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生防菌株 LB-1 培养液对黄瓜的抑病促生作用

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摘要:LB-1 为新筛选的生防近缘毛壳(*Chaetomium subaffine*)菌株。为明确 LB-1 培养液的抑病促生效果,本研究以黄瓜为供试植物,分别采用灌根和叶面喷施的方式,测定了 LB-1 培养液对黄瓜枯萎病和黄瓜白粉病的抑制效果;通过种子萌发、盆栽苗和生化检测试验,分析了 LB-1 培养液对黄瓜的促生作用。结果发现,LB-1 培养液对黄瓜枯萎病抑制作用效果甚微,但对黄瓜白粉病生防效果明显,黄瓜叶片接菌 24 h 后叶面喷施 LB-1 培养液对白粉病的防效高达 48.86%。LB-1 培养液浸润催芽处理 24 h 的黄瓜种子萌发率和根长均显著高于对照($P=0.05$),且 LB-1 培养液浸种、灌根、叶面喷施处理均能促进黄瓜幼苗的生长发育。LB-1 没有产嗜铁素、产氢氰酸、固氮和溶磷能力,但能够产生吲哚乙酸(Indoleacetic acid, IAA)。表明生防菌株 LB-1 培养液能有效抑制黄瓜白粉病的发生,促进黄瓜种子萌发和植株生长,而且其促生作用可能通过产生 IAA 来实现。

关键词:近缘毛壳;LB-1 培养液;黄瓜;白粉病;枯萎病;促生作用

Disease suppression and growth-promoting effects of the culture broth of a biocontrol strain LB-1 on cucumber

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Abstract: LB-1 is a newly screened biocontrol strain of *Chaetomium subaffine*. The disease suppression and growth-promoting effects of LB-1 culture broth were clarified with cucumber as donor plant in this study. The disease suppression effects on *Fusarium* wilt and powdery mildew of cucumber were detected using root-irrigation and foliar spraying methods, respectively; the growth-promoting effect on cucumber was evaluated through seed germination test, pot seedling experiment and biochemical assay. The results showed that LB-1 culture broth has little inhibitory effect on cucumber *Fusarium* wilt, but significant biocontrol effect on cucumber powdery mildew was obtained. The control efficacy on cucumber powdery mildew was reached to 48.86% when cucumber seedlings being foliar sprayed with LB-1 culture broth 24 h after the inoculation of *Sphaerotheca fuliginea*. The seed germination rate, root length of cucumber being treated with LB-1 culture broth for 24 h were significantly higher ($P=0.05$) than those of control cucumber seed. The growth and development of cucumber seedlings could be promoted when being treated with seed soaking, root irrigation or foliar spraying of LB-1 culture broth. No obvious siderophore production, hydrogen cyanide production, nitrogen fixation and phosphate solubilization abilities were detected from LB-1, but its indole acetic acid (IAA) production ability was prominent. These indicated that LB-1 culture broth could inhibit the occurrence of cucumber powdery mildew, promote the seed germination and seedling growth of cucumber, and its growth-promoting effect might be obtained through IAA production.

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Key words: *Chaetomium subaffine*; LB-1 culture broth; cucumber; powdery mildew; *Fusarium* wilt; growth-promoting effect

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以化学农药为主导的植物病害防治策略引起的环境污染、抗药性增强等问题日趋凸显,使得生防菌成为植物病害防治中最具研发潜力的领域[1]。毛壳菌(*Chaetomium* spp.)是子囊菌亚门、毛壳菌属的真菌,也是近年来新发掘的生防菌类群[2]。已报道的生防毛壳菌有球毛壳(*C. globosum*)[3,4]、角毛壳(*C. cupreum*)[5]、螺旋毛壳(*C. spirale*)[6]等。进一步的研究发现这些毛壳菌对植物病害的生防作用主要通过菌体重寄生、菌丝快速生长的生存竞争和产生抑菌物质实现,其中以产生抑菌物质的研究报道最多[3,7],而我国 Xu 等[8]认为,毛壳菌的生防作用并不是通过单一途径实现,而是营养竞争、重寄生及产生抑菌物质的协同作用结果。

许多生防菌既能抑菌,又能通过自身的代谢产物或其他方式促进植物的生长发育[9,10]。生防菌的促生作用可通过产生植物激素、产生嗜铁素、固氮、溶磷等途径实现[11,12]。吲哚乙酸(indole acetic acid, IAA)是一类刺激植物生长的常见内源性激素,能够促进植物的生长并提高植物的抗逆能力[13]。铁是生物体的必须元素,在铁有限的条件下,促生菌可产生嗜铁素,促进植物铁的吸收而起到促生作用[14]。氮和磷是作物生长发育必不可少的养分和代谢参与物质,促生菌能够起到固氮或者将难溶性磷转化为可溶性磷,促进植物吸收、生长的作用[15]。Abdallah 等[16]发现分离自曼陀罗(*Datura stramonium*)的两菌株 *Stenotrophomonas maltophilia* S37 和 *Bacillus mojavensis* S40 对番茄枯萎病菌(*Fusarium oxysporum* f. sp. *lycopersici*)有抑制作用,而且能够明显促进番茄植株的株高、根长、鲜重等生长指标。Zamoum 等[17]发现放线菌(*Sreptomyces asterosporus*)SNL2 对番茄枯萎病菌有拮抗作用,同时能够产生 IAA、嗜铁素,促进番茄根、茎的生长。我国 Kang 等[18]、Yang 等[19]也分别发现了兼具有抗病和促生作用的解淀粉芽孢杆菌(*Bacillus amyloliquefaciens*)B1619、成团泛菌(*Pantoea vagans*)ASR16 和短小芽孢杆菌(*B. pumilus*)ALR33,而且菌株 ASR16 和 ALR33 均能产生 IAA 和嗜铁素,具有溶磷能力。

黄瓜是我国最为常见的果蔬之一。枯萎病和白粉病是黄瓜生产上发生普遍的病害。生产中黄瓜枯萎病的防治尚缺乏有效抗病品种,轮作抗病也因耕地资源的限制而不易实施[20],而白粉病的防治虽然可以利用抗病品种,但施用化学农药仍然是生产中该病的主要防治手段[21]。因此,生防菌研发是这两种病害无公害控制的重要途径。已报道的黄瓜枯萎病生防菌有木霉菌(*Trichoderma* spp.)[22]、枯草芽孢杆菌(*B. subtilis*)等[23]。白粉病生防菌主要有白粉寄生孢(*Ampelomyces quisqualis*)[24]、哈茨木霉(*T. harzianum*)[25]、枯草芽孢杆菌[26]、蜡蚧轮枝菌(*Verticillium lecanii*)[27,28]等。但是,毛壳菌对黄瓜枯萎病和白粉病生防作用的研究鲜有报道。

LB-1 是我们实验室从龙柏鳞叶小枝中新筛选分离的一株近缘毛壳(*C. subaffine*)生防菌株,能够拮抗多种植物病原真菌,而且其培养液的抑菌效果尤为显著[29]。基于此,本研究以黄瓜为供试植物材料,检测分析了 LB-1 培养液对黄瓜枯萎病和和白粉病的抑制效果和对黄瓜生长的促进作用,以期为生防菌株 LB-1 应用于黄瓜生产提供参考。

1 材料与方法

1.1 供试植物材料和菌种

黄瓜种子由山东寿光蔬菜产业集团提供;生防菌株 LB-1 和黄瓜枯萎病菌(*F. oxysporum* f.sp. *cucumerinum*)为潍坊学院生物与农业工程学院保存;黄瓜白粉病菌(*Sphaerotheca fuliginea*)采自潍坊市区黄瓜植株。

1.2 LB-1 培养液抑病效果检测

1.2.1 培养液的获取 参照 Tan 等的方法[5],接种 LB-1 菌饼于 PDB 培养液中(菌饼直径 6 mm,接种量为每 50 毫升接种一个菌饼,25℃、130 r·min^{-1}的条件下振荡培养 15 d。培养液经双层纱布过滤,滤液在 4℃、12 000 r·min^{-1}条件下离心 15 min,上清液即为生防菌株 LB-1 培养液。

1.2.2　对黄瓜枯萎病的抑制效果检测　供试黄瓜种子催芽后播种于小花盆(直径 15 cm)的灭菌土中,置于温室隔离培育(17℃~25℃,自然光照)至黄瓜苗长出 2 片真叶后,灌根接种黄瓜枯萎病菌孢子悬浮液(1×10^5 个·mL^{-1}),5 d 后浇灌 LB-1 培养液于黄瓜植株根部,此后每隔 7 d 浇灌一次,每次每盆 80 mL,连续浇灌 3 次。接种 45 d 后开始调查发病情况,计算病情指数和防效。处理和对照各 20 盆黄瓜苗(每盆 1 株)。黄瓜枯萎病分级参照 Qin 等的标准[30]:0 级,无症状;1 级,叶片轻微萎蔫;2 级,植株轻度萎蔫;3 级,植株明显萎蔫或矮化;4 级,植株严重萎蔫或倒伏枯死。

1.2.3　对黄瓜白粉病的抑制效果检测　供试黄瓜种子催芽后播种于小花盆(直径 15 cm)的灭菌土中,置于温室隔离培育(17℃~25℃,自然光照)至长出 8~10 片叶之后,做 2 种处理:(1)黄瓜植株叶面喷施 LB-1 培养液 2 次,每次间隔 2 d,第二次喷洒 1 d 后,采用孢子粉沉降法接种黄瓜白粉病菌,并在接种 1 d 后再喷洒一次;(2)黄瓜植株先接种白粉病菌,并于接种 1、4 和 7 d 后叶面喷施 LB-1 培养液各 1 次。对照黄瓜苗接种前、后 1 d 分别用无菌水喷施一次。不同处理的黄瓜苗隔离培养至对照植株叶片明显发病后,每株取中上部 3 片叶子,根据国家标准 GB/T 17980.30-2000 对叶片进行病情分级,统计计算病情指数和防效。每一处理设 20 盆黄瓜苗(每盆 1 株),喷施时以叶片完全湿润为好。

1.3　LB-1 培养液促生作用检测

1.3.1　对黄瓜种子萌发的促进作用检测　将供试黄瓜种子用 2% NaClO 表面消毒 10 min,无菌水冲洗 5 次后,均匀摆放在铺有灭菌滤纸的培养皿中,25℃培养条件下设 3 种处理:(1)LB-1 培养液浸润 24 h 后改用蒸馏水浸润;(2)LB-1 培养液浸润;(3)蒸馏水浸润(对照)。每处理设 100 粒种子,催芽培养 5 d 后统计分析不同处理的黄瓜种子萌发率、根长。

1.3.2　对黄瓜苗生长的促进作用检测　将供试黄瓜种子用 2% NaClO 表面消毒 10 min,无菌水冲洗 5 次后,做 4 种处理:(1)浸种。LB-1 培养液浸润催芽 24 h 后,播种在装有灭菌土(直径 15 cm)的小花盆中培养,待黄瓜苗长出 2 片真叶后用自来水浇灌,每盆每次浇灌 80 mL,浇灌 5 次,每次间隔 5 d;(2)灌根处理。正常催芽处理 24 h 的黄瓜种子播种在装有灭菌土的小花盆中(直径 15 cm),待黄瓜苗长出 2 片真叶后用 LB-1 培养液浇灌,每盆每次浇灌 80 mL,浇灌 5 次,每次间隔 5 d;(3)叶面喷施。正常催芽处理 24 h 的黄瓜种子播种于装有灭菌土的小花盆中(直径 15 cm),待黄瓜苗长出 2 片真叶后用 LB-1 发酵液叶面喷施黄瓜苗,同时根部自来水浇灌 60 mL,处理 5 次,每次间隔 5 d;(4)对照。正常催芽处理 24 h 的黄瓜种子播种在装有灭菌土的小花盆中(直径 15 cm),待黄瓜苗长出 2 片真叶后用自来水浇灌,每盆浇灌 80 mL,浇灌 5 次,每次间隔 5 d。所有黄瓜苗培养 45 d 后,测定不同处理的黄瓜苗株高、根长、单株叶片数、叶面积、茎长、茎围、地上部和地下部鲜重、地上部和地下部干重等生长指标。每个处理设 10 盆,每盆 2 株。

1.3.3　促生物质检测　IAA 检测参照 Manivannan 等的方法[31]:0.5 mmol·L^{-1} 三氯化铁溶解于 35% 的高氯酸中,再与等量的 LB-1 培养液混合,遮光静置 30 min,观察颜色变化。混合液由黄色变为淡红色,表明有 IAA 的产生。

嗜铁素检测参照 Kejela 等的方法[32]:PDA 培养基、CAS 染液(铬天青 5 mmol·L^{-1}、0.1 mmol·L^{-1}、十六烷基三甲基溴化铵 4 mmol·L^{-1})和磷酸盐缓冲液分别灭菌后,向 1 000 mL 培养基中加入 CAS 染液、磷酸缓冲液各 50 mL,制成检测培养基平板,接种直径 6 mm 的 LB-1 菌饼,25℃、自然光照条件下培养,观察菌落周围颜色变化。颜色从蓝色变为淡红色,表明有嗜铁素产生。

可溶性磷的检测参照 Husen 等的方法[33]:制作 Pikovskaya 培养基(葡萄糖 1 g,三磷酸钙 0.5 g,硫酸铵 0.05 g,氯化钾 0.02 g,硫酸镁 0.01 g,酵母提取物 0.05 g,硫酸锰 0.000 2 g,硫酸亚铁 0.000 2 g,琼脂 2 g,水 100 mL),高压灭菌后,在其中加入适量溴酚蓝染液,混匀后制成培养基平板。接种 LB-1 菌饼(直径 6 mm),25℃、自然光照条件下培养,观察菌落周围透明带的形成。

固氮能力检测参照 Li 等的方法[34]:制作检测培养基平板(葡萄糖 1 g、磷酸氢二钾 0.02 g、硫酸钾 0.02 g、氯化钠 0.02 g、碳酸钙 0.5 g、硫酸镁 0.02 g、琼脂粉 2 g,水 100 mL),接种 LB-1 菌饼(直径

6mm),25℃、自然光照条件下培养,观察菌落生长 情况。

Table 1 Effects of LB-1 culture broth on *Fusarium* wilt and powdery mildew on cucumber plants

Treatment	Disease	Disease index	Control efficacy /%
Root irrigation	*Fusarium* wilt	5.40 a	2.70
Control	*Fusarium* wilt	5.55 a	/
Foliar spraying before inoculation	Powdery mildew	37.80 b	30.39
Foliar spraying after inoculation	Powdery mildew	27.77 c	48.86
Control	Powdery mildew	54.30 a	/

Note: Different letters indicate a signficant difference at the 5% level.

氢氰酸的检测参照 Abdallah 等的方法[16]:制作含有 4.4 g·L^{-1}甘氨酸的培养基平板并接种 LB-1 菌饼(直径 6 mm),25℃、自然光照条件下培养 2 d 后,把在碱性苦味酸溶液中浸泡后的灭菌滤纸嵌入灭菌培养皿盖中,继续培养 4 d,观察培养皿盖中滤纸颜色变化,分析氢氰酸的产生。

1.4 数据分析

试验数据均采用 SAS 8.1 软件进行统计分析,应用最小显著差异法(LSD)进行差异显著性检验。

2 结果与分析

2.1 LB-1 培养液对黄瓜枯萎病和白粉病的作用效果

LB-1 培养液对黄瓜盆栽苗枯萎病和白粉病的作用效果如表 1 所示。由表 1 可见,LB-1 培养液灌根处理的黄瓜苗枯萎病对照和处理的病情指数相当,但接种前、后叶面喷施 LB-1 培养液,均能对黄瓜白粉病的发生产生明显抑制作用,而且接菌后叶面喷施处理对黄瓜苗白粉病的防效高达48.86%,病情指数显著低于接菌前处理(P=0.05)。

2.2 LB-1 培养液对黄瓜植株的促生作用

2.2.1 对黄瓜种子萌发的影响 黄瓜种子萌发实验中,LB-1 培养液+蒸馏水、LB-1 培养液和对照三种方式处理的黄瓜种子在第 4 d 后萌发情况差异明显(图 1),因此在第 5 d 对不同处理的黄瓜种子萌发率、根长进行统计分析,结果如表 2 所示。

由图 1、表 2 可知,与对照相比,LB-1 培养液浸润 24 h 后,再用蒸馏水浸润处理,能够显著提高黄瓜种子的萌发率,促进根的伸长。但是,LB-1 培养液持续浸润处理则可抑制黄瓜种子萌发和根的伸长。

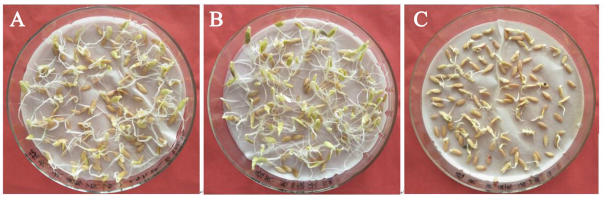

Fig. 1 Effect of LB-1 culture broth on cucumber seed germination

A: Distilled water; B: LB-1 culture broth and distilled water; C: LB-1 culture broth.

Table 2 Germination rate and root length of cucumber seed treated with LB-1 culture broth

Treatment	Germination rate /%	Root length /mm
LB-1 culture broth+ water	85.00 a	42.78±3.81 a
LB-1 culture broth	73.33 c	2.68±1.44 b
Control (distilled water)	80.33 b	39.01±3.38 a

Note: Different letters indicate a signficant difference at the 5% level.

2.2.2 对黄瓜苗生长的影响 盆栽苗检测结果发现,与对照黄瓜植株相比,LB-1 培养液浸种、灌根和叶面喷施后,对黄瓜植株的株高、根长、茎长、茎围、单株叶片数、叶面积、茎围、地上部和地下部鲜重、地上部和地下部干重等生长指标均可产生不同程度的促进作用。其中,灌根处理的促生效果优于其他处理方式(表 3)。

2.2.3 促生物质的产生 LB-1 产促生物质的实验检测结果如图 2 所示。由图 2-A 可知,LB-1 培养液与 IAA 检测液混合后,混合液变为淡红色(右侧

试管),而不加 LB-1 培养液的对照仍为黄色(左侧试管),表明LB-1培养液中含有IAA。LB-1在含有 CAS 的培养基上生长 4 d 后,菌落周围培养基蓝色没有发生变化,表明没有嗜铁素产生(图 2-B)。LB-1 接种在溶磷和固氮检测培养基上培养 4 d 后,溶磷检测培养基上 LB-1 菌落周围没有出现透明圈(图 2-C),固氮培养基上 LB-1 生长衰弱(图 2-D),表明 LB-1 没有溶磷和固氮能力。LB-1 在含有甘氨酸的培养基中培养后,培养皿盖上苦味酸浸泡过的滤纸颜色依然为鲜黄色,没有发生变化,表明 LB-1 不能够产生氢氰酸(图 2-E)。

Table 3　Growth parameters of cucumber plants being treated with LB-1culture broth

Growth parameters	Treatments ofLB-1cultre broth			
	Seed soaking	Foliar spraying	Root irrigation	Control
Plant height/cm	22.24 ± 4.08 b	28.78 ± 3.40 b	32.87 ± 6.92 a	18.93 ± 4.40 c
Root length/cm	5.56 ± 0.85 c	6.75 ± 0.67 b	7.80 ± 0.92 a	5.25 ± 1.14 c
Stem length/cm	5.89 ± 1.16 a	6.86 ± 0.92 a	7.60 ± 1.00 a	5.74 ± 1.46 a
Stem girth/cm	1.98 ± 0.45 a	1.77 ± 0.22a	1.99 ± 0.30a	1.77 ± 0.27 a
Leaf area/cm^2	56.29 ± 14.68 b	59.68 ± 7.29 b	66.29 ± 13.79 a	43.14 ± 13.73 c
Fresh plant weight/g	10.29 ± 4.29 b	11.13 ± 1.96 b	13.34 ± 5.78 a	6.42 ± 2.44 c
Fresh root weight/g	0.43 ± 0.19 b	0.53 ± 0.14 b	0.93 ± 0.44 a	0.33 ± 0.13 c
Dry plant weight/g	0.87 ± 0.46 c	1.10 ± 0.22 b	1.48 ± 0.68 a	0.67 ± 0.29 c
Dry root weight/g	0.08 ± 0.03 b	0.06 ± 0.02 b	0.10 ± 0.05 a	0.04 ± 0.02 c

Note: Different letters indicate a signficant difference at the 5% level.

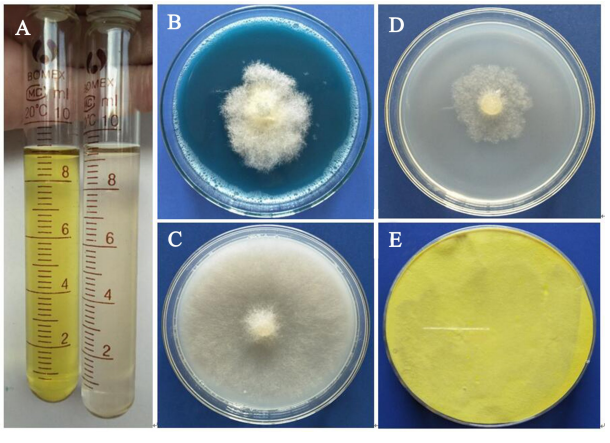

Fig. 2　Growth promoting factors produced by strain LB-1

A: IAA production; B: Siderophore production; C: Phosphate solubilization; D: Nitrogen fixation; E: HCN production.

3　讨论与结论

生防菌可通过生存空间和营养竞争、产生抑菌物质、诱导植物产生抗病性、重寄生等方式对植物病原菌产生抑制作用,其中产生抑菌物质是生防菌较为常见的生防途径[35,36]。已发现的毛壳菌生防作用途径有重寄生、产生抑菌物质等[37,38]。Park 等[39]研究发现,球毛壳菌株 F0142 培养液对稻瘟病菌(*Pyricularia oryzae*)和小麦叶锈病菌(*Puccinia recondita*)有抑制作用,而且从菌株 F0142 培养液中分离出了抗生物质 chaetoviridins。本研究前期发现,新筛选的近缘毛壳菌株 LB-1 培养液抑菌效果显著[29]。因此,本文对 LB-1 无细胞培养液的抑菌作用进行了研究。盆栽苗试验结果发现 LB-1 培养液叶面喷施对黄瓜白粉病生防效果显著,但灌根对黄瓜枯萎病的作用效果甚微。这表明 LB-1 培养液中含有抑菌成分,LB-1 可通过产生抑菌物质发挥生防作用,但土壤的复杂环境可能会对其抑菌效果产生一定影响。因此,菌株 LB-1 应用于植物病害生物防治时,其培养液叶面喷施可作为优选施用途径。实验过程中,未发现 LB-1 培养液对供试黄瓜植株表现出毒性,因此培养液及其抑菌物质是菌株 LB-1 值得深入研发的安全生防组分。

生防菌促生作用研究中,植株生长指标,促生长物质 IAA、氢氰酸、嗜铁素的产生及固氮、溶磷能

力是最为常见的检测指标[40]。本研究发现,LB-1 培养液能够促进黄瓜种子萌发和根的伸长,而且对黄瓜幼苗生长指标有促进作用;促生物质检测发现,LB-1 培养液中含有 IAA,但是 LB-1 不具有产生氢氰酸、嗜铁素、固氮、溶磷等促生活性,这表明 LB-1 促生作用可能通过产生 IAA 来实现。

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