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松材线虫对其携带的细菌繁殖的影响*

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Effects of pine wood nematode on propagation of its carrying bacteria. LIN Feng, ZHAO Boguang (*College of Forestry Resources and Environment, Nanjing Forestry University, Nanjing* 210037, *China*). -*Chin. J. Appl. Ecol.*, 2005, 16(12):2476~2478.

In this paper, the aseptic eggs of *Bursaphelenchus xylophilus* were obtained after treated with 30% H_2O_2, and cultured with *Pinus thunbergii* callus. Ten *B. xylophilus*-carrying bacterial strains directly isolated from diseased *P. thugbergii* and *P. massoniana* in six epidemic provinces *i.e.*, GcM6-2A *Pseudomonas putida*, GcM6-1A *P. putida*, ZpB1-2A *P. putida*, HeM2A *Pseudomonas* sp., HeM1A *Pseudomonas* sp., HeM142B *Pseudomonas* sp., GcM1-3A *P. cepacia* and HM3 *Pantoeu* sp., ZpB4-2B *Staphylococcus sciuri* and ZpB2-3A *Enterobacter amnigenus*, were collected, and the effects of axenic *B. xylophilus* (ABx) on their propagation were studied. The results showed that pine wood nematode (PWN) promoted the propagation of 7 bacterial strains in *Pseudomonas* and 1 bacterial strain in *Pantoeu* sp., including *Pseudomonas putida*, *P. putida*, *P. putida*, *Pseudomonas* sp., *Pseudomonas* sp., *Pseudomonas* sp., *P. cepacia* and *Pantoeu* sp., but inhibited *Staphylococcus sciuri* and *Enterobacter amnigenus*, which could explain the phenomenon that *Pseudomonas* was the prevailing genus of the bacteria carried by PWN, and might provide essential nutrition to the bacteria. The close relationship between PWN and bacterial strains in *Pseudomonas* might account for the pine wood nematode disease.

Key words Pine wood nematode disease, Pathogenic bacteria, Complex infection, Propagation.
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1 引 言

松材线虫病(*Bursaphelenchus xylophilus*)是松树的一种毁灭性病害,在日本[14,15]、中国[1,23]、韩国和北美[2]、尼日利亚[13]和葡萄牙[17]等国家蔓延,造成了巨大经济损失,其中以日本和中国受害最重[30].一直认为松材线虫是引起该病的唯一病原[14,16,18,23],但近十几年来的研究发现,细菌在致病过程中可能起着重要作用[7~12,19],相继从病木和松材线虫体上分离到能对黑松苗有致萎活性的细菌[5,6,12,20,21,25].赵博光等[25]首次根据实验提出松材线虫病是线虫和细菌共同侵染引起的复合侵染病害的假说,并在以后的试验中得到了验证[4,20,21,24,26~29].关于松材线虫对其细菌繁殖的影响研究鲜有报道.本试验采用从感病松树上分离并鉴定了的细菌菌株中选取假单胞属7株、其它属的细菌菌株3株,在无菌条件下研究松材线虫对这些细菌菌株繁殖的影响,旨在解释这些细菌得以在病木和线虫体上存在的原因,为进一步弄清松材线虫与其携带细菌之间的关系打下基础.

2 材料与方法

2.1 供试材料

2.1.1 松材线虫来源与培养 从采集与野外自然感病枯死的黑松木段上取样,分离方法见文献[27],将分离并鉴定的线虫培养在长满灰葡萄孢(*Botrytis cinerea*)的PDA平板上培养,冰箱保存.

2.1.2 无菌松材线虫卵的获得 取适量松材线虫,用两层纱布包好,置于贝曼漏斗中,加适量的水,在常温下分离6~24 h;鉴定后离心、去上清液,留下约2 ml,移到琼脂培养基上,用脂膜封口,放入25 ℃的恒温箱中培养;12~18 h后,用水冲洗琼脂培养基,滴管吸取松材线虫水至离心管内,于离心机中离心5 min(1 500 转·min^{-1}),吸去上清液,留松材线虫水约1.5 ml,无菌条件下移至另离心管中,线虫水和30%双氧水按1:1比例混合,将离心管置于15 ℃左右的水中静置10 min;用无菌蒸馏水稀释线虫水3~5遍[28];分别用180目和200目的筛子过滤;于45倍昆虫解剖镜下挑取卵数个于NA培养基上,检查线虫卵是否带细菌;如果带菌,则放弃本次实验.

2.1.3 无菌松材线虫的培养 挑取100个无菌线虫卵于装着黑松愈伤组织(约1 g)和2 ml无菌水的小培养皿中(内径约为1 cm),于28 ℃温箱中培养3 d,备用.

2.1.4 各细菌菌株的获得 根据松材线虫病在我国的发生、发展、分布和蔓延,选择我国松材线虫病发生的、有代表性的疫区,在2001年春秋两次进行采样.采集了感染松材线虫病的黑松、马尾松病木,采用木块组织分离法,分离松材线虫所携带的细菌58株.利用传统的细菌鉴定方法和自动化细菌鉴定系统相结合的手段,对这些细菌进行了鉴定[20,29].笔者从中选取10个菌株,将这些细菌菌株于NA培养基上斜面

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培养,冰箱低温保存备用.

2.2 研究方法

2.2.1 黑松愈伤组织的诱导和继代培养[3,28]　将黑松种子浸泡数小时,去壳,于 75% 乙醇溶液中表面消毒 3 次,每次 40 s,用无菌水冲洗 3 次,置于 0.1% 升汞溶液中浸泡 4 min,再用无菌水冲洗 5 次,在无菌操作台上剥去胚乳得到成熟胚,将其移入灭过菌的装有 MS 诱导培养基的锥形瓶中,封口,于 28 ℃ 恒温箱中暗培养、两星期后,将诱导出黑松愈伤组织,将生长良好(为乳白色)的愈伤组织移至继代培养基上.在无菌操作台上,将愈伤组织切成黄豆大小,移至装有 1/2MS 继代培养基的锥形瓶中,每个锥形瓶接入 3～4 块,于 28 ℃ 恒温箱中暗培养,每隔一个月左右继代一次;

2.2.2 松材线虫对其携带细菌繁殖的影响试验　接种环挑取菌苔 5 环(直径约为 1 cm),悬浮于 10 ml 无菌水中,即得各种细菌悬浮液,备用,用微量移液器移取 2 ml 无菌水于小培养皿(内径约为 1 cm)内;先后向小培养皿中加入 2 条雄虫、约 0.01 g 黑松愈伤组织和 0.05 ml 细菌悬浮液;每种细菌做 4 个重复,将小培养皿放入直径为 7.5 cm 的培养皿中,封口,于 28 ℃ 恒温箱中培养;从第 2 天到第 7 天,每天取出一小培养皿,做平板菌落计数;处理的细菌菌株及其对照(不加线虫)的每日细菌的单菌落数目分别取 LOG 值后作两条曲线,并进行方差分析(SPSS12.0).

3　结果与讨论

图 1 结果表明,松材线虫对 HeM2A 假单胞菌、HeM1A 假单胞菌、GcM6-2A 恶臭假单胞、GcM6-1A 恶臭假单胞菌、ZpB1-2A 恶臭假单胞菌、GcM1-3A 洋葱假单胞菌以及 HM3 泛菌属 2 的繁殖有显著促进作用($P<0.01$),其细菌数量的对数值曲线全部或大部分情况下高于对照曲线,即加入松材线虫后能显著地促进该菌繁殖,也促进 HeM142B 假单胞菌的繁殖,但方差分析显示无显著的促进作用.由松材线虫对 ZpB4-2B 松鼠葡萄球菌和 ZpB2-3A 河生肠杆菌的繁殖影响的曲线可见,大部分情况下,加入无菌松材线虫的曲线低于没有加入松材线虫对照繁殖曲线,经方差分析表明,加入无菌松材线虫后抑制了两株菌株的繁殖.无论是促进或抑制,各细菌生长的高峰期为处理后的第 3 天或第 4 天.这与细菌在自然状态下的生长趋势相一致.

从松材线虫体上或从病死木分离得到的细菌以假单胞菌属(*Pseudomonas* spp.)的一些种为主[4～6,20～22].对松材线虫病发生过程中病树体内细菌种群的变化研究结果表明,在非接种枝上,以假单胞杆菌为主的细菌种群在松材线虫病发生过程中出现早、比例高[21,22].本试验中,松材线虫对供试的 7 株假单胞菌株的繁殖都有促进作用,且这些菌株对无菌黑松切根苗都有强致萎活性[20,29].接种试验证明这些细菌均为强致病菌,同时松材线虫又能促进其繁殖;相关试验也证明,除 HeM142B 菌株外,其余 6 个假单胞菌株均能显著地促进松材线虫的繁殖[27].因此,这些细菌偶然污染的可能性很小.假单胞菌属不仅是松材线虫病侵染过程中的优势属,

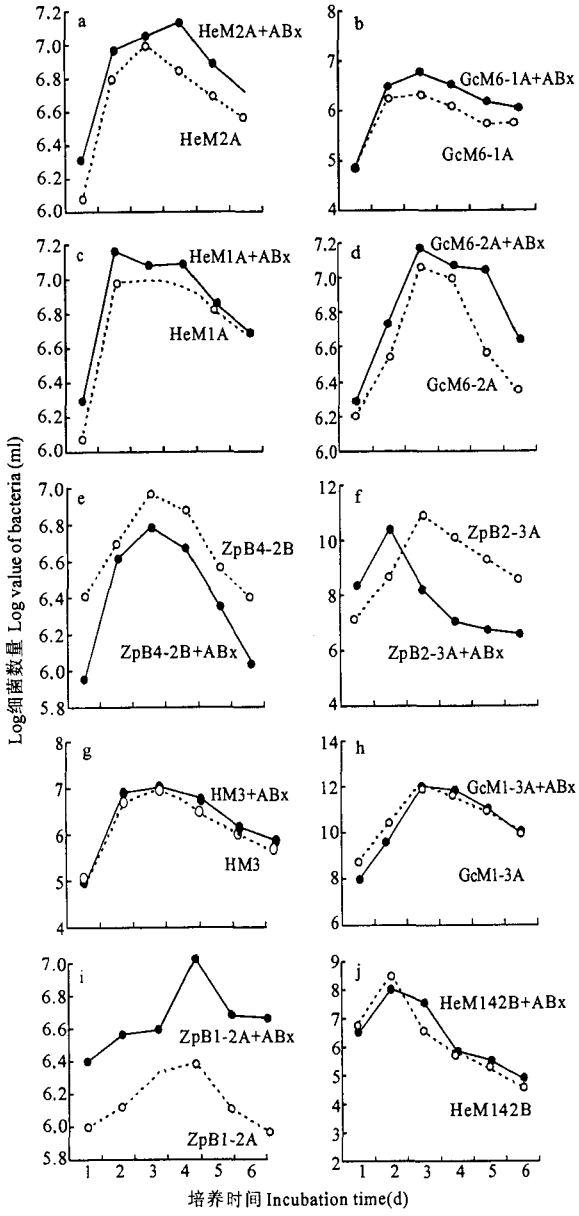

图 1　无菌松材线虫对其携带细菌繁殖速率的影响

Fig. 1 Effects of PWN on the reproductive rates of its carried bacterial strains.

在产生毒素导致寄主萎蔫甚至死亡中也起着重要的作用.此外,松材线虫对泛菌属的某些菌株如本试验中的 HM 3 泛菌属 2 的繁殖也有促进作用.这可能是在感病松树上也可分离到较多泛菌[20]的原因之一.松材线虫对 ZpB4-2B 松鼠葡萄球菌及 ZpB2-3A 河生肠杆菌的繁殖有抑制作用,这两株菌株对黑松切根苗也无致萎活性[20].因此,这些细菌可能是松材线虫偶然携带的,也可能在松材线虫病致病过程中起某种作用.另外,松材线虫提供何种物质促进其携带细菌的繁殖,仍需深入研究.

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