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杧果细菌性角斑病菌粗毒素的生物活性、理化特性及致病作用

张大智[1,2],詹儒林[1],柳 凤[1*],赵艳龙[1],何衍彪[1],常金梅[1]

([1]中国热带农业科学院南亚热带作物研究所·农业部热带果树生物学重点实验室,广东湛江 524091;
[2]海南大学环境与植物保护学院,海口 570228)

摘 要:【目的】探讨杧果细菌性角斑病菌粗毒素的生物活性、理化特性及其在致病过程中的作用,以期为明确该病菌的致病机制及病害防控提供有效依据。【方法】以'台农一号'杧果叶片为试验材料,利用离体叶片针刮法对其粗毒素生物活性进行了研究,通过测定杧果叶片防御酶活性、可溶性蛋白含量、总糖含量和叶绿素含量的变化分析其对杧果的致病生理机制;同时以不同培养液、培养时间、培养温度及培养液pH为条件,对该菌产毒条件进行了优化。【结果】在Watanabe培养滤液中得到淡黄色粗毒素,是一类具有热稳定性的非蛋白类物质。毒素对杧果叶片有浸解作用,随着浓度的上升,对杧果叶片的损伤程度不断加重,形成的病斑与病原菌症状一致。生理生化研究发现粗毒素侵染杧果叶片后,能激活杧果体内过氧化物酶(POD)、多酚氧化酶(PPO)、过氧化氢酶(CAT)和苯丙氨酸解氨酶(PAL)活性。POD、PPO、CAT与PAL分别在接种病菌48、72、36 h后达到顶峰,酶活性分别是965.33、8 456、1 341.13和4.02×10^4 $U\cdot g^{-1}\cdot min^{-1}$,是健康叶片的1.35、2.20、1.08和4.19倍。可溶性蛋白含量、总糖含量随着处理时间的延长而显著升高,叶绿素含量呈现相反的变化趋势,随着处理时间的延长不断降低。毒素通过摇瓶震荡培养,优化出病菌的最佳产毒条件是:Watanabe培养液(pH 7~8),24 ℃培养120 h。【结论】因该粗毒素对杧果叶片产生明显的浸解作用,表明其能改变细胞膜的通透性,导致杧果叶片组织电解质的渗漏,对叶片细胞膜具有损伤作用,最终导致病害的发生。由此推断,毒素可能属于非寄主专化性毒素(NHST),同时可能是杧果细菌性角斑病菌的致病因子之一。

关键词:杧果;细菌性角斑病;毒素;酶活性;致病作用

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Biological activity, physical and chemical properties and pathogenic effect of crude toxic produced by pathogen causing mango bacterial leaf spot

ZHANG Dazhi[1,2], ZHAN Rulin[1], LIU Feng[1*], ZHAO Yanlong[1], HE Yanbiao[1], CHANG Jinmei[1]

([1]South Subtropical Crop Research Institute, CATAS· Key Laboratory of Tropical Fruit Biology, Ministry of Agriculture, Zhanjiang 524091, Guangzhou, China; [2]College of Environment and Plant Protection, Haikou University, Haikou 571737, Hainan, China)

Abstract:【Objective】Mango (*Mangifera indica* L.) fruit is a popular and economically important fruit grown worldwide throughout the tropics and subtropics, due to its excellent qualities such as bright color, sweet taste and luscious flavor, and nutritional composition such as vitamins, minerals, fiber, and other phytochemical compounds. Mango bacterial leaf spot, a bacterial disease caused by *Xanthomonas campestris* pv. *Mangiferaeindicae*, is very difficult to control, and it usually becomes a limiting factor for mango industries while fungal disease and other pests can be managed at acceptable levels. It affects all aerial parts of the mango plant and can be very destructive in areas where high temperatures and rainfalls occur concomitantly. The bacterial pathogen can infect wounds and natural openings. Leaf and fruit symptoms

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作者简介:张大智,男,硕士,研究方向为植物细菌病害。Tel:18789769058,E-mail:zhangdazhi102@163.com

*通信作者 Author for correspondence. Tel:13729009837, E-mail:liufengneau@163.com

are most common, but twig and branch cankers may occur when infection is severe. Leaf symptoms begin as small water-soaked spots delineated by veins, becoming raised, black, sometimes with a chlorotic halo. Several months after infection, leaf lesions dry and turn light brown ash-gray. In addition, fruit symptoms appear as small water-soaked spots on the lenticels. These spots later become star-shaped, erumpent and exude an infectious gum. Often, a "tear stain" infection pattern is observed on the fruit. Severe fruit infections will cause premature fruit drop. Less severe infections reduce fruit quality and allow entry of other pathogens. The mango bacterial leaf spot pathogen virulence factors include the cell wall degrading enzymes (CWDEs), toxins, extracellular polysaccharide and hormones, etc. The toxins are secreted by the plant pathogens outward role in plant cell walls or plant tissue. The purpose of this experiment was to explore the biological activities, physical and chemical properties of the crude toxin produced by pathogens and their pathogenic effects were studied during the disease developing based on the characteristics of the pathogen with the decomposition of the host tissue. This study aims to reveal the pathogenic mechanisms of toxin in the mango bacterial leaf spot, providing the basic theory of evidence for effective disease control.【Methods】'Tainong No.1' mango leaves for the test material, the method of *in vitro* leaves needle scraping was used for the toxin biological activity assay. By measuring the change of mango leaves defense enzyme, soluble protein, total sugar and chlorophyll contents and analyzing it to the mango pathogenic physiological mechanisms. At the same time in different mediums, time, temperature and pH of the culture medium conditions were optimized.【Results】The filtrate liquid which was extracted in the Watanabe culture, was not a kind of toxin of protein substance which showed the most abilities. Under the 185-800 nm wavelength ultraviolet scanning, when the toxin concentration was 35%, the absorbance at 264 nm was the highest, OD_{264} was 2.933. Using fumaric acid (FUA) and succinic acid (SUA) as the prototypes, the samples in the 302 nm and 302 nm UV light have three yellow spots, the measured Rf value of FUA was 0.361, SUA was 0.944, the samples were 0.074, 0.097 and 0.542, and the contrast (methanol) was 0. What we learn from this is that the toxins have more than three types of organic acids, but not FUA and SUA. The mango bacterial leaf spot pathogen secretes crude toxin on the mango leaf cell membrane causing damage, with the increase of crude toxin concentration, the permeability of the mango leaf after retting reductive monosaccharide and relative conductivity also gradually rises. The assay of biological activity of the liquid showed it could induce the symptoms of mango bacterial leaf spot. Studies on the physiological and biochemical indexes of the crude toxin, showed that mango leaves were inoculated by crude toxin. The results also showed that the activities of peroxidase (POD), polyphenoloxidase (PPO), cate-lase (CAT) and phenylalanineammonialyase (PAL) were increased in the inoculated leaves. The POD activity increased significantly within 12 to 48 h after inoculation, POD reached its maximum level activity at 965.33 $U \cdot g^{-1} \cdot min^{-1}$ after inoculation for 48 h, while the control, inoculation mango leaves with culture medium without pathogen, the inoculation activity value was 1.35 times that of the control. PPO reached its maximum level activity at 5 354 and 8 456 $U \cdot g^{-1} \cdot min^{-1}$ after inoculation for 24 and 72 h, which were 1.96 and 2.20 times that of the control. CAT and PAL reached their maximum level activity at 1 341.13 and 4.02×10^4 $U \cdot g^{-1} \cdot min^{-1}$ after inoculation for 36 h, which were 1.08 and 4.19 times that of the control. After being sharply lower, PAL again reached its maximum level activity at 2.06×10^4 $U \cdot g^{-1} \cdot min^{-1}$ after inoculation for 60 h, which was 2.84 times that of the control. And the soluble protein and sugar content were significantly increased, while the content of chlorophyll decreased. After shaking the culture, the optimum conditions of producing toxin were as follows: Watanabe filtrate culture, pH value 7 to 8, 24 ℃, 120 h.【Conclusion】The crude toxin obviously produced retting on the mango leaves, which shows that it can change cell membrane permeability, lead to the organization of mango leaf electrolyte leakage, and cause the damage effects of blade

cell membrane, eventually giving rise to the occurrence of diseases. The results indicate that the crude toxin is maybe one of the pathogenicity factors of mango bacterial leaf spot pathogen, which may belong to non-host specific toxins (NHST).

Key words: Mango; Bacterial leaf spot; Toxin; Enzyme activity; Pathogenic effect

杧果（*Mangifera indica* L.）为漆树科杧果属植物，广泛种植于印度、中国、墨西哥、泰国、巴基斯坦及菲律宾等热带及亚热带国家（地区）[1]。杧果细菌性角斑病（mango bacterial leaf spot）1915年在南非首次报道，随后在印度、巴基斯坦、巴西等国均有发生，该病主要危害杧果叶片、枝条、花和果实等部位，出现明显的“十字交叉”病斑[2]。在我国被称为中国杧果之乡的田东县，杧果细菌性角斑病造成的损失较为严重[3]，在田东县新州杧果场，凯特杧的发病率为80.2%[4]。植物病原细菌的致病因子主要包括酶、毒素、胞外多糖和激素等，其中毒素（toxin）又称为攻击素（aggressins）、毒性物质（toxin substances）及植物毒素（phytotoxin），是由植物病原菌分泌，干扰寄主正常生理功能的一类化合物[5]。引起植物症状的细菌毒素主要有2类，一类是由假单胞菌引起的褪绿症状，另一类是由欧文氏菌属、黄单胞菌属及棒形杆菌属引起的萎蔫症状。黄单胞菌属毒素分为水稻白叶枯病菌毒素和甘蔗白纹枯病菌毒素2类。毒素可导致寄主叶片组织内具有清除活性氧作用的防御酶系如过氧化物酶（POD）、多酚氧化酶（PPO）、过氧化氢酶（CAT）和苯丙氨酸解氨酶（PAL）的活性发生变化，这是致病毒素入侵寄主后的明显反应之一[6]。目前，国内外学者在杧果细菌性角斑病病原菌的病原学[7-8]、发病条件[9]、致病因子[10]、检测技术及防治方法[11]上取得一些研究进展，但并不能根除病害，主要原因之一是病原菌的致病机制尚不清楚。杧果细菌性角斑病病菌是否分泌的毒素以及毒素在病原菌致病过程中的作用目前尚无人探讨。笔者以‘台农一号’杧果叶片为试验材料，初步探索杧果细菌性角斑病病菌毒素的活性、产生条件和基本理化性质，同时通过人工接种病菌毒素后，比较接种与健康杧果叶片体内防御酶系活性变化的差异，旨在阐明杧果细菌性角斑病病菌毒素的生物活性、理化性质及作用机制，为明确该病害的致病机制及病害防控提供有效依据。

1　材料和方法

1.1　材料

病原菌：杧果细菌性角斑病病菌野油菜黄单胞菌杧果致病变种（*Xanthomonas campestris* pv. *mangiferaeindicae*），为本试验分离获得，编号为XCM07，科赫氏法则验证其对杧果具有致病性。

主要培养基：改良Watanbe培养液[12]、Wakimoto培养液[13]、种子培养液和发酵培养液[14]用于毒素的诱导。

植物材料：‘台农一号’，叶龄接近中期的健康植株。

1.2　病原菌培养及毒素的提取

菌株接种于100 mL Watanabe液体培养基，此时的液体培养基作为种子液，28 ℃、130 r·min^{-1}恒温振荡培养，48 h后按照3%的接种量转接于1 500 mL Watanabe液体培养基，28 ℃、130 r·min^{-1}恒温摇床振荡培养72 h，8 000 r·min^{-1}离心20 min后取上清液。取6倍体积的丙酮浸泡浓缩液，此浓缩液经50 ℃旋转蒸发为原体积的1/10，摇均匀后放置过夜，经46 ℃旋转蒸发除去浸泡液。将得到的液体pH调至7.0后加入等体积的氯仿，抽提3次，取水相，将水相pH调至3.0后加入等体积的乙酸乙酯，抽提3次，收集乙酸乙酯相，合并后于46 ℃旋转蒸发，取2 mL无水乙醇溶解残留物得到粗毒素[15]。

1.3　粗毒素生物活性的测定

用无菌水清洗新鲜杧果叶片，消毒针轻刮叶片造成伤口，取20 μL浓缩后的粗毒素（1.12 g·L^{-1}）接种于伤口处[16]，以培养液和无水乙醇为对照，置于28 ℃光照培养箱中培养，24 h后观察叶片处理部位的发病情况。

1.4　杧果细菌性角斑病病菌毒素的基本性质

杧果细菌性角斑病病菌毒素的热稳定性、非蛋白和蛋白部分测定参考伏颖[17]的方法，生物活性检测采用离体叶片针刮法，每处理3次重复，3 d后观察叶片症状，测量病斑直径（用直尺测量病斑扩展的宽度，而不是指刮伤的长度），比较病菌毒素热稳定性、非蛋白和蛋白部分的生物活性。取微量毒素稀释于95%（φ）乙醇中，浓度分别为5%、15%、25%和35%，对照采用95%乙醇，用UV-1700（日本岛津）紫外分