菌的功能

EFB 在真菌中如此普遍存在，那其存在具有怎样的生物学意义呢？许多学者为了回答这一问题，从形态学、生理学、病理学和生态学的角度开展了大量的工作，在宿主形态、宿主萌发和生长、病原菌毒素和固氮、菌根菌共生体、内生真菌共生体等方面分别给出了出乎意料的答案。

EFB 能影响真菌宿主的孢子形状（Lumini *et*

*al.* 2007；Araldi-Brondolo *et al.* 2017），调节真菌宿主生殖关键 GTP 酶的基因 *ras2* 的方式来控制其产孢能力（Partida-Martinez *et al.* 2007c；Lackner *et al.* 2011b；Moebius *et al.* 2014；Mondo *et al.* 2017），提高其孢子产量（Pakvaz & Soltani 2016；Guo *et al.* 2017），也能使其菌丝更粗壮、更茂盛（Arendt *et al.* 2016；Araldi-Brondolo *et al.* 2017）。

EFB 对真菌宿主孢子萌发和菌丝生长有显著影响。在孢子萌发阶段，有些 EFB 定殖能够刺激孢子的萌发（Mayo *et al.* 1986；Xavier & Germida 2003；Li *et al.* 2010），而有些则起着抑制的作用（Xavier & Germida 2003）。在菌丝生长阶段，EFB 能提高真菌宿主对碳源的利用量，极大地促进其生长速度，显著地提高其生物量（Varese *et al.* 1996；Kai *et al.* 2012；Guo *et al.* 2017；Shaffer *et al.* 2017；Desirò *et al.* 2018），产生抗生素协助宿主真菌抵抗细菌病害（Aslani *et al.* 2018），宿主真菌从 EFB 水平转移获得的基因具有防御外来 DNA 或病毒的功能（Sun *et al.* 2019）。然而也有 EFB 抑制宿主真菌的情况，比如，*Mycoavidus cysteinexigens* 消耗了宿主真菌 *Mortierella elongata* 的脂肪酸等初级代谢产物，使得宿主真菌可以利用的物质减少而生长受到抑制（Uehling *et al.* 2017）。

在病原菌方面，EFB 发挥着重要的作用。一直以来，*Rhizopus microsporus* 被认为能产生一种植物毒素——小孢根霉素（rhizoxin），导致水

稻幼苗枯萎病 (Noda *et al.* 1980)。实际上, 小孢根霉素并非由真菌 *R. microsporus* 本身合成的, 而是由其菌丝内的 *Paraburkholderia rhizoxinica* 所合成 (Partida-Martinez & Hertweck 2005; Partida-Martinez *et al.* 2007b)。与小孢根霉素类似, 长期被误认为是真菌毒素的小孢根霉毒素 (rhizonin) 也是由 *B. endofungorum* 所产生 (Steyn *et al.* 1983; Partida-Martinez *et al.* 2007a, 2007b)。此外, 导致玉米受害组织肿大成瘤的玉米致病性真菌 *Ustilago maydis* 具有固定大气氮的能力, 该菌本身并没有这种能力, 而是依赖作为其 EFB *Bacillus pumilus* 的固氮性能 (Ruiz-Herrera *et al.* 2015)。目前, 在人类病原菌 *Rhizopus* spp. 中, 并未检测到 (Ibrahim *et al.* 2008; Partida-Martinez *et al.* 2008), 有研究将产生各种毒素的细菌植入人类机会性病原菌 *Rhizopus* spp. 并建立起共生关联, 但与在植物病原菌的情况不同, 这些真菌-EFB 的共生关联没有对其真菌宿主的致病性产生任何影响 (Ibrahim *et al.* 2008)。

与菌根真菌共生的 EFB, 在真菌宿主的代谢、繁殖和抗逆性中发挥着重要的作用。EFB 可为真菌宿主提供生物能量、光合作用产生的糖化合物和维生素等必需因子 (Kluge 2002; Ghignone *et al.* 2012; Schüßler 2012; Salvioli *et al.* 2016; Bonfante & Desirò 2017), 诱发真菌宿主降解毒性活性氧化物质 (Salvioli *et al.* 2016; Vannini *et al.* 2016), 促使真菌宿主菌丝延伸更长距离以寻找植物的根 (Lumini *et al.* 2007), 提高真菌宿主的适应性 (Guo *et al.* 2017)。此外, *R. microsporus* 为了适应其 EFB, 许多与脂质代谢有关的基因上调, 其中以编码双酰基甘油激酶的基因上调最为明显, 导致三酰甘油和磷脂酰乙醇胺显著增加, 二者比例最后约为 1:1, 从而利于宿主真菌的生长 (Lastovetsky *et al.* 2016)。

EFB 也可提高菌根的形成量 (Lumini *et al.* 2007), 影响菌根的形态 (Bianciotto *et al.* 2004),

促进菌根磷酸盐的吸收和运输 (Ruiz-Lozano & Bonfante 1999; Cruz *et al.* 2008), 帮助菌根抑制病原菌的侵入, 提高菌根的呼吸 (Cruz *et al.* 2008), 显著地增加植物宿主枝条重量, 促进植物生长 (Guo *et al.* 2017)。对于定殖于丛枝菌根真菌中的 MRE, 虽然在菌根真菌生物学中的作用尚不清楚, 但它们在菌根真菌宿主分类群中的广泛分布表明, MRE 可能调节菌根真菌对陆地生态系统的影响 (Desirò *et al.* 2015)。

EFB 可对内生真菌及其植物宿主产生各种作用, 以有益作用为主, 个别情况下也具有负面影响。有益方面, EFB 提高真菌的植物细胞壁降解酶活性从而有助于内生真菌与植物共生关系的建立 (Anca *et al.* 2009; Arendt 2015); EFB 可提高其宿主真菌竞争力, 抑制其他植物内生真菌和植物内生细菌生长, 对真菌宿主起辅助保护作用 (Pakvaz & Soltani 2016; Shaffer *et al.* 2018); 可提高植物对病原微生物的抗性 (Hoffman *et al.* 2013; Pakvaz & Soltani 2016); 刺激真菌宿主产生更多的植物激素吲哚-3-乙酸 (IAA), 促进植物生长, 从而提高 EFB-内生真菌-植物共生体的适应性 (Hoffman *et al.* 2013); 还可延长某些种子的活力期, 促进种子萌发 (Shaffer *et al.* 2018)。另一方面, EFB 的负面作用主要表现在, 其存在有时会降低种子内生真菌的定殖率, 有时缩短某些种子的活力期, 有时抑制种子萌发等 (Shaffer *et al.* 2018)。

除上述已经明确的生物学功能之外, 有研究发现 *Mortierella elongata* 菌丝中存在由 EFB 产生的功能未知的毒素 (Sato *et al.* 2010)。*M. alpina* 的 EFB 能够产生群感效应信号分子并将其释放到培养液中, 但这些信号分子在共生中的生物学作用仍然未知 (Kai *et al.* 2012)。

## 6 真菌的治愈、EFB 的分离和重新植入

分别对宿主真菌和 EFB 进行纯培养, 是研究真菌-EFB 共生生物学的基础, 许多学者也对此进

行了大量的研究。通过特定方法的处置,可将真菌菌丝内的 EFB 去除,使原本与 EFB 共生的真菌回到独立生活的状态,这一过程被研究人员亲切地称之为“治愈”(Cure)。关于真菌治愈,在绝大多数研究中取得了成功 (Partida-Martinez & Hertweck 2005; Lumini *et al.* 2007; Sharma *et al.* 2008; Sato *et al.* 2010; Kai *et al.* 2012; Hoffman *et al.* 2013; Arendt *et al.* 2016; Guo *et al.* 2017; Mondo *et al.* 2017; Desirò *et al.* 2018), 仅有 2 次是未能治愈成功,失败的原因可能是 EFB 在真菌宿主内部受到某种保护 (Sharma *et al.* 2008; Guo *et al.* 2017)。为治愈真菌宿主,可在培养基中添加各种抗生素,包括青霉素、庆大霉素、卡那霉素、链霉素、克林霉素、壮观霉素、羧苄青霉素、硫酸链霉素、四环素、多西环素、米诺环素、苯唑西林、替卡西林、美洛西林、氨苄西林钠、环丙沙星、利福平等,并在抗生素环境中进行连续继代培养。真菌的治愈通过显微镜观察和 PCR 分析进行验证 (Barbieri *et al.* 2000; Hoffman & Arnold 2010; Desirò *et al.* 2015; Mondo *et al.* 2017; Desirò *et al.* 2018)。除用抗生素外,孢子继代连续培养也能达到治愈真菌的效果 (Lumini *et al.* 2007)。

EFB 可通过真菌孢子或菌丝表面消毒,破碎,匀浆液过滤和离心,EFB 沉淀重悬,最后适温培养来进行分离 (Jargeat *et al.* 2004; Partida-Martinez *et al.* 2007a; Izumi *et al.* 2008; Kai *et al.* 2012; Ohshima *et al.* 2016; Naito *et al.* 2017)。大体步骤如下:(1) 首先将孢子或者菌丝用 4% 氯胺 T、30%  $H_2O_2$  或者 85% NaCl 消毒,然后用抗生素(比如链霉素、庆大霉素和青霉素)溶液冲洗;(2) 在提取缓冲液 (250mg/L 蔗糖, 25mg/L 氯化钾, 20mg/L 氯化镁, 1mg/L 二硫苏糖醇, 1% 吗啡乙磺酸) 或者磷酸盐溶液 (0.14mg/L 氯化钠, 0.003mg/L 氯化钾, 0.01mg/L 磷酸盐缓冲液) 中通过机械剪切、挤压或者震荡的方法破碎孢子,释放 EFB;(3) 对匀浆液进行过滤 (0.2 $\mu$ m

或 2–8 $\mu$ m 孔滤膜),离心 (500r/min 或 1 000r/min),取上清液再次离心 (4 000r/min),沉淀 EFB,去除上清液;(4) 将 EFB 沉淀重悬于提取缓冲液 (10 $\mu$ L),然后加入分离缓冲液 (250mg/L 蔗糖, 0.5% 聚乙二醇, 0.1% 聚蔗糖, 0.1% 牛血清蛋白) 进行稀释;(5) 将 EFB 稀释液涂布于营养琼脂平板上在合适温度 (25 $^{\circ}$ C、28 $^{\circ}$ C 或者 30 $^{\circ}$ C 等) 培育直至细菌菌落出现。多数 EFB 可以在普通培养基上生长,比如胰蛋白酶肉汤 (Tryptic soy broth, TSB) 培养基 (10g 胰蛋白酶, 15g 细菌琼脂, 1L 蒸馏水)、Luria–Bertani (LB) 培养基、胰酶解酪蛋白 (Trypticase soy agar, TSA) 培养基 (5g 大豆蛋白胨, 3g 酵母提取物, 15g 琼脂, 1L 蒸馏水)。个别 EFB 需要添加特殊成分才能正常生长,比如 *Mycoavidus cysteinexigens* 需要在酵母提取物培养基中加入半胱氨酸,因其基因组中缺少参与半胱氨酸生物合成的关键基因 (Fujimura *et al.* 2014; Ohshima *et al.* 2016)。

迄今为止,关于 EFB 重新植入宿主真菌中的研究较少。用带有绿色荧光蛋白 (GFP) 标记的 EFB,与宿主真菌混合培养,成功地将 *Ralstonia solanacearum* 重新植入真菌 *Aspergillus flavus* 中 (Spraker *et al.* 2016)。或者 GFP 标记并混合培养后,采用激光束转化技术成功地将该 EFB 引入治愈的真菌中 (Partida-Martinez *et al.* 2007c)。用氯化镁洗涤真菌,然后将其与 EFB 共同制备悬浮液,在固体培养基上共培养,不但可成功将 EFB 重新植入其原真菌宿主 (Arendt *et al.* 2016; Baltrus *et al.* 2018),还可以将其植入非原宿主真菌 (Arendt *et al.* 2016)。将 EFB 接种到含有 10mL/L 甘油和 100 $\mu$ g/mL 环己酰亚胺的 LB 平板中,同时将治愈真菌接种到 1/2PDA 平板,分别培养一定时间之后,在一个新的 1/2PDA 平板上取出 1 小块琼脂,嵌入接有 EFB 的 LB 小琼脂块,再将生长有治愈真菌的 1/2PDA 琼脂块倒置覆盖其上,继续培养之后,可成功将 EFB 重新引入其原真菌宿主 (Mondo *et al.* 2017)。

## 7 真菌内共生细菌研究展望

近年来,关于 EFB 的研究取得了巨大进步。真菌和 EFB 的关联比以前认为的更为重要和广泛,正如 Margulis (1998) 对于生物共生现象的感叹,“独立生命趋向于结合在一起,在更高、更大的组织层面上作为一个新的整体重新出现”。

对 EFB 的研究目前涉及的真菌物种总计 84 种(表 1-表 4)。考虑到地球真菌高达 150–1 200 万种的生物多样性(Hawksworth 2012; Wu *et al.* 2019),有理由推断仍有大量的真菌-细菌共生关系有待探索。传统的真菌分离方法中,普遍添加各种抗生素得到纯培养物,然后通过继代培养方法进行保藏,这两个步骤都有可能对真菌宿主进行了无意识的治愈从而降低 EFB 的普遍性(Partida-Martinez & Hertweck 2005; Lumini *et al.* 2007; Arendt *et al.* 2016; Desirò *et al.* 2018)。因此,在今后的研究中,从自然界获得新真菌菌株时,应将 EFB 的检测作为一种常规手段,而且尽量减少抗生素使用,尽可能永久保藏初始菌种而少用继代培养。

分离得到的 EFB 也可表现出显著的生物学功能。Sharma *et al.* (2008) 从牧豆树和枣树根部的菌根菌 *Piriformospora indica* 中发现 EFB *Rhizobium radiobacter*, 将其成功分离并回接到大麦幼苗后,可改善幼苗的生长状况,并使其获得对白粉病菌的抗性。由此可见,EFB 具有潜在的农业应用价值,有必要对其分离纯培养。但是许多 EFB 是专性共生体,严格依赖其真菌宿主而存活并进行生长,无法在纯培养物中培育,这阻碍了相应的生物学实验(Naumann *et al.* 2010; Desirò *et al.* 2015)。目前的宏基因组学技术,突破了纯宿主和纯 EFB 的限制,也有助于解析关联体双方的营养需求,配制 EFB 特殊培养基(Ohshima *et al.* 2016; Naito *et al.* 2017)。

在真菌-细菌共生关系形成和演化过程中,两者表现出了独特的生存策略,相互影响,并进

一步作用于相关植物。真菌宿主为 EFB 提供生长场所和生活史中特定阶段的必要营养(Anca *et al.* 2009; Schüßler 2012),而 EFB 控制真菌宿主的产孢(Partida-Martinez *et al.* 2007c; Mondo *et al.* 2017), EFB 提供的毒力因子还作用于植物,可造成严重的农作物病害(Partida-Martinez & Hertweck 2005; Partida-Martinez *et al.* 2007a; Lackner & Hertweck 2011)。同时,携带 EHB 并导致水稻幼苗枯萎病的 *Rhizopus microsporus*, 其近缘类群毛霉目真菌也是传统食品发酵菌和酒类真菌。此类真菌在世界很多地区,尤其是亚洲,大量用于人们的食品和饮品,因此其安全性至关重要。由于小孢根霉素和小孢根霉毒素对真核动物都具有毒性,所以对这些毛霉目真菌的 EFB 介导的毒性开展深入研究对食品安全具有重要的意义(罗晓妙 2012; 吴荣等 2013)。最后,小孢根霉素具有抑制有丝分裂的特性,因此在肿瘤生物学中具有广阔的应用前景(Scherlach *et al.* 2012)。

以上真菌-细菌共生关系的初步研究结果都依赖于传统方法,现代基因组学方法解释了真菌-细菌共生关系的更多生物学功能,比如 EFB 控制宿主真菌的产孢行为(Mondo *et al.* 2017),水平基因转移导致的宿主真菌新功能和 EFB 种群多样化(Sun *et al.* 2018),专性内生的特性导致 EFB 基因组明显缩减(Baltrus *et al.* 2017; Sharmin *et al.* 2018), EFB 编码表面活性剂线性脂肽利于穿透细胞膜(Niehs *et al.* 2018)。随着多组学技术的广泛深入应用,将来必定会对这一共生关系的更多细节给出更为明确的答案。

子囊菌门和担子菌门真菌天生具有丰富且规则的隔膜,会对 EFB 在菌丝中的移动产生物理限制,阻碍或减缓 EFB 向营养丰富的部位迁移。而毛霉菌门真菌缺少或仅有很少的不规则隔膜,利于 EFB 沿菌丝“高速公路”移动(Partida-Martinez & Hertweck 2005; Desirò *et al.* 2015)。有研究已成功将 EFB 重新植入毛霉菌门原真菌宿主

*Rhizopus* 或者 *Geosiphon*, 并发挥出 EFB 的各种生物学功能 (Mollenhauer *et al.* 1996; Partida-Martinez *et al.* 2007c; Schüßler 2012; Mondo *et al.* 2017)。因此, 毛霉菌门真菌作为模式生物, 通过 EFB 重新植入, 用于研究微生物之间的信息交流、侵染、适应性与共生关系。

有研究发现 EFB 会分泌一些物质降解真菌宿主孢子壁纤维素和几丁质, 促使 EFB 更容易进入到孢子内部 (Walley & Germida 1996; Filippi *et al.* 1998; Gazzanelli *et al.* 1999; Moebius *et al.* 2014)。因此, 在 EFB 重新植入的研究中, 可以考虑用纤维素酶和几丁质酶预处理真菌孢子或者菌丝, 加快植入速度, 提高植入成功率。

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