

Xanthomonas fuscans subsp. *fuscans* causing wilt symptoms in bean plants (*Phaseolus vulgaris*) in Brazil

Xanthomonas fuscans subsp. *fuscans* causando sintomas de murcha em feijoeiro (*Phaseolus vulgaris*) no Brasil

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ABSTRACT: Common and fuscous blights of bean are diseases widely distributed in the world. The most commonly observed symptoms are spots on leaves, stems, pods and seeds. In December 2009, bean plants cv. Uirapuru showing symptoms of wilt similar to those induced by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* were observed in a commercial crop located in the county of Itararé, State of São Paulo, Brazil. The plants were at the last cycle stage with mature pods and these symptoms were noted in the majority of the growing area. Optical microscopic observations of discolored vascular tissue from diseased stems revealed the presence of bacterial masses oozed from infected tissue, indicating that the disease was caused by bacterial pathogen. Isolations on nutrient agar showed circular, convex, yellow colonies with smooth edges. The causal bacterium was Gram-negative and produced a dark brown pigment in culture medium. Biochemical, cultural and physiological tests confirmed its identity as *Xanthomonas fuscans* subsp. *fuscans* (syn. *Xanthomonas campestris* pv. *phaseoli* “var. *fuscans*”). The pathogenicity of the isolates was confirmed by artificial inoculations. Systemic infection has been reported in the literature but these kinds of symptoms are not currently observed in Brazilian fields. Bacterial strains were deposited on the Phytobacteria Culture Collection of Instituto Biológico (IBSBF - www.biologico.sp.gov.br/bacterias/php) under accession numbers 2813 and 3028.

KEYWORDS: bacterial blight; systemic infection; *Xanthomonas axonopodis* pv. *phaseoli*.

RESUMO: O crestamento bacteriano comum do feijoeiro é uma doença amplamente distribuída no mundo e os sintomas comumente observados são manchas nas folhas, hastes, vagens e sementes. Em dezembro de 2009, plantas de feijoeiro cv. Uirapuru com sintomas de murcha similares aos produzidos por *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* foram observadas em campos comerciais localizados no município de Itararé, estado de São Paulo, Brasil. As plantas encontravam-se no final do ciclo, com as vagens já formadas. Os sintomas foram notados em quase a totalidade da área cultivada. Observações ao microscópio óptico de fragmentos de tecido do sistema vascular das hastes de plantas doentes evidenciaram intenso fluxo bacteriano, confirmando tratar-se de doença bacteriana. Isolamentos realizados em meio nutriente ágar produziram colônias de coloração amarelada, brilhantes, convexas, lisas. A bactéria agente causal era Gram-negativa e produtora de pigmento marrom escuro em meio de cultura. Testes bioquímicos, culturais e fisiológicos confirmaram sua identidade como *Xanthomonas fuscans* subsp. *fuscans* (sin. *Xanthomonas campestris* pv. *phaseoli* “var. *fuscans*”). A patogenicidade dos isolados foi confirmada por inoculações artificiais em mudas de feijão cv. Carioca e os reisolamentos efetuados resultaram em colônias semelhantes às originais. Embora descrita na literatura, a infecção sistêmica não é usualmente observada nos plantios de feijoeiro em nosso país. Linhagens bacterianas encontram-se depositadas na Coleção de Culturas do Instituto Biológico (IBSBF) sob os números 2813 e 3028.

PALAVRAS-CHAVE: crestamento bacteriano; infecção sistêmica; *Xanthomonas axonopodis* pv. *phaseoli*.

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Common and fuscous blights of bean are serious and widespread diseases in most bean-growing areas of the world. The disease can be highly destructive during extended periods of warm and humid weather, causing reductions in both yield and seed quality. In Brazil, it has been the cause of several great losses in productive growing areas, especially during the summer. The most commonly observed symptoms of infection are initially small, water soaked leaf spots becoming necrotic, light brown, usually surrounded by chlorotic haloes. Lesions often coalesce and cause extensive tissue damage. These symptoms could be seen in the stems, pods and seeds. Systemic infection can also occur on susceptible cultivars (BIANCHINI et al., 2005).

According to the literature, the pathogen associated with this disease was classified as *Xanthomonas campestris* pv. *phaseoli* at the 1980's. However, since some bacterial strains can cause darkening of the culture medium, it was also named *X. campestris* pv. *phaseoli* "var. *fuscans*". In 1995, VAUTERIN et al. (1995) published a reclassification of the genus *Xanthomonas* and this bacterium was allocated as *X. axonopodis* pv. *phaseoli*. SCHAAD et al. (2005, 2006) proposed *Xanthomonas fuscans* subsp. *fuscans* for the fuscous blight pathogen.

The pathogen can survive on the soil surface and as an epiphyte on several weeds and it is usually introduced to new regions via contaminated seed. Secondary spread can occur by wind-blown rain, overhead irrigation, contaminated equipment, or people and animals.

Two other bacteria have already been described causing wilt symptoms in beans: *Ralstonia solanacearum* and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. In Brazil,

Ralstonia solanacearum was reported for the first time in 1980 in the State of Rio de Janeiro (AKIBA et al., 1980) and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, in 1997, in the State of São Paulo (MARINGONI; ROSA 1997).

In December 2009, bean plants cv. Uirapuru showing wilt symptoms similar to bacterial wilt induced by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* were observed in commercial fields located at the county of Itararé, State of São Paulo, Brazil. The plants were at the last stage of the cycle with mature pods (Fig. 1) and these symptoms were noted in the majority of the growing area.

Optical microscopic observations of longitudinal sections of stems with discolored vascular tissue revealed the presence of intense bacterial masses streamed from the infected tissue, indicating that the disease was caused by bacterial pathogen.

Bacteria isolation was performed by macerating small pieces of diseased tissues in sterile distilled water and the resulting suspension was streaked on nutrient agar medium (NA) (LEVINE, 1954).

Detection of the causal agent was also made in seed samples collected from the pods of the diseased plants received for analysis. Bacteria were extracted from seeds by soaking the samples in sterile saline solution (0.85% NaCl) for 16 h at 4°C. Aliquots of 100 µL of the seed leachate were plated on NA medium containing 10 µg/L of the fungicide chlorothalonil.

After incubation at 28°C for 48–72 h, yellow, mucoid, glistening, convex, smooth bacterial colonies, characteristic of the genus *Xanthomonas*, were observed in both isolations, from diseased tissue and seeds (Fig. 1B).

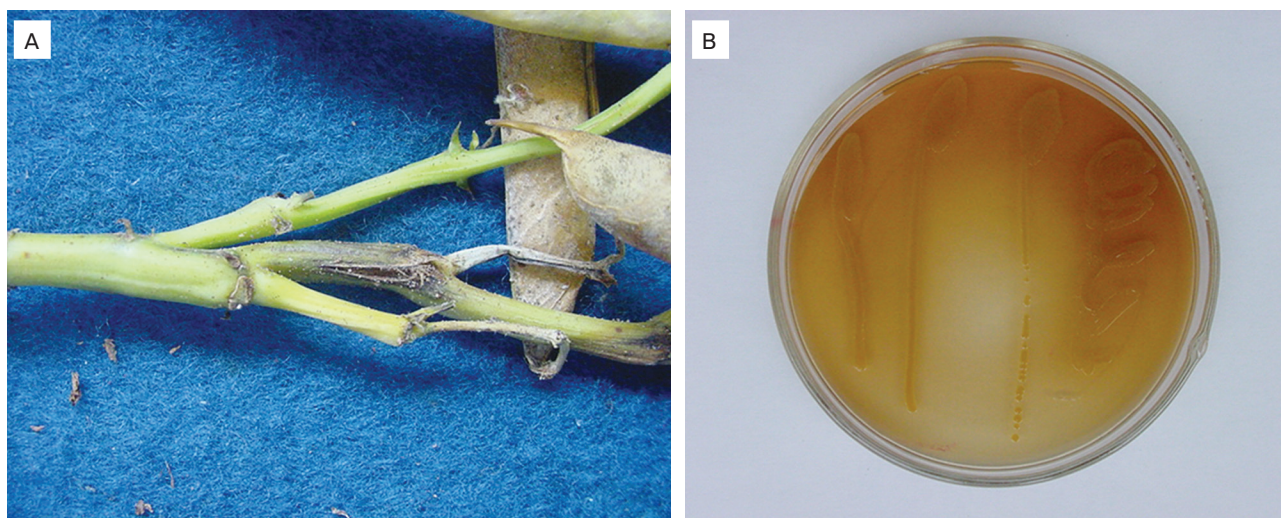


Figure 1. *Xanthomonas fuscans* subsp. *fuscans* in bean. (A) Natural infection. Longitudinal section of stem showing vascular discoloration of the vessels; (B) purified colonies onto culture medium. Note the darkening of the medium.

The bacterial isolates were tested for pathogenicity through inoculations performed by spraying suspensions containing ca. 10⁸ CFU/mL on previously wounded leaves of 15-day-old bean seedlings cv. Carioca. Control plants were similarly treated using sterile distilled water. The inoculated plants were kept under moist chamber conditions for 72 h.

Symptoms of necrotic lesions on leaves and petioles could be seen 10–15 days after inoculation. Control plants remained healthy. Re-isolations resulted in bacterial colonies similar to the originals.

Results of the cultural, biochemical and physiological tests (BRADBURY, 1986; SCHAAD et al., 2001) confirmed the identity of the bacterium as *Xanthomonas fuscans* subsp. *fuscans* (syn. *Xanthomonas campestris* pv. *phaseoli* “var. *fuscans*”) (Table 1).

According to the literature, common bean cv. Uirapuru was susceptible to curtobacterium wilt and common bacterial blight diseases. However, the bacterial isolates were Gram-negative, induced hypersensitivity reaction in tomato leaves and produced a diffusible dark brown pigment in the culture medium, indicating that *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* could not be the causative agent of the observed symptoms but *Xanthomonas fuscans* subsp. *fuscans*.

Bacterial strains were deposited at the Phytobacteria Culture Collection of Instituto Biológico (IBSBF - www.biologico.sp.gov.br/bacterias/php) under accession numbers 2813 and 3028.

Table 1. Biochemical and physiological characteristics of *Xanthomonas fuscans* subsp. *fuscans* (X.f.f.) and the bean isolates.

Characteristics	Cultures	
	X.f.f.	Bean isolates
Catalase	+ ¹	+
Oxidase	- ¹	-
Oxidative/fermentative reaction	O ¹	O
Hypersensitivity reaction on tobacco	-	-
Hypersensitivity reaction on tomato	+	+
Asparagine growth	-	-
H ₂ S from cystein	+	+
Indol production	-	-
Nitrate reduction	-	-
Christensen’s urease	-	-
Tween 80 hydrolysis	+	+
Starch hydrolysis	+	+
Gelatin hydrolysis	-	-
Acid production from:		
arabinose, cellobiose, fructose	+	+
galactose, glycerol, mannose	+	+
sucrose, threulose	+	+
adonitol, dulcitol, inulin	-	-
alpha-m-D-glucoside, inositol	-	-
rhamnose, raffinose	-	-
salicin, sorbitol	-	-
Organic acid utilization:		
acetate, citrate, succinate	+	+
benzoate, malonate, oxalate	-	-
tartrate	-	-
Acetoin production	-	-
Growth at 41 °C	-	-

O: oxidative; +: positive reaction; -: negative reaction

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