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广东南瓜细菌性叶枯病及其病原鉴定

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摘要:在广东省雷州市发生一种南瓜(*Cucurbita moschata*)叶枯病,病株叶片边缘开始出现水渍状病斑,逐步发展成大病斑,后期病斑焦枯;在叶片上也可形成近圆形水渍状病斑,伴有黄色晕圈,后期病斑联合形成不规则大枯斑;叶柄和匍匐茎被侵染后呈水渍状腐烂。从病斑上分离到一种细菌,在 KB 培养基上,菌落为椭圆形,乳白色,半透明,边缘参差不齐,紫外灯照射下产生荧光反应。致病性测定结果表明,该病原细菌可侵染 6 个南瓜品种引起与田间症状相同的叶枯病。生理生化试验结果表明,该病原细菌与丁香假单胞丁香致病变种(*Pseudomonas syringae* pv. *syringae*)的特性一致。应用假单胞菌属特异引物 Ps-for/Ps-rev 和丁香假单胞丁香致病变种组群特异性引物 Group III-F/Group III-R,可从该病原细菌中扩增出预期大小分别为 1 018 bp 和 750 bp 的目的片段。应用丁香致病变种 *syrB* 基因特异性引物 B1/B2,可从该病原菌中扩增出预期大小为 750 bp 的丁香霉素基因片段。基于 16S rDNA 与 *gyrB* 基因序列系统进化分析均表明,南瓜叶枯病菌株与已报道的 *P. syringae* pv. *syringae* 菌株 HS191(CP006256)亲缘关系最近,二者聚类在一起形成一个小分支。人工接种条件下,该病原细菌还可侵染西葫芦、丝瓜、茄子、番茄、菜豆、扁豆等植物。这些结果表明,引起广东省南瓜叶枯病的病原为丁香假单胞丁香致病变种(*Pseudomonas syringae* pv. *syringae*)。这是首次在中国发现丁香假单胞丁香致病变种引起南瓜叶枯病。

关键词:南瓜细菌性叶枯病;丁香假单胞丁香致病变种;病原鉴定;生物学特性

Identification of the pathogen of pumpkin bacterial leaf blight disease in Guangdong

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Abstract: A leaf blight disease on pumpkin (*Cucurbita moschata*) occurred in Leizhou, Guangdong. The water-soaked lesions appeared at the edge of the leaves of infected-pumpkins, and gradually formed large lesions. The nearly-circular water-soaked lesions also appeared on leaves with yellow haloes. The petioles and stolons were also infected by the pathogen and exhibited water-soaked rot. A bacterium was isolated from the diseased spots. The colonies on KB medium were elliptical, milky white, translucent with uneven edges, and produced fluorescence under ultraviolet light. Pathogenicity test showed that the bacterium could infect 6 varieties of pumpkin and cause leaf blight symptoms being similar to those in the fields. The physiological and biochemical analysis showed that characteristics of the bacterium were in accordance with *Pseudomonas syringae* pv. *syringae*. PCR amplification with *Pseudomonas* specific primers Ps-for/Ps-rev and *P. syringae* pv. *syringae* specific primers Group III-F/Group III-R produced the expected 1018 bp and 750 bp fragments, respectively. An expected 750 bp product encoding the syringomycin was amplified from the isolates of the bacterium with *P. syringae* pv. *syringae syrB* gene specific primers B1/B2. Both of the phylogenetic analyses based on the 16S rDNA and *gyrB* gene

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sequences showed that the bacterium isolates were very nearly related to the *P. syringae* pv. *syringae* isolate HS191 (CP006256) and they clustered in one branch. By artificial inoculation, the bacterium could also infect zucchini, loofah, eggplant, tomato, common bean and hyacinth bean. These results reveal that the pathogen of bacterial leaf blight disease on *C. moschata* in Guangdong is *P. syringae* pv. *syringae*. This is the first report of *P. syringae* pv. *syringae* causing bacterial leaf blight on pumpkin in China.

Key words: pumpkin bacterial leaf blight; *Pseudomonas syringae* pv. *syringae*; pathogen identification; biological characteristics

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南瓜(*Cucurbita moschata*)是广东广泛种植的葫芦科作物之一。2014 年,在广东省雷州市的南瓜(品种:黄金二号)上发生一种细菌性叶枯病,田间病株叶片边缘开始出现水渍状病斑,逐步向中间发展成大病斑,后期病斑焦枯、皱缩,病健交界明显;在叶片上也会形成近圆形水渍状病斑,逐渐发展成不规则大枯斑,后期病斑联合,病斑四周有时也会出现黄化,叶片焦枯;叶柄和匍匐茎也会被侵染呈水渍状腐烂,湿度大时有菌脓溢出。该病害已给雷州市南瓜生产带来严重的损失。

目前,已报道的南瓜细菌病害包括由 *Xanthomonas cucurbitae* 侵染引起的南瓜细菌性叶斑病[1,2]、*Acidovorax citrulli* 侵染引起的南瓜细菌性果斑病[3,4]、*Pseudomonas syringae* pv. *lachrymans* 侵染引起的南瓜细菌性角斑病[5,6]和 *Erwinia tracheiphila* 引起的南瓜细菌性枯萎病[7,8]。Park 等(2015)报道了 *Pseudomonas syringae* pv. *syringae* 引起韩国的绿皮南瓜(*Cucurbita moschata*)细菌性叶斑病[9]。为了明确引起广东南瓜叶枯病的病原,本文对分离获得的病原细菌进行了致病性测定、生理生化特性及分子特征分析,旨在为该病害的防治提供科学依据。

1 材料与方法

1.1 病样采集与病原菌分离

南瓜病样采自于广东省雷州市,采用常规组织分离方法[10]分离病原菌。切取病健交界处组织约 0.5 cm×0.5 cm,在 75%乙醇和 0.1%升汞溶液中依次表面消毒 1 min,灭菌水清洗 3 次,用灭菌吸水纸吸干。消毒后的病组织放在无菌的培养皿中,加入 100 μL 灭菌水,将组织捣碎,静置 10 min。用接种环蘸取悬浮液在金氏 B(KB)平板上划线,28℃下培养 2 d;挑取典型单菌落划线进一步纯化,选取单菌落,保存于-80℃冰箱备用。

1.2 致病性测定

1.2.1 *接种液制备* 选取菌株 LZ-5、LZ-8 和 LZ-15,在 KB 培养基平板上划线,28℃培养 2 d;挑取单菌落接种到 NA 液体培养基中,180 r · min^{-1} 震荡培养 24 h,5 000 r · min^{-1} 离心 6 min 收集细菌,用灭菌水配成 3×10^8 CFU · mL^{-1} 细菌悬液。

1.2.2 *烟草过敏性反应* 取播种于营养钵灭菌土壤中 6~7 片叶龄的普通烟幼苗,采用浸润注射法将细菌接种液注射到普通烟叶片下表皮叶肉细胞间,每个菌株接种 10 片烟草叶,以注射接种 NA 液体培养基和清水作为阴性对照,28℃下 24~48 h 后观察过敏性反应。

1.2.3 *南瓜致病性测定* 采用摩擦法、喷雾法和针刺法 3 种方法,分别接种黄金二号南瓜(江淮园艺)、巨型大南瓜(北京市芳萱苑种子有限公司)、真甜谢花面南瓜(北京市芳萱苑种子有限公司)、日本田栗南瓜(山西农科品资种业有限公司)、龙鑫丹印度南瓜和密本 3 号中国南瓜(广东省农业科学院蔬菜研究所)等 6 个南瓜品种,每种方法中每个品种接种 10 株(4~5 片真叶期);以 NA 液体培养基和清水作为阴性对照。接种后 28℃保湿 48 h,之后放至温室中观察病情。植株发病后,再从每个处理中随机采集 3 株上的病斑,分离病原菌,并与接种菌株进行比较。

1.2.4 *对其他植物的致病性测定* 供试寄主植物包括葫芦科的西葫芦(*Cucurbita pepo*)、葫芦(*Lagenaria siceraria*)、甜瓜(*Cucumis melo*)、黄瓜(*Cucumis sativus*)、丝瓜(*Luffa cylindrica*)、苦瓜(*Momordica charantia*)、冬瓜(*Benincasa hispida*)、节瓜(*Benincasa hispida* var. *chieh-gua*),茄科的甜椒

(*Capsicum annuum* var. *grossum*)、辣椒(*Capsicum annuum* var. *longum*)、番茄(*Solanum lycopersicum*)、茄子(*Solanum melongena*),十字花科的白菜(*Brassica rapa* subsp. *chinensis*)、菜心(*Brassica rapa* subsp. *parachinensis*),豆科的刀豆(*Canavalia gladiata*)、豇豆(*Vigna unguiculata*)、菜豆(*Phaseolus vulgaris*)、豌豆(*Pisum sativum*)、蚕豆(*Vicia faba*)、红豆(*Adenanthera microsperma*)、扁豆(*Lablab purpureus*)。采用喷雾法接种上述寄主植物。

1.3　病原菌的生理生化特性测定

革兰氏反应(KOH 测验)、41℃条件下生长、TTC 培养基上生长、耐盐度、Hugh & Leifson 试验(OF)、糖酵解反应、硝酸还原反应、氧化酶测验、精氨酸双水解、淀粉水解、明胶液化、果聚糖的产生、丙二酸产碱以及对 30 种不同碳水化合物的利用等试验方法参照《植物病原细菌鉴定实验指导》(第三版)[11]和《植病研究方法》(第三版)[10]。以丁香假单胞菌丁香致病变种(*P. syringae* pv. *syringae*)Pss1(中国农业科学院植物保护研究所赵廷昌研究员惠赠)作为对照菌株。每个菌株设三次重复。

1.4　分子特征分析

1.4.1　16S rDNA 和 *gyrB* 看家基因序列克隆与分析　采用 EasyPure Plant Genomic DNA Kit(北京全式金生物技术有限公司)提取菌株 LZ-5、LZ-8 和 LZ-15 的基因组 DNA,应用 16S rDNA 通用引物 27f/1541r[12] 和 *gyrB* 基因通用 GYR-1/GYR-1R[13]进行 PCR 扩增及序列测定。利用 NCBI 中 BLAST 程序进行序列同源性搜索,进一步利用软件 MEGA 6.0 UPGMA(The unweighted pair group method with arithmetic averages)方法分别构建 16S rDNA 和 *gyrB* 基因序列系统发育树。

1.4.2　特异性引物 PCR 检测　应用 *Pseudomonas* 属成员的专化引物 Ps-for/Ps-rev[14]、丁香假单胞致病变种特异性引物 Group IA-F/Group IA-R、Group IB-F/Group IB-R、Group II-F/Group II-R、Group III-F/Group III-R 和 Group IV-F/Group IV-R[15]及丁香霉素 *syrB* 基因特异引物 B1/B2[16]等 7 对特异引物(表 1)分别对菌株 LZ-5、LZ-8 和 LZ-15 的基因组进行 PCR 扩增,取 10 μL PCR 产物在 1.5%琼脂糖凝胶上电泳。

Table 1　The primers used in this study

Primer	Target gene	Primer sequence (5′-3′)	Tm/℃	Expected size/ bp	Reference
27f	16S rDNA	AGAGTTTGATCCTGGCTCAG	48	1 400	[12]
1541r		AAGGAGGTGATCCAGCCGCA			
GYR-1	*gyrB*	CAYGCNGGNGGNAARTTYGA	48	1 200	[13]
GYR-1R		CCRTCNACRTCNGCRTCNGT			
Ps-for	16S rDNA	GGTCGAGAGGATGATCAGT	48	1 018	[14]
Ps-rev		TTAGCTCCACCTCGCGGC			
Group IA-F	*hrpZ*	CAGCTTGCCCAGGAGCTGA	67	880	[15]
Group IA-R		ATGTTGACCAGCAGCAAGGC			
Group IB-F		TTGGCTCAAGAGTTGACCCG	60	850	[15]
Group IB-R		GCGCGTTGACCAGCAAGTTG			
Group II-F		GCTGTGATCGATCAGCTGGT	60	1 000	[15]
Group II-R		TCAGGCCACAGCCTGGTTAG			
Group III-F		AGCTGGCCGAGGAACTGATG	60	750	[15]
Group III-R		AACTGGTCAAGATCCTGAGC			
Group IV-F		ATGCTCGCAAAATCGATGGC	62	780	[15]
Group IV-R		TGACTGGCCGTATTGCCATT			
B1	*syrB*	CTTTCCGTGGTCTTGATGAGG	60	750	[16]
B2		TCGATTTTGCCGTGATGAGTC			

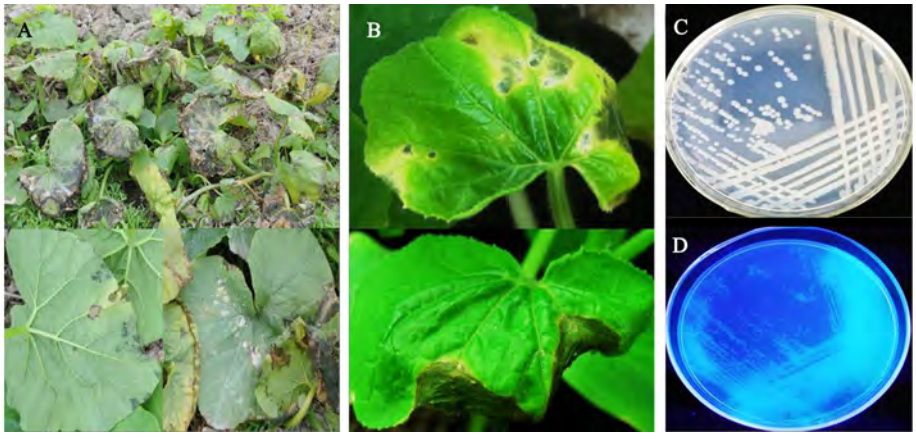

Fig. 1 The symptom of the diseased pumpkin plants and the morphological characteristics of the bacterium on medium plates

A: The diseased pumpkin (Variety: Huangjin No. 2) in the fields; B: Inoculated pumpkin (Variety: Huangjin No. 2) at 7 dpi; C: Bacterial colonies on KB medium at 28℃, 24 h; D: Fluorescence of the colonies on KB medium under UV light.

2 结果与分析

2.1 病原菌的形态特征

从田间病样中分离到菌落形态较一致的细菌，进一步纯化得到 20 个菌株，编号为 LZ-1～LZ-20。在 KB 培养基上，菌落椭圆形，乳白色，半透明，边缘参差不齐，无流动性，隆起，中间下凹，紫外灯照射下产生荧光反应（图 1-C、D）。

2.2 病原菌致病性测定结果

选取 LZ-5、LZ-8 和 LZ-15 为代表菌株进行致病性测定。烟草过敏性测定结果显示，3 个菌株均可引起烟草产生过敏性反应。采用喷雾、摩擦、针刺接种方法将 3 个菌株分别接种在 6 个南瓜品种的叶片上，均出现感病症状，以喷雾接种法植株发病较快。接种后 6～10 d（dpi），植株开始表现症状，叶片上主要产生淡黄色椭圆斑点，病斑初期为针尖大小的褪绿小点，随后逐渐扩大为椭圆形水渍斑，病斑周围有时产生黄色晕圈；或者从叶缘开始出现 V 字形水浸状病斑，最后扩展至整个叶片（图 1-B、图 2-A）。从接种发病叶片病斑上分离到的细菌菌株与接种用的 3 菌株形态特征一致。

2.3 对其他植物的致病性测定结果

室内人工喷雾接种结果表明：该病原细菌可侵染西葫芦、丝瓜、番茄、茄子、扁豆和菜豆（表 2、图 2），但不侵染甜瓜、葫芦、黄瓜、苦瓜、冬瓜、节瓜、白菜、菜心、辣椒、甜椒、烟草、刀豆、豇豆、豌豆和蚕豆。

2.4 病原菌生理生化特性

该病原菌为革兰氏阴性菌。生理生化试验结果表明，LZ-5、LZ-8 和 LZ-15 这 3 个菌株表现一致，菌株均不能在 41℃生长，具有运动性、耐盐性（5%），可在 TTC 培养基上生长，氧化酶、硝酸还原、精氨酸双水解、淀粉水解反应均为阴性，OF 为氧化型反应阳性，可利用葡萄、山梨醇、蔗糖作碳源，不能利用纤维素二糖、D-酒石酸盐、D-海藻糖、乳糖、麦芽糖，能使明胶液化，水解七叶苷、果聚糖形成反应均为阳性，可利用 D-甘露醇、肌醇、甜菜碱、赤藓糖醇、L-乳酸，不能利用核糖醇、L-酒石酸盐、DL-高丝氨酸作为碳源。这些特征与对照丁香假单胞丁香致病变种（*Pseudomonas syringae* pv. *syringae*）菌株 Pss1 的特征[11, 17]一致，但该病原菌还可以利用 L-酪氨酸和 L-甘氨酸，与对照菌株 *P. syringae* pv. *syringae* Pss1 特性不同（表 3）。

2.5 分子特征鉴定结果

2.5.1 丁香假单胞致病变种特异性引物扩增结果

利用 *Pseudomonas* 属特异引物 Ps-for/Ps-rev 对菌株 LZ-5、LZ-8、LZ-15 和对照菌株 Pss1 的 DNA 进行 PCR 扩增，均扩增出预期片段大小为1 018 bp

的产物(图 3-A)。Group IA-F/Group IA-R、Group IB-F/Group IB-R、Group II-F/Group II-R、Group III-F/Group III-R 和 Group IV-F/Group IV-R 等 5 对丁香假单胞特异性引物 PCR 检测结果显示,仅引物 Group III-F/Group III-R 能扩增出预期大小为 750 bp 的产物(图 3-B),其余引物均不能扩增出预期片段。丁香霉素 *syrB* 基因特异性引物 B1/B2 可从 LZ-5、LZ-8 和 LZ-15 菌株与对照菌株 *P. syringae* pv. *syringae* 扩增出预期片段大小 752 bp 产物(图 3-C)。可见,LZ-5、LZ-8 和 LZ-15 菌株属于 *P. syringae* pv. *syringae* 或 *P. syringae* pv. *aptata*。

2.5.2　16S rDNA 和 *gyrB* 基因序列分析　对 LZ-5、LZ-8 和 LZ-15 菌株的 16S rDNA 和 *gyrB* 基因进行 PCR 扩增测序,分别获得片段大小为 1 447 bp 和 1 193 bp 的近全长序列(GenBank 登录号分别为 KY054830 ~ KY054832 和 KY054833 ~ KY054835),相同基因序列间的同源率均为 100%。BLAST 结果显示,3 个菌株的 16S rDNA

Table 2　Symptoms of the host plants by artificial inoculation

Host plant	Variety	Symptoms
Solanum melongena	Nongfeng	At 14-16 dpi, black irregular spots (diameter 2mm ~ 4mm) appeared on the leaves. Then the spots expanded into big water-soaked lesions.
Cucurbita pepo	Cuiyou	At 7-8 dpi, a small number of circular chlorotic spots (diameter <2mm) with yellow halo appeared on the leaves, and the minority water-soaked lesions appeared at the edge of leaves. The disease developed slowly.
Phaseolus vulgaris	Qingfeng	At 7-8 dpi, the brown circular spots (diameter 1mm ~3mm) appeared on the leaves, veins, petioles and stalks.
	Chunqiu	At 10-12 dpi, sporadic brown circular spots (diameter 1mm ~3mm) appeared on the leaves, veins, petioles and stalks. The disease developed slowly.
Solanum lycopersicum	Xinxing101	At 14-16 dpi, black irregular spots (diameter 2mm ~ 4mm) appeared on the leaves. Later, the spots expanded into big water-soaked lesions.
Lablab purpureus	Qingfeng	At 7-8 dpi, brown circular spots (diameter 1mm ~ 3mm) with yellow halo appeared on the leaves, and showed water-soaked lesions on the back sides.
Luffa cylindrica	Yueyou2	At 7-8 dpi, elliptical water-soaked spots (diameter 2mm ~ 4mm) appeared on the leaves and the center of the spots were broken easily.

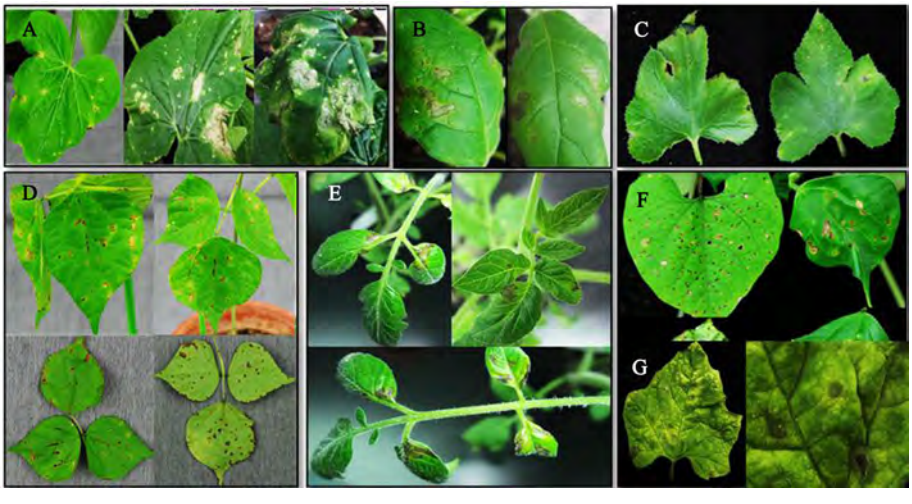

Fig. 2　Symptoms of the host plants by artificial inoculation

A: *Cucurbita moschata*; B: *Solanum melongena*; C: *Cucurbita pepo*; D: *Phaseolus vulgaris*; E: *Solanum lycopersicum*; F: *Lablab purpureus*; G: *Luffa cylindrica*.

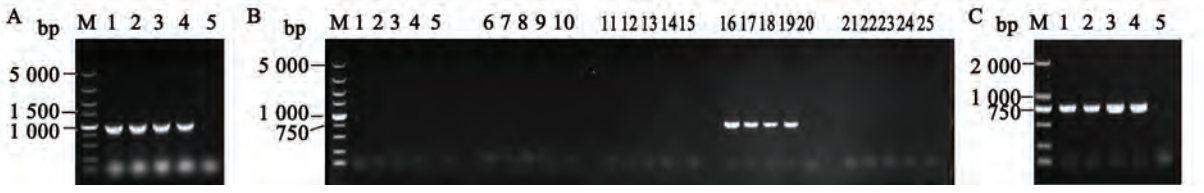

Fig. 3 PCR results of the DNAs of three representative strains with specific primers

A: Primers Ps-for/Ps-rev (Lane 1-5: LZ-5, LZ-8, LZ-15, Pss1, ddH_2O; M: DL5000™ DNA marker);
B: Primers Group IA-F/Group IA-R (Lane 1-5: LZ-5, LZ-8, LZ-15, Pss1, ddH_2O;
M: DL5000™ DNA marker); Group IB-F/Group IB-R (Lane 6-10: LZ-5, LZ-8, LZ-15, Pss1, ddH_2O); Group II-F/Group II-R (Lane 11-15: LZ-5, LZ-8, LZ-15, Pss1, ddH_2O); Group III-F/Group III-R (Lane 16-20: LZ-5, LZ-8, LZ-15, Pss1, ddH_2O);
Group IV-F/Group IV-R (Lane 1-5: LZ-5, LZ-8, LZ-15, Pss1, ddH_2O);
C: Primers B1/B2 (Lane 1-5: LZ-5, LZ-8, LZ-15, Pss1, ddH_2O; M: DL2000™ DNA marker).

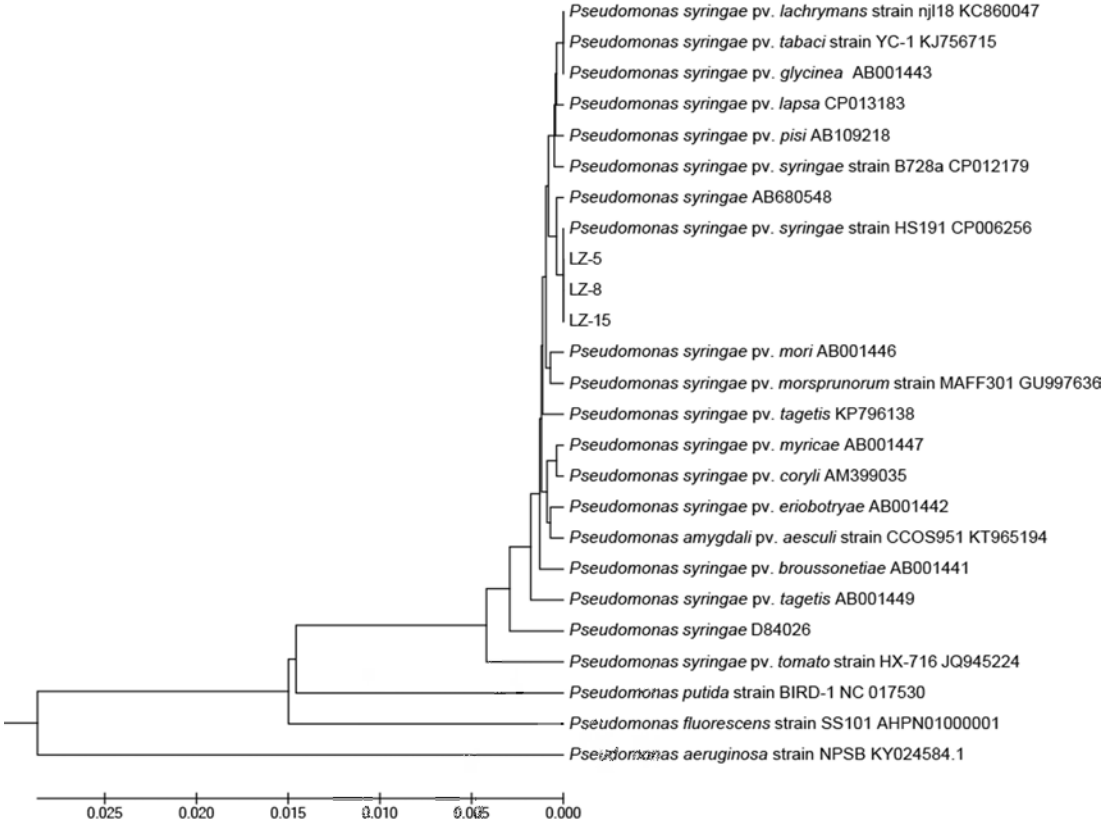

Fig. 4 Phylogenetic tree of three representative strains and other 22 strains based on the 16S rDNA sequences

和 *gyrB* 基因序列与已登录 GenBank 中 *P. syringae* pv. *syringae* 的相应序列同源率最高，达 99%以上。基于 16S rDNA 基因序列的系统发育树显示(图 4)，供试菌株与 *P. syringae* pv. *syringae* 菌株 HS191 (CP006256) 形成一个小分支，进一步与 *P. syringae* 其他菌株聚类形成一个大分支。基于 *gyrB* 基因序列的系统发育树也显示(图 5)，供试菌株与 *P. syringae* pv. *syringae* HS191 (CP006256) 形成一个小分支，与 *P. syringae* 其他菌株聚类形成一个大分支。

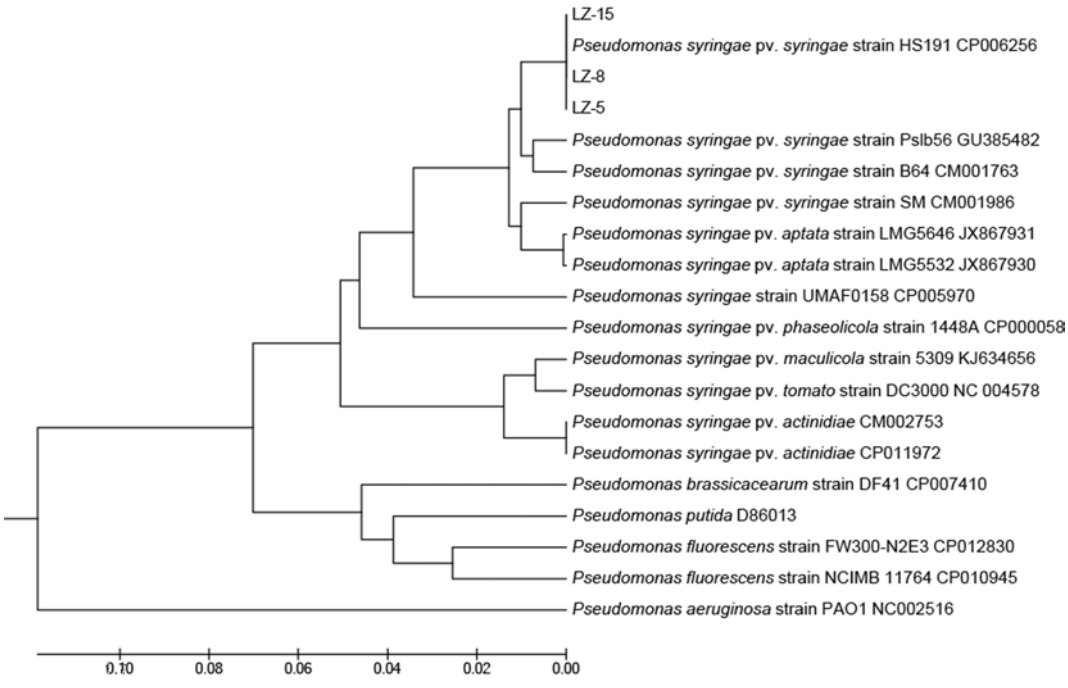

Fig. 5　Phylogenetic tree of three representative strains and other 17 strains based on *gyrB* sequences

Table 3　Physiological and biochemical characteristics of three representative strains in the study

Test	Strain LZ5,LZ-8 and LZ-15	*Pseudomonas syringae* pv. *syringae* strain Pss1
Growth at 41℃	-	-
Moveability	+	+
Gelatin liquefaction	+	+
Tolerance of NaCl	5%	5%
Oxidase	-	-
Starch hydrolysis	-	-
Nitrate deoxidize	-	-
Levan formation	+	+
Arginine dihydrolase	-	-
OF test	O	O
Growth on TTC agar	+	+
Adonito	-	-
Betaine	+	+
Ascorbate	+	+
Erythrito	+	+
DL-homoserine	-	-
Inositol	+	+
Lactate	+	+
L-leucine	-	-
Malonate	+	+
Citricacid	+	+
Mannitol	+	+
D-sorbitol	+	+
Sucrose	+	+

Continued Table 3

D(-)tartrate	-	-
L(+)tartrate	-	-
Aesculin hydrolysis	+	+
Cellobiose	-	-
D-fucose	-	-
D-glucose	+	+
D-raffinose	+	+
Lactose	-	-
L-fucose	-	-
Maltose	-	-
Rhamnose	-	-
D-serine	-	-
L-threonine	-	-
D-ribose	+	+
L-tyrosine	+	-
L-glycine	+	-
L-glutamicacid	+	+

Note: +: Positive; -: Negative; OF: Oxidative-fermentative metabolism of glucose; O: Oxidative reaction; F: Fermentative reaction.

3 结论与讨论

本文通过对引起广东南瓜细菌性叶枯病的病原菌形态特征、致病性、生理生化特性、16S rDNA、*gyrB* 基因序列分析以及特异性 PCR 检测,鉴定出该病原菌为丁香假单胞丁香致病变种 *P. syringae* pv. *syringae*,这是国内首次发现丁香假单胞丁香致病变种引起南瓜叶枯病。

丁香假单胞致病变种分类复杂,利用 DNA-DNA 杂交和多位点序列分析(MLSA)可将其分为 60 个致病变种[18],统称为丁香假单胞复合种(*P. syringae* species complex, Pssc)[19]。本研究分离的南瓜叶枯病原菌菌株在 KB 培养基上能产生荧光色素,生理生化测定菌株氧化酶、精氨酸双水解酶、硝酸还原反应均为阴性,果聚糖反应阳性等性状与丁香假单胞杆菌性状一致。同时,该病原菌能使明胶液化并水解七叶苷,可利用肌醇、赤藓糖醇、L-乳酸等,不利用核糖醇、L-酒石酸盐,这些特征与丁香假单胞丁香致病变种(*P. syringae* pv. *syringae*)的特征[11]相符。但该病原菌可以利用 L-酪氨酸和 L-甘氨酸,与对照菌株 *P. syringae* pv. *syringae* Pss1 特性不同,推测可能因为 *P. syringae* pv. *syringae* 菌株间存在个体差异所致。

应用丁香假单胞致病变种 5 对特异性引物 Group IA-F/Group IA-R、Group IB-F/Group IB-R、Group II-F/Group II-R、Group III-F/Group III-R 和 Group IV-F/Group IV-R 进行 PCR 检测,仅 Group III-F/Group III-R 可扩增出预期条带,表明该病原菌属于丁香假单胞丁香致病变种的第三致病变种组群(Group III)。另外,丁香霉素 *syrB* 基因特异引物 B1/B2 也可扩增出预期产物,说明该病原菌为 *P. syringae* pv. *syringae* 或 *P. syringae* pv. *aptata*。利用 16S rDNA 与 *gyrB* 基因序列分析进一步将病原菌鉴定为 *P. syringae* pv. *syringae*。

植物病原细菌鉴定方法包括以细菌生理生化特性为主的常规鉴定、BIOLOG 碳源自动分析鉴定、全细胞脂肪酸分析鉴定和分子生物学鉴定等。基于与标准数据库的参比对微生物种类进行鉴定的 BIOLOG 鉴定系统和全细胞脂肪酸分析鉴定,可将细菌鉴定到种以上水平[20],但目前还不能实现鉴定到致病变种等种下水平。克隆菌株的 16S rDNA 及其看家基因,与已登录 GenBank 的序列进行同源性比较与亲缘关系分析,对常规生理生化特性鉴定结果进行验证,结果稳定、可靠。本文采用常规的生理生化特性测定方法[10, 11]鉴定出引起广东南瓜细菌性叶枯病的病原菌为 *P. syringae* pv. *syringae*,通过对病原菌的 16S rDNA、*gyrB* 基因序列同源性与系统进化分析以及特异性 PCR 检测等分子生物学方法得到确认,从而明确广东南瓜细菌性叶枯病的病原菌为 *P. syringae* pv. *syringae*。我

们也尝试用 BIOLOG 鉴定系统对该病原细菌进行鉴定,但结果的重复性差,且只能鉴定到种的水平(结果未显示)。

丁香假单胞丁香致病变种寄主范围较广,可侵染 40 多种植物引起病害[19],包括南瓜[9]、西葫芦[21, 22]、西瓜[23]、笋瓜[24]和哈密瓜[22]等多种葫芦科作物。绿皮南瓜(*Cucurbita moschata*)被侵染的症状为叶斑[9],西葫芦叶片被侵染的症状表现为深色、水渍状带有黄色边缘的斑点[22],本研究结果与其相同。在西瓜上症状表现为叶片上产生中心棕色、周边暗黑色的圆形至不规则形状的斑点[23],在笋瓜上症状为果实产生疣状突起[24],哈密瓜上产生浅棕色不规则坏死斑[22]。在国内,*P. syringae* pv. *syringae* 引起的病害包括小麦细菌性叶枯病[25],水稻细菌褐斑病,丁香疫病,果树溃疡病、疫病[26, 27],苜蓿细菌性茎疫病[28],三七细菌性叶斑病[29],番茄茎髓黑腐病[30]和辣椒细菌性叶斑病[31]。本文发现为害广东南瓜的 *P. syringae* pv. *syringae* 菌株,除为害南瓜外,人工接种还能侵染西葫芦、丝瓜、番茄、茄子、扁豆和菜豆,但不能侵染辣椒、豌豆,与前人研究结果不同[32, 33]。近年来,南瓜在广东种植面积不断增加。由于南瓜生长期适逢广东高温、多雨季节,极易引起细菌性叶枯病的发生与流行。本文鉴定出引起广东省雷州市南瓜叶枯病的病原为丁香假单胞丁香致病变种,并测定了该病原菌的寄主范围,这些研究结果为该病害的防治提供了理论依据。

参考文献

[1] Lamichhane J R, Varvaro L, Balestra G M. Bacterial leaf spot caused by *Xanthomonas cucurbitae* reported on pumpkin in Nepal [J]. New Disease Reports, 2010, 22: 20.

[2] Babadoost M, Ravanlou A. Outbreak of bacterial spot (*Xanthomonas cucurbitae*) in pumpkin fields in Illinois [J]. Plant Disease, 2012, 96(8): 1222.

[3] Nascimento A R P, Mariano R L R, Silva E I. Alternative hosts of *Acidovorax avenae* subsp. *citrulli* [J]. Horticultura Brasileira, 2004, 22(3): 511-515.

[4] Burdman S, Walcott R. *Acidovorax citrulli*: generating basic and applied knowledge to tackle a global threat to the cucurbit industry [J]. Molecular Plant Pathology, 2012, 13(8): 805-815.

[5] Cortesmonllor A, Rodriguezmarcano A. Angular leaf spot of some cucurbits in Puerto Rico [J]. Journal of Agriculture of the University of Puerto Rico, 1991, 75: 195-198.

[6] Bhat N A. Survival of *Pseudomonas syringae* pv. *lachrymans* incitant of angular leaf spot of cucumber under temperate conditions of Kashmir valley [J]. Indian Phytopathology, 2009, 4(4): 429-434.

[7] Shapiro L R, Scully E D, Straub T J, *et al*. Horizontal gene acquisitions, mobile element proliferation, and genome decay in the host-restricted plant pathogen *Erwinia tracheiphila* [J]. Genome Biology and Evolution, 2016, 8(3): 649-664.

[8] Rojas E S, Dixon P M, Batzer J C, *et al*. Genetic and virulence variability among *Erwinia tracheiphila* strains recovered from different cucurbit hosts [J]. Phytopathology, 2013, 103(9): 900-905.

[9] Park K S, Kim Y T, Kim H S, *et al*. Selection of the antibacterial agents for control against *Pseudomonas syringae* pv. *syringae* causing leaf spot disease on green pumpkin (*Cucurbita moschata*) [J]. The Korean Journal of Pesticide Science, 2015, 19(2): 119-124.

[10] Fang Z D. Plant Pathology Research Methods (3rd Ed.) (in Chinese) [M]. Beijing: China Agriculture Press (北京:中国农业出版社), 1998: 165-211.

[11] Schaad N W, Jones J B, Chun W. Laboratory guide for identification of plant pathogenic bacteria (3rd Ed.) [M]. Beijing: China Agricultural Science and Technology Press (北京:中国农业科学技术出版社), 2011: 69-81.

[12] Villa J E, Tsuchiya K, Horita M, *et al*. Phylogenetic relationships of *Ralstonia solanacearum* species complex strains from Asia and other continents based on 16S rDNA, endoglucanase, and *hrpB* gene sequences [J]. Journal of General Plant Pathology, 2005, 71(1): 39-46.

[13] Izumi S, Yamamoto M, Suzuki K, *et al*. Identification and detection of *Pseudomonas plecoglossicida* isolates with PCR primers targeting the *gyrB* region [J]. Journal of Fish Diseases, 2007, 30(7): 391-397.

[14] Widmer F, Seidler R J, Gillevet P M, *et al*. A highly

selective PCR protocol for detecting 16S rRNA genes of the genus *Pseudomonas* (sensu stricto) in environmental samples [J]. Applied and Environmental Microbiology, 1998, 64(7): 2545-2553.

[15] Inoue Y, Takikawa Y. The *hrpZ* and *hrpA* genes are variable, and useful for grouping *Pseudomonas syringae* bacteria [J]. Journal of General Plant Pathology, 2006, 72(1): 26-33.

[16] Sorensen K N, Kim K H, Takemoto J Y. PCR detection of cyclic lipodepsinonapeptide-producing *Pseudomonas syringae* pv. *syringae* and similarity of strains [J]. Applied and Environmental Microbiology, 1998, 64(1): 226-230.

[17] Young J M, Triggs C M. Evaluation of determinative tests for pathovars of *Pseudomonas syringae* van Hall 1902 [J]. Journal of Applied Bacteriology, 1994, 77(2): 195-207.

[18] Young J M. Taxonomy of *Pseudomonas syringae* [J]. Journal of Plant Pathology, 2010, 92(1S): 5-14.

[19] Lamichhane J R, Messéan A, Morris C E. Insights into epidemiology and control of diseases of annual plants caused by the *Pseudomonas syringae* species complex [J]. Journal of General Plant Pathology, 2015, 81(5): 331-350.

[20] Xie J Y, Wang Y L. Application and study of the BIOLOG automated bacterial identification system (in Chinese) [J]. Microbiology China (微生物学通报), 1996, 23(5): 264-267.

[21] Balaz′ J, Iličic R, Maširevic S, *et al*. First report of *Pseudomonas syringae* pv. *syringae* causing bacterial leaf spots of oil pumpkin (*Cucurbita pepo*) in Serbia [J]. Plant Disease, 2014, 98(5): 684.

[22] Langston D B, Sanders F H, Brock J H, *et al*. First report of a field outbreak of a bacterial leaf spot of cantaloupe and squash caused by *Pseudomonas syringae* pv. *syringae* in Georgia [J]. Plant Disease, 2003, 87(5): 600.

[23] Dutta B, Gitaitis R D, Driver J E, *et al*. First report of bacterial leaf spot on watermelon caused by *Pseudomonas syringae* pv. *syringae* in Georgia [J]. Plant Disease, 2016, 100(2): 518.

[24] Sharrock K R, Hawthorne B T, Young J M. *Pseudomonas syringae* pv. *syringae* causes wart-like eruptions on fruit of buttercup squash (*Cucurbita maxima*) [J]. New Zealand Journal of Crop and Horticultural Science, 1997, 25(3): 203-211.

[25] Zhang X M, Guo J H, Liu H Z. The investigation and identification of wheat bacterial diseases (in Chinese) [J]. Plant Protection (植物保护), 2004, 30(4): 60-62.

[26] Qiu W, Huai Y, Xu F S, *et al*. Pear blossom blast caused by *Pseudomonas syringae* pv. *syringae* in China [J]. Agricultural Sciences in China, 2008, 7(9): 1091-1096.

[27] Rudolph K, Burr T J, Mansfield J, *et al*. *Pseudomonas syringae* pathovars and related pathogens [M]. Kluwer Academic Publishers, 1997: 465-469.

[28] Zhang Z F, Nan Z B. Research progress on alfalfa bacterial diseases (in Chinese) [J]. Acta Prataculturae Sinica (草业学报), 2014, 23(4): 330-342.

[29] Zhou L H, Han Y, Ji G H, *et al*. First report of bacterial leaf spot disease caused by *Pseudomonas syringae* pv. *syringae* on *Panax notoginseng* [J]. Plant Disease, 2013, 97(5): 685.

[30] Zhao T C, Chen X, Li Q. Pathogen identification of tomato bacterial stem necrosis (in Chinese) [C]. The 8th Youth Conference of Chinese Society for Plant Pathology (中国植物病理学会第八届青年学术研讨会), 2008: 63-71.

[31] Guan J J, Li K M, Luo M, *et al*. The occurrence and pathogen identification of bacterial leaf spot of processing pepper in Xinjiang (in Chinese) [J]. Acta Phytopathologica Sinica (植物病理学报), 2015, 45(3): 239-247.

[32] Gardan L, Samson R, Mitrev S. Characterization of bacterial strains of *Pseudomonas syringae* pv. *syringae* isolated from pepper leaf spot in Macedonia [J]. Journal of Plant Pathology, 2000, 82(3): 227-231.

[33] Popovic T, Ivanovic Z, Trkulja N, *et al*. First report of *Pseudomonas syringae* pv. *syringae* on pea (*Pisum sativum*) in Serbia [J]. Plant Disease, 2015, 99(5): 724.

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