Eur J Plant Pathol (2009) 124:695–700
DOI 10.1007/s10658-009-9441-3

Clove-transmissibility of *Pseudomonas salomonii*, the causal agent of 'Café au lait' disease of garlic

Marie-Agnès Jacques · Nathaëlle Luçon · Sandrine Houdault

Received: 25 August 2008 / Accepted: 29 January 2009 / Published online: 28 February 2009

Abstract Bacterial blight of garlic, caused by *Pseudomonas salomonii*, results in leaf and sheath necrosis and sometimes leads to soft rot and plant death. The epidemiology of this bacterial disease, known as 'Café au lait' disease, is poorly understood and no resistant cultivars are currently available. To develop control strategies for this disease, we investigated principal sources of inoculum. The pathogen was isolated from bulbs from plants with typical vegetative symptoms of bacterial blight. Subsequent development of typical foliar symptoms on plantlets originating from symptomatic bulbs demonstrated transmission of the pathogen in the planting material. In one of three field experiments the contamination rate of planting stock influenced the disease incidence in field-grown garlic. The importance of planting stock as a source of inoculum was demonstrated here and should be evaluated relative to other potential sources such as crop debris, soil or alternate hosts in order to develop successful control strategies.

Keywords *Allium sativum* · Bacterial dispersion · Epidemiology · Inoculum · Seedborne disease

M.-A. Jacques (✉) · N. Luçon · S. Houdault
UMR077 PaVé, Centre INRA,
42, rue George Morel, BP 60057,
F-49071 Beaucouzé, France
e-mail: marie-agnes.jacques@angers.inra.fr

A bacterial blight of garlic, known as 'Café au lait', has been recorded in France since 1976. The disease appeared first in southwestern France (Samson 1982) and has since expanded to all growing areas in France (Jacques et al. 2000; Girard et al. 1994), and has been reported in Italy (Calzolari and Bazzi 1985). However, little is known about its distribution in other garlic production areas. The causal agent of this disease was initially assigned to biovar I of *Pseudomonas fluorescens* (Samson 1982; Calzolari and Bazzi 1985) and more recently Gardan et al. (2002) showed that it belongs to a new species, *P. salomonii*.

Diseased plants develop lesions at the base of leaf blades followed by yellowing and wilting of the leaf, during April and May in the traditional garlic-culture areas of southwestern France. These characteristic symptoms most frequently appear on internal leaves (Samson 1982). In addition to aerial leaf symptoms, papery leaves of bulbs become torn and dark brown. Brown soft rot of the pseudostem and total decay of plants are rare (Jacques et al. 2000). Bulb formation is usually not affected by bacterial infection and symptoms are restricted to discolouration at the base of dry sheathing leaves, decreasing commercial quality of the garlic bulbs.

For controlling bacterial diseases of plants, resistance and sanitation are the most frequently and efficiently employed, especially in areas such as France, where antibiotic use against plant pathogenic bacteria is prohibited. Additionally, resistance to

bacterial blight of garlic has not been described. Therefore, identification of the main sources of bacterial inoculum is an essential first step in developing control strategies for this bacterial disease. To date, only one study has focused on the epidemiology of bacterial blight of garlic. Caubel and Samson (1984) evaluated the impact of stem nematode on bacterial blight and demonstrated that increased penetration of *P. salomonii* into garlic bulbs occurred in the presence of *Ditylenchus dipsaci* although the nematode was not always associated with diseased plants. They observed that a higher frequency of brown colouration of planting material, hypothesised as an indicator of bulb bacterial contamination, was not correlated with an increased frequency of symptoms on plants developing from those bulbs. Additionally, levels of bacterial infection of garlic was affected neither by fumigation of the soil nor by nematicide application.

The objectives of this study were to determine whether *P. salomonii* is clove-borne and to evaluate the relationship between the incidence of clove contamination and disease in subsequent plantings.

To investigate *P. salomonii* transmission by planting material, we selected bulbs naturally infected with *P. salomonii* by repetitive observations and analyses of foliar symptoms and isolation of *P. salomonii* from harvested bulbs. During May 1999, two series of 25 plants per row of the garlic (*Allium sativum*) cvs Germidour (three fields), Messidrome (one field), Corail (one field), and Jolimont (one field) grown by commercial growers using standard practices, were marked for study. Café au lait incidence was visually evaluated by recording foliar symptoms three times at 15-day intervals. Isolations from the margin of typical leaf lesions were made to confirm the presence of the pathogen. To isolate the pathogen, aliquots from leaf fragments macerated in phosphate buffer (K_2HPO_4 8.75 g, KH_2PO_4 6.75 g l^{-1}, pH 7) were streaked on King's medium B (King et al. 1954) containing cycloheximide (50 mg l^{-1}). After incubation for 2 days at 25°C fluorescent colonies morphologically resembling *P. salomonii* (by comparison with a control culture of *P. salomonii* type strain CFBP2022 stored in the French Collection of Plant Pathogenic Bacteria, Angers, France) were characterised by conventional biochemical tests: HR on tobacco, hydrolysis of aesculin and gelatin, levan production, and nitrate reduction (Gardan et al. 2002), and by an agglutination test with a specific polyclonal antiserum (Samson 1982).

Typical symptoms initially included lesions at the base of a single leaf blade followed by yellowing and wilting of one to two older leaves. Later, symptoms were observed on younger leaves (Table 1). *Pseudomonas salomonii* was consistently isolated from the margin of lesions. Symptoms on individual tagged plants during the first weeks of May were difficult to differentiate from senescence in late June, which appeared to cause the disease to go into remission. At harvest, bulbs from plants that had symptoms in May were discoloured and brown, but bulbs from plants that developed symptoms later in the season were not consistently discoloured. Additionally, the incidence of discoloured bulbs at harvest was sometimes higher than the incidence of foliage symptoms (Table 1). Bacterial blight symptoms on garlic were restricted to the leaves and their dry sheathing bases; no symptoms related to the disease were observed on the storage leaf of the clove.

To evaluate survival of *P. salomonii* on stored bulbs, symptomless and discoloured bulbs were harvested from the previously described commercial plots and stored until dry in a greenhouse. The discoloured papery sheathing leaves of cloves were evaluated for the presence of *P. salomonii* after harvest and monthly during 4 months of storage. Frequency of *P. salomonii* isolation was between 25% and 67% depending on the length of storage (data not shown). *Pseudomonas salomonii* was also isolated from discoloured papery leaves of bulbs from asymptomatic plants. This indicated that infection of the papery sheathing leaves was not consistently correlated with foliage infection (data not shown). *Pseudomonas salomonii* was not isolated from any of the apparently healthy papery sheathing leaves on five bulbs of each cultivar.

Next, the development of bacterial blight symptoms and contamination of plantlets were evaluated for plants derived from contaminated planting material. Bulbs with discoloured papery leaves from which *P. salomonii* was isolated at harvest, were vernalised for 3 weeks at 4°C. For each cultivar and original commercial field plot, one clove was selected from between four to 14 contaminated bulbs for planting (12/13/99) in a commercial soil substrate (Neuhaus humine substrat S NF 11-44-551, Proveg, La Rochelle, France). Plants were grown in a green-

Table 1 Incidence of garlic bacterial blight of field-grown garlic evaluated on leaves at three sampling dates and on bulbs at harvest

Plot	Cultivars	Organ [a]	Sampling time				
			5/05/99[b]	5/18/99	6/1/99	Veg. [c]	Harvest [d]
I	Germidour	Old leaves / bulb	28	4	4	68	86
		Young leaves	16	44	20		
II	Germidour	Old leaves / bulb	10	8	52	76	48
		Young leaves	4	8	0		
III	Germidour	Old leaves / bulb	20	16	24	56	60
		Young leaves	0	20	32		
IV	Messidrome	Old leaves / bulb	76	32	ND [e]	96	76
		Young leaves	0	48	36		
VI	Corail	Old leaves / bulb	4	0	8	60	60
		Young leaves	0	4	36		
VII	Jolimont	Old leaves / bulb	52	6	2	74	80
		Young leaves	6	22	32		

Lesions at the base of a leaf blade followed by yellowing and wilting of leaves, and dark-brown colouration of papery leaves of bulbs were recorded as symptoms of bacterial blight.

[a] Leaf age was evaluated by leaf position on the plants. Symptoms were evaluated on leaves during vegetation and on bulbs at harvest.

[b] Percentage of plants showing symptoms of bacterial blight at the specified sampling date independently from results of previous notations. Symptoms were observed on samples of 25 plants per plot.

[c] Percentage of plants that showed bacterial blight symptoms at least at one sampling date during vegetation (Veg.).

[d] Observations were made at harvest on the 06/9/99 for plots I to III and on the 06/22/99 for the other plots.

[e] Not determined.

house, watered daily and supplemented with 0.3 g l^{-1} NPK (18/14/18) once a week. Isolation of *P. salomonii* from individual cloves ranged between 25% and 64% indicating that not all cloves in contaminated bulbs were contaminated (Table 2). Typical symptoms of garlic bacterial blight (yellowing and wilting of leaves) were observed on 22% of the plantlets from these cloves. *Pseudomonas salomonii* was isolated from all symptomatic plantlets demonstrating clove transmission of this plant pathogenic bacterium. All leaves from individual asymptomatic plants were weighed and ground (Stomacher 80; Seward, London, UK) for 2 min at maximum power in 5 to 40 ml of phosphate buffer according to the weight of the sample. Aliquots of 100 µl of samples and ten-fold serial dilutions were spread on

Table 2 Transmission of *P. salomonii* and garlic bacterial blight by planting stock material in the greenhouse

Plot	Cultivar	Number of samples	% at planting of brown bulbs from which *P. salomonii* was isolated	Percent contaminated plantlets 134 dap	% plantlets with symptoms 134 dap
I	Germidour	6	33	83	33
II	Germidour	9	56	78	11
III	Germidour	8	38	75	25
IV	Messidrome	5	60	80	20
VI	Corail	4	25	75	0
VII	Jolimont	14	64	86	50

Cloves from bulbs showing brown papery leaves were analysed to determine *P. salomonii* contamination at planting (*i.e.* after 140 days storage) and 134 days after planting (dap) in the greenhouse. Aerial symptoms were recorded 134 dap and aerial parts of plants were analysed to determine asymptomatic *P. salomonii* contamination.

King's medium B containing cycloheximide using a spiral system (DS, Interscience, Saint-Nom-la-Brètèche, France). Plates were incubated and colonies were characterised as described above. Plantlets from healthy cloves (three cloves per cultivar) were asymptomatic and no contamination with *P. salomonii* was detected (data not shown).

Contamination by *P. salomonii* of the new generation of cloves was evaluated in field trials simultaneously with the greenhouse experiment, using the previously described contaminated planting material. Cloves from contaminated and healthy bulbs were planted in experimental field plots (11/17/99; at INRA, UMR PaVé, Beaucouzé France) and cultivated according to commercial practices. Plots had not previously been planted with garlic (for at least 10 years). Field plots consisted of four 0.80 m wide 6 m long beds and each bed was planted with two rows spaced 0.40 m apart at a rate of ten cloves per m. Two beds were planted with healthy cloves and two additional beds with contaminated cloves. Forty cloves of each cultivar and sanitary status per replicate were planted in a randomised four block design.

No symptoms were observed on aerial parts of any plants during the growing season. However, at harvest the percentage of brown protective leaves was higher in plots grown from contaminated cloves (70.9%) than in plots from healthy cloves (23.6% of plants). These significant differences were observed for each cultivar (Table 3). Moreover, *P. salomonii* was isolated from discoloured protective leaves in 44.8% and 40% of the initially contaminated and healthy planting material, respectively. The hypothesis of random error could not be rejected with a χ^2 test, using Statbox Pro software (Grimmer Logiciels, Optima France) which suggested that plants were contaminated regardless of their initial sanitary status. This also indicated that *P. salomonii* spread in the field plot among beds; indeed, as mentioned above healthy bulbs from healthy mother plants did not harbour *P. salomonii*.

We hypothesise that dispersion of the pathogen in the field was favoured by rainy autumn and winter seasons that allowed good *P. salomonii* soil establishment and its movement in humid soil. More frequent presence of the pathogen in the rhizosphere than in the phyllosphere of garlic was monitored (data not shown) suggesting that *P. salomonii* is primarily a soil inhabitant organism. Moreover, we correlated the dispersion of the bacteria in soil with increasing water potentials in experiments conducted under controlled conditions (data not shown).

Finally, experiments were established to evaluate the relationship between contamination of the planting stock and disease incidence under field conditions in locations were the disease is frequent. Two series of field experiments at two locations that varied in climatic conditions were conducted each with the

Table 3 Isolation of *P. salomonii* from brown protective leaves of cloves at harvest after field planting of either contaminated or healthy cloves

Cultivars	Status of planting material [a]	% of brown leaves [b]	χ^2 value (significance) [c]	% of positive *P. salomonii* isolation	χ^2 value (significance)
Germidour	Contaminated	72.5	29.7 (S**)	31	Nd
	Healthy	10		0	
Germidour	Contaminated	64.3	12.6 (S**)	44.5	0.134 (NS)
	Healthy	14.3		50	
Corail	Contaminated	60	6.1 (S*)	54.17	0.011 (NS)
	Healthy	30		41.67	
Jolimont	Contaminated	85	17.1 (S**)	50	0.021 (NS)
	Healthy	37.5		46.67	
Total	Contaminated	70.9	64.5 (S**)	44.8	0.018 (NS)
	Healthy	23.6		40	

[a] Every clove from a bulb with only white protective leaves was considered non-contaminated. Cloves from bulbs with dark brown leaves were considered contaminated even if each clove did not display brown colouration.

[b] A sample of 40 cloves was planted per cultivar and per status except for plot II for which 28 cloves were planted per status.

[c] For one degree of freedom, critical values of χ^2 statistics for P=0.05, P=0.01 and P=0.001 are 3.84, 6.64 and 10.83, respectively. S (*) indicates that differences are significant at P=0.05, S(**) significant at P=0.001; NS not significant; Nd not determined.

same planting material. Experimental field plots were planted with garlic (cv. Messidrome, gifted by Prosemail, Loriol, France) in Moissac (CEFEL experimental farm, Tarn et Garonne) southwestern France, and in Balandran (CTIFL experimental station, Gard) southeastern France, in 2000 and 2001 and cultivated according to commercial practices. Each year, a different site on the farm that had not been planted with garlic for at least 10 years was used to establish the plots. Closest garlic field plots were separated by 100 m. Plots were sown on the 12/4/00 and 11/14/01 in Moissac and on 12/20/00 and 11/07/01 in Balandran. Plots were harvested on the 06/25/01 and 06/17/02 in Moissac and on 07/02/01 and 06/25/02 in Balandran. Field plots consisted of five 0.90 m wide × 22 m long beds for each environmental condition tested and each bed was planted with three rows spaced 0.40 m apart at a rate of ten cloves per m. External beds and 2 m at both ends of each bed were considered as buffers. Treatments consisted of three levels of Café au lait contamination based on rate of contaminated cloves in lots. Highly, low and not contaminated lots contained around 70%, 15% and 0% of suspected contaminated cloves, respectively.

Bacterial contamination of planting material was first evaluated visually according to colouration of the bulb and then confirmed by microbiological analysis. Only when every clove from a bulb had white protective leaves was the bulb considered non-contaminated. Cloves from bulbs with dark brown protective leaves were considered contaminated even if each clove was not brown and discoloured. Setting-up lots of known contamination rates was obtained by mixing defined quantities of non-contaminated cloves with contaminated cloves. A sample of 50 cloves was taken from each lot before planting and analysed to confirm the visual evaluation and contamination of cloves by *P. salomonii*. Visual assessment of aerial symptoms was recorded at 15–30 day intervals from March to June and a final assessment was done at harvest.

Garlic bacterial blight symptoms developed late in 2000–2001 growing season and no difference was detected in fields planted with the high rate (>70%) and low rate (<20%) of contaminated cloves regardless of location. Garlic bacterial blight incidence remained low at harvest at both sites (Table 4). In the 2001–2002 growing season, a clear difference in garlic bacterial blight incidence among plots with different initial clove contamination levels was detected in Moissac. Plots planted with the high rate of *P. salomonii* contamination showed a bacterial blight incidence of 39% at harvest while low contaminated plots showed only 11% of bacterial blight incidence and 9% for the control plot.

Both in Balandran and Moissac, November 2000 to March 2001 were rainy months compared to the past 10 years with a total of 430 mm and 401 mm compared to the 303 and 254 mm in Moissac and in Baladran, respectively. November 2001 to January 2002 were drier than usual. Three important rainstorms occurred in May 2002 in Moissac resulting in 149 mm of water in three events during a 15 day-period. No such rainstorms were recorded in either

Table 4 Impact of clove contamination on garlic bacterial blight incidence on garlic at harvest in two experimental sites (Moissac and Balandran, France) for two consecutive experiments

Experimental site	2000/2001		2001/2002	
	Percent of estimated clove contamination[a]	Blight incidence[b]	Percent of estimated clove contamination	Blight incidence
Moissac	75	15	70	39
	19	13	26	11
	nd[c]	nd	0	9
Balandran	71	8	70	11
	18	8	26	12
	nd	nd	0	0

[a] Percentage of cloves with brown protective leaves.

[b] Percentage of plants with garlic bacterial blight symptoms in the field

[c] not determined

location in 2001 or in Balandran in 2002. Temperature recordings did not depart from standards.

Development of bacterial blight is highly dependent both on the presence of high populations of the pathogen and on favourable environmental factors. Bacterial blight epidemics developed only in one plot out of the 10 studied, one of the two most contaminated. Several rainstorm events could have favoured symptom expression in this plot where inoculum pressure was high (70% of contaminated cloves). It is known that the momentum of rain drops favours disease initiation by increasing bacterial ingress and multiplication in plant tissue (Hirano et al. 1996). At the other site, no disease developed in the plots regardless of the inoculum pressure, presumably due to the environmental conditions being unfavourable for disease development.

Here we present the first evidence that bacterial blight of garlic is transmitted by planting stock, thus providing a significant target for management of this disease. The use of pathogen-free planting material is an important management strategy, as once established bacterial diseases are difficult to eliminate. Thermotherapy has been used to clear planting stock from *Aceria tulipae* and *D. dipsaci* (Courtin et al. 2000) and the same treatment may be effective for disinfesting the planting stock from *P. salomonii.* Knowledge about the epidemiology of this disease will help to develop management strategies including the production of *P. salomonii*-free planting stock. The role of other sources of inoculum needs to be evaluated in comparison with the importance of contamination of the planting stock as sources of inoculum for bacterial blight of garlic. Although there are numerous potential sources, including crop debris, soil, weeds, irrigation water and alternate hosts, none of these have been reported to be contaminated by *P. salomonii*.

Acknowledgements This work was financially supported by ANIAil, Prosemail and Oniflhor. We thank P. Erard, (CTIFL, Balandran) and F. Leix-Henry, (CEFEL, Montauban) for field cultivations and sampling, and ANIAil for the gift of garlic seeds. We thank N., Bourhis, J.-P. Leroux, and C. Bureau for assistance, and C. Bull, for the critical review and English editing of the manuscript.

References

Calzolari, A., & Bazzi, C. (1985). Un marciume fogliare dell'aglio causato da *Pseudomonas fluorescens*. *Informatore Fitopatologico*, *7*, 35–38.

Caubel, G., & Samson, R. (1984). Influence du nématode des tiges, *Ditylenchus dipsaci* (Kühn) Fil. dans le développement de la bactériose "café au lait" de l'ail (*Allium sativum* L.) occasionnée par un biovar de *Pseudomonas fluorescens* Migula. *Agronomie*, *4*, 311–313. doi:10.1051/agro:19840312.

Courtin, O., Fauvel, G., & Leclant, F. (2000). Temperature and relative humidity effects on egg and nymphal development of *Aceria tulipae* (K.) (Acari: Eriophyidae) on garlic leaves (*Allium sativum* L.). *Annals of Applied Biology*, *137*, 207–211. doi:10.1111/j.1744-7348.2000.tb00061.x.

Gardan, L., Bella, P., Meyer, J. M., Christen, R., Rott, P., Achouak, W., et al. (2002). *Pseudomonas salomonii* sp. nov., pathogenic on garlic, and *Pseudomonas palleroniana* sp. nov., isolated from rice. *International Journal of Systematic and Evolutionary Microbiology*, *52*, 2065–2074. doi:10.1099/ijs.0.02225-0.

Girard, J.-C., Nicole, J.-F., & Cheron, J.-J. (1994). A disease of garlic caused by a fluorescent pseudomonad closely related to *Pseudomonas fuscovaginae*. In M. Lemattre, S. Freigoun, K. Rudolph, & J. G. Swings (Eds.), *Plant pathogenic bacteria* (pp. 87–92). Paris: Les colloques n° 66 INRA.

Hirano, S. S., Baker, L. S., & Upper, C. D. (1996). Raindrop momentum triggers growth of leaf-associated populations of *Pseudomonas syringae* on field-grown snap bean plants. *Applied and Environmental Microbiology*, *62*, 2560–2566.

Jacques, M.-A., Luçon, N., & Josi, K. (2000). La maladie café au lait de l'ail. *PHM-Revue Horticole*, *414*, 36–38.

King, E. O., Ward, M. K., & Raney, D. E. (1954). Two simple media for the demonstration of pyocianin and fluorescein. *Journal of Laboratory and Clinical Medicine*, *44*, 301–307.

Samson, R. (1982). A biovar of *Pseudomonas fluorescens* pathogenic to *Allium sativum*. In CIAT (Ed.),*Plant pathogenic bacteria* (pp. 60–64). Colombia: Proceedings of the Vth international conference on plant pathogenic bacteria Cali.