

EPPO Datasheet: *Acidovorax citrulli*

Last updated: 2020-09-25

IDENTITY

Preferred name: *Acidovorax citrulli*

Authority: (Schaad et al.) Schaad, Postnikova, Sechler, Claflin, Vidaver, Jones, Agarkova, Ignatov, Dickstein & Ramundo

Taxonomic position: Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae

Other scientific names: *Acidovorax avenae* subsp. *citrulli* (Schaad, Sowell, Goth, Colwell & Webb) Willemse, Goor, Thielemans, Gillis, Kersters & De Ley, *Paracidovorax citrulli* (Schaad et al.) Du et al., *Pseudomonas avenae* subsp. *citrulli* (Schaad, Sowell, Goth, Colwell & Webb) Hu, Young & Triggs., *Pseudomonas pseudoalcaligenes* subsp. *citrulli* Schaad et al.

Common names: bacterial fruit blotch

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EPPO Categorization: A1 list

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EPPO Code: PSDMAC



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Notes on taxonomy and nomenclature

Two evolutionary lineages have been identified, dividing the *A. citrulli* species into two genetically different groups: Group I and Group II. The two groups can be distinguished by DNA sequence polymorphism of the housekeeping gene *gltA* (Walcott et al., 2004); such genetic diversity is reflected in differences of pathogenicity on cucurbit hosts. A third genetic group, including a singleton, was described in China (Yan et al., 2013). Feng et al. (2009), based on multilocus sequence typing analysis (MLST), identified two major clonal complexes: CC1, appeared earlier and with a wider host range, whereas CC2 has a wider worldwide distribution among cucurbits.

HOSTS

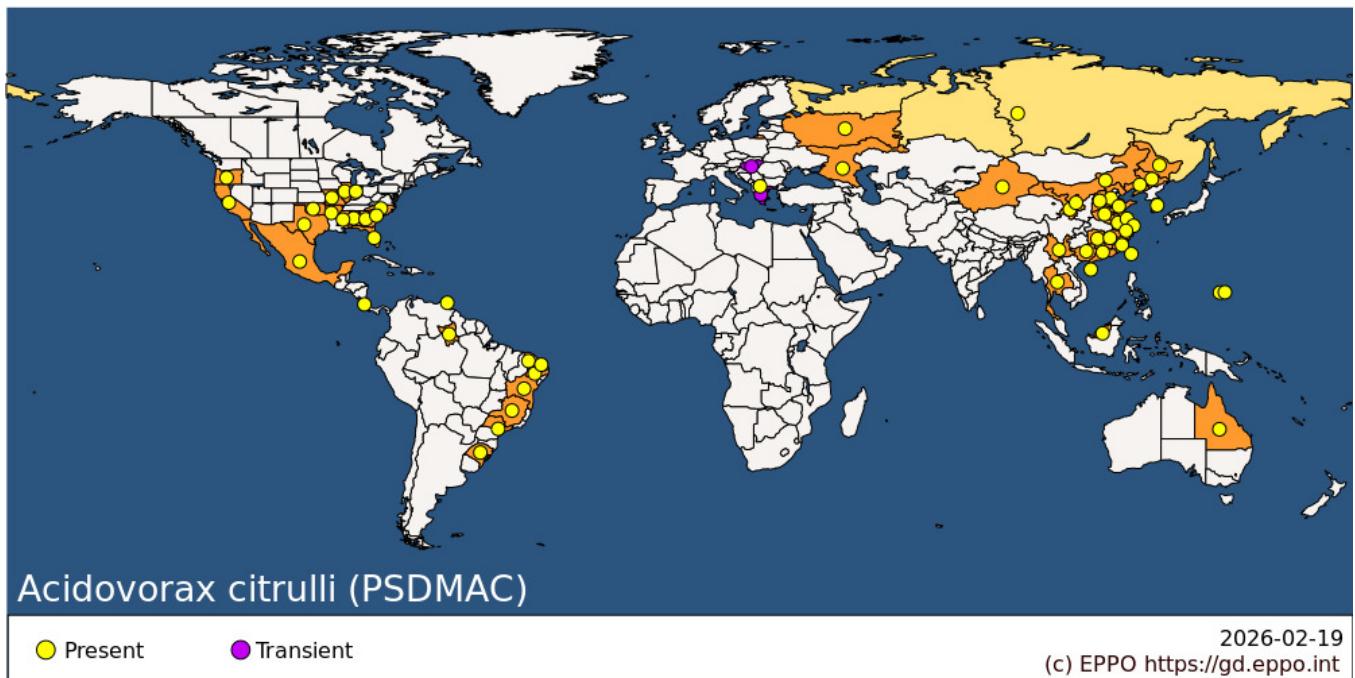
The bacterial fruit blotch caused by *A. citrulli* may affect several cultivated cucurbits belonging to the *Cucurbitaceae* family. Differences in host plant susceptibility are reported for different species, or different cultivars belonging to the same species. Watermelon (*Citrullus lanatus*) (Schaad et al., 1978) and melon (*Cucumis melo*) (Isakeit et al., 1997) are the major host plant species. Citron melon (*C. lanatus* var. *citroides*, syn. *C. caffer*) (Isakeit et al., 1998), pumpkin and squash (*Cucurbita* spp.) and cucumber (*Cucumis sativus*) may also be infected (Langston et al., 1999; Martin and Horlock, 2002; Martin and O'Brien, 1999; Burdman & Walcott, 2012). Differential host susceptibility is reported and related to *A. citrulli* grouping: Group I is moderately aggressive on most cucurbits, whereas Group II is specifically more aggressive on watermelon than on other cucurbit hosts (Walcott et al., 2004). Intraspecific susceptibility to *A. citrulli* is also reported: watermelon genotypes with pale green skin are remarkably more susceptible than dark green varieties; among melons (*C. melo*), cantaloupes and honeydew melons are more susceptible than other genotypes (Walcott et al., 2000; Walcott et al., 2004). Betel vine (*Piper betle*), a non-cucurbit plant species, was reported to be an additional host for *A. citrulli* in Taiwan: isolates from betel vine were also pathogenic on melon, watermelon and *Benincasa hispida* (Deng et al., 2010).

Host list: *Citrullus lanatus* var. *citroides*, *Citrullus lanatus*, *Cucumis melo* var. *inodorus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita moschata*, *Cucurbita pepo*, *Piper betle*, *Solanum lycopersicum*, *Solanum melongena*

GEOGRAPHICAL DISTRIBUTION

The bacterial fruit blotch of cucurbits was first observed in 1965, when an unknown phytopathogenic bacterium was

isolated from necrotizing watermelon seedlings in Georgia, USA (Webb and Goth, 1965). Four years later, rotting of watermelon fruits associated with leaf spots was reported by Crall and Schenk (1969) in Florida. Schaad *et al.* (1978) classified the causal organism as *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, later reclassified into the new genus *Acidovorax* (Willems *et al.*, 1992). The disease was initially considered of low phytopathogenic interest, until a severe outbreak was reported in the Mariana Islands (Wall and Santos, 1988). Later on, severe outbreaks were observed in several States in the USA, from Indiana, to Delaware, to Texas (Latin & Rane, 1990; Evans & Mulrooney, 1991; Somodi *et al.*, 1991; Black *et al.*, 1994). In the late 1990s, the bacterial fruit blotch was reported on more cucurbit hosts, other than watermelon, and in different areas worldwide, possibly due to an increasing trade of seeds (Langston *et al.*, 1999; Martin & O'Brien, 1999; Walcott *et al.*, 2004). Disease outbreaks have been reported in all continents, except Africa. In China, the disease was first reported in 2006, but it dramatically increased in importance during the following years (Yan *et al.*, 2013), whereas in the USA frequent outbreaks are mainly reported in the south-east and, occasionally, in California (Kumagai *et al.*, 2014). In the EPPO region, the pathogen is not considered as established. However it has been repeatedly reported in Greece (Holeva *et al.*, 2009; 2010) and in Hungary (Palkovics *et al.*, 2008); sporadic outbreaks have also been reported from Turkey, Italy, North Macedonia and Serbia (Demir, 1996; Mirik, 2006; Mitrev & Arsov, 2020; Popovi? & Ivanovi?, 2015).



EPPO Region: Greece (mainland), Hungary, North Macedonia, Russian Federation (Central Russia, Southern Russia)

Asia: China (Anhui, Fujian, Gansu, Guangdong, Guangxi, Hainan, Hebei, Heilongjiang, Henan, Hunan, Jiangsu, Jiangxi, Jilin, Liaoning, Neimenggu, Ningxia, Shandong, Shanghai, Shanxi, Xinjiang, Yunnan, Zhejiang), Korea, Republic of, Malaysia (Sarawak), Taiwan, Thailand

North America: Mexico, United States of America (Alabama, Arkansas, California, Florida, Georgia, Illinois, Indiana, Mississippi, Missouri, North Carolina, Oklahoma, Oregon, South Carolina, Texas)

Central America and Caribbean: Costa Rica, Trinidad and Tobago

South America: Brazil (Bahia, Ceara, Minas Gerais, Pernambuco, Rio Grande do Norte, Rio Grande do Sul, Roraima, Sao Paulo)

Oceania: Australia (Queensland), Guam, Northern Mariana Islands

BIOLOGY

A. citrulli overwinters in cucurbit seeds, in plant debris left in the fields after harvesting and in volunteer plants (Bahar & Burdman, 2010). In seeds, *A. citrulli* may colonize both the embryo and the cotyledons: the embryo is infected when the bacteria penetrate the flower through the stigma, whereas the cotyledons are infected when they penetrate the fruitlets through the lenticels, or via the xylem vessels (Walcott *et al.*, 2003). *A. citrulli* is a vascular pathogen, and it is seed-borne and seed-transmitted. The main source of primary inoculum is seed: infected seeds

may easily develop symptomatic seedlings during nursery production of plantlets, especially in the conditions of high humidity and temperature typical in glasshouses. Cucurbits (especially melons and watermelons) are frequently grafted on *Cucurbita* spp. hybrids, to enhance crop tolerance to soil-borne fungi and nematodes, and increase crop productivity. Grafting may result in a very efficient dissemination of the bacterium among seedlings during nursery production, thus symptomless seedlings are reported to be another source of inoculum.

A. citrulli generally infects the plant by colonizing the xylem, from the infected seedling to the adult plant. Symptoms may develop on aerial parts during warm and humid periods and high rainfall, where secondary inocula may be produced and efficiently dispersed (Wall & Santos, 1988). Evasion and short distance dissemination from the plant lesions is aided by rain, and sprinkler irrigation, thus causing additional cycles of the disease. Areas characterized by a dry climate are usually not at high risk for disease outbreaks (Schaad *et al.*, 2003). Secondary inocula penetrates through stomata and lenticels and, possibly, through the stigma. There is no definitive indication that pollinating insects may have a role in flowers' inoculation, although Fessehaie *et al.* (2005) suggested a possible role for honeybees in watermelon seed infection through blossom inoculation. Plant debris, especially rotting fruits where high numbers of bacteria are present, may help pathogen survival from season to season. Volunteers, very commonly present in cucurbit fields after harvest, may ensure the field contamination from one production cycle to the next.

DETECTION AND IDENTIFICATION

Symptoms

Disease symptoms may develop on all aerial parts, except flowers: cotyledons, leaves, stems, fruits. Infected flowers do not show any alterations (Bahar & Burdman, 2010). Fruits (especially watermelons and cantaloupes) are far more susceptible to infections than other plant parts: therefore, it may happen that the disease remains undetected during the production cycle until fruits are reaching maturity. On cotyledonal leaves, during nursery production, lesions initially appear as water-soaked spots, rapidly developing into large rotting and necrotizing areas. In the field, stem and foliar symptoms barely develop and remain very mild and may easily be overlooked: some necrotic stripes and cracks may develop along the stems, very rarely causing significant damages to the plant. Necrotic spots, which are round or angular, may appear on leaves, together with necrotic lesions affecting the leaf margins. A significant chlorosis may appear on melon leaves, when the necrotic areas coalesce. On fruits, initial symptoms appear on melons and watermelons as water-soaked spots, initiating from lenticels: later, those spots enlarge, deepen in the flesh and rot, becoming brown. On watermelons, small water-soaked areas appear, then quickly enlarge, with a tendency to form small cracks that later necrotize. Such lesions deepen into the flesh, causing large soft rotting areas affecting large portions of the fruits. On honeydew melons, rots may be confused with those caused by *Pectobacterium carotovorum* subsp. *carotovorum*, with the significant difference that lesions by pectolytic bacteria typically initiates from wounds.

Morphology

A. citrulli is a Gram negative and rod-shaped bacterium, with average dimensions of 0.5 x 1.7 μm . It is motile due to a single polar flagellum. It forms tiny, creamy-whitish, circular colonies on nutrient-sucrose-agar medium (NSA) or on King's B medium, where it does not produce any fluorescent pigment. It grows more slowly than other saprophytes which are likely to develop during isolation from symptomatic tissue: a 1-2 mm large colony requires 3-4 days to develop on the above media.

Detection and inspection methods

Visual inspections should be done during the production of seedlings, in order to detect any symptom related to the presence of the pathogen. Early disease detection in transplant nurseries is possible, since *A. citrulli* causes large necrotic areas on cotyledonal leaves. Diseased plants are usually grouped in small patches randomly distributed on the production tables. Inspection in nurseries should first try to locate such patches. During crop production in the field or under protected environments (tunnels, greenhouses, etc...), leaf and vine symptoms are barely visible and, may be easily confused with fungal diseases, *e.g.* anthracnose (*Colletotrichum orbiculare*). Brown and rotting spots on fruits are more easily visible but, again, they may be confused with fungal symptoms, such as anthracnose. *Didymella bryoniae*

, the causal agent of the gummy stem blight and black rot, may also cause fruit rots, but necrotizing tissue is dark and dry, instead of wet and soft.

Detection from symptomatic plant material (e.g. vines, leaves, fruits) is done either through direct isolation onto semi-selective agar media, PCR tests or serological tests on plant extracts. Detection from seeds can be performed using a real time-PCR test. Alternatively, a sweat box test (followed by a confirmation) can be done. For more details regarding detection and identification of *A. citrulli* in different plant material, see EPPO Standard PM 7/127.

PATHWAYS FOR MOVEMENT

Long distance dissemination occurs through the trade of infected seeds (Hopkins and Thomson, 2002a). Symptomless, infected seedlings may be an additional pathway for pathogen dissemination.

Splash dispersal during rain or irrigation with sprinklers disseminates *A. citrulli* within the crop and between adjacent crops during the growing season, if secondary inoculum is available on the crop, *i.e.* symptoms are present on plant parts (especially fruits) that allow pathogen growth and spread. Human-aided, short distance dissemination is also possible (and quite efficient) through grafting: infected plant material and contaminated grafting tools may allow pathogen survival and plant-to-plant transmission. Infected fruits do not represent a significant pathway for introduction of the pathogen to new areas.

PEST SIGNIFICANCE

Economic impact

A. citrulli strains are pathogenic to various species of cucurbits, including watermelon, melon, squash, pumpkin and cucumber: significant economic losses have been reported in watermelon and melon. The disease is favoured by heavy rainfalls, high humidity and warm temperatures: when these conditions are met, severe outbreaks may happen with heavy losses, up to 90% (Burdman *et al.*, 2005; Walcott, 2005; Bahar & Burdman, 2010). During the first outbreak on Mariana Islands, entire watermelon fields were destroyed by the pathogen (Wall and Santos, 1988). Usually, disease incidence is 5-50%, with possible total crop loss under ideal conditions for the bacterium (Latin and Hopkins, 1989; Latin and Rane, 1990). Therefore, *A. citrulli* has a great potential to cause significant economic losses to cucurbit crops. Pale-skinned watermelon cultivars, cantaloupe and honeydew melons are particularly sensitive to the pathogen when suitable agro-environmental conditions are met. Due to its destructive nature, disease outbreaks quite often lead to litigation against seed companies and to international controversies (Schaad *et al.*, 2003), thus adding additional costs connected to expensive lawsuits (Walcott, 2005). Therefore, *A. citrulli* represents a constant economic threat to the cucurbit industry, including growers, seed producers and transplant nurseries.

Control

Strategies able to avoid *A. citrulli* infection of seeds are the main means to avoid crop damage during the growing season. Therefore, certification schemes (for seeds and transplants) and seed testing are the major strategies to ensure a healthy crop. The goal of pathogen-free seeds or transplants may be achieved by a thorough inspection of the plant material before its introduction into the greenhouse or field. A widely used method for the detection of *A. citrulli* in contaminated seeds is the seedling grow-out assay (SGO): this method consists of sowing about 30 000 seeds of each evaluated lot in a disease conducive environment. Seedlings are then inspected for symptoms, which will result in rejection of the entire seed lot if even one seedling is proven to be infected (ISF, 2018). The SGO test is labour intensive and time/space-consuming; it requires a minimum of 2-3 weeks for completion and should be done in special greenhouse facilities.

Since *A. citrulli* is seed transmitted, seed treatments have also been suggested to disinfect seeds: such methods were able to decrease the microbial populations colonizing seeds epiphytically, but none of the seed treatments was able to eliminate the pathogen in its endophytic locations (Rane and Latin, 1992; Hopkins *et al.*, 1996; Hopkins *et al.*, 2001; Giovanardi *et al.*, 2015). Seed sanitation with different methods (use of bactericidal chemicals, seed coating with antimicrobial compounds or biocontrol agents, heat treatment) did not prove to be sufficiently effective against the pathogen, probably because of its location in the embryo.

In nurseries or in transplant houses, *A. citrulli* is controlled through several applications of combined ionized copper and peroxyacetic acid in the irrigation water, together with foliar sprays of acybenzolar-S-methyl (Hopkins *et al.*, 2009). Glasshouses should be divided into more sectors using transparent panels, to avoid cross contamination of seedling sub-lots during irrigation. Together with the highest hygiene standards, such an approach may ensure the phytosanitary quality of plantlets prior to transplanting.

There is no effective pesticide to control *A. citrulli* during the growing season: the pathogen is systemic, colonizing any aerial part of the plant and copper compounds are largely ineffective to kill the pathogen in its endophytic stage. To avoid possible dissemination of secondary inoculum in the field, sprinkle irrigation is not recommended: plants should preferably be irrigated using a subsurface irrigation system. Crop rotation with non-cucurbit species is highly recommended, since the pathogen may remain latent into the crop from season to season, producing sudden and dramatic outbreaks when weather conditions are suitable. Plant debris should not remain in the fields, but be cleaned and burned on site when they are dry. Volunteers should be rogued. In case of an outbreak, all plants should be destroyed on site with an herbicide and dry plant residues should be burned.

Resistant cucurbit lines with high commercial value are not available so far, but tolerant cultivars are available for melons and watermelons: such cultivars are currently incorporated into breeding programmes (Hopkins and Thompson, 2002b; Bahar *et al.*, 2009). Carvalho *et al.* (2012) identified tolerant watermelon genotypes and Wechter *et al.* (2011) found possible sources of *A. citrulli* resistance in *Cucumis* spp. plant introductions and in *C. ficifolius*. A large study was done to screen for resistance 1344 *Citrullus* spp. and *Praecitrullus fistulosus* accessions: results indicated that *C. lanatus* var. *citroides* possesses some resistant traits possibly useful to breed resistant watermelon varieties (Hopkins and Thompson, 2002b). Later, it was seen that quantitative inheritance of resistance did not allow a useful level of such resistance to be maintained, along with the fruit quality traits (Hopkins and Levi, 2008).

Phytosanitary risk

A. citrulli is a major threat for cucurbits in the EPPO region in particular in the Southern part of the region (MacLeod *et al.*, 2012), for watermelon. In conditions conducive to *A. citrulli* (warm climate and heavy rainfalls), the disease is destructive, leading to up to 90% of crop loss. Cucurbit seeds are frequently produced in regions where the pathogen is endemic (e.g. the USA and China). Despite the implementation of routine seed testing, sporadic disease outbreaks continue to occur on a range of cucurbit hosts in several countries worldwide. The sporadic disease outbreaks that occurred in the past (Turkey, Italy, Serbia) were successfully eradicated thanks to prompt action, but this highlights the risk of further outbreaks. The seed industry may also be affected: as *A. citrulli* is a regulated pest in several countries, its detection in a seed producing area, even in the absence of severe symptoms on plants, will result in the rejection of any seed lot produced.

PHYTOSANITARY MEASURES

A. citrulli is a seed-borne and seed-transmitted bacterium, therefore seeds represent the major source of primary inoculum. Seed is the major pathway for *A. citrulli*'s long distance dissemination; therefore, seed and seedling certification schemes should be implemented. Seed and seedlings should be produced in pest free areas or in pest-free sites of production. During production, fields should be under official surveillance and plants tested if any symptoms are detected during inspections. Seedling production in nurseries should be done under strict hygiene measures, especially if grafting is planned. Alternatively, seed lots should be tested to guarantee pest freedom of the lot.

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