Xanthomonas campestris

Subjects: Genetics & Heredity

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Xanthomonas campestris pv. campestris (Xcc) is a Gram-negative, aerobic, vascular, and motile bacterium with a single flagellum, which causes the disease identified as black rot in Brassica crops.

Keywords: black rot ; plant–pathogen interaction ; plant secondary metabolites

1. Introduction

Xanthomonas campestris pv. *campestris* (*Xcc*) is a Gram-negative, aerobic, vascular, and motile bacterium with a single flagellum, which causes the disease identified as black rot in *Brassica* crops [1]. *Xcc* enters in the plant through the stomas, the hydathodes, and wounds. Once inside, the pathogen travels through the vascular system, invading the xylem and colonising the mesophyll. Disease symptoms are developed in the host plants in warm and humid conditions [2]. The infection is characteristic because it causes V-shaped necrotic lesions in the edges of leaves and necrosis and darkening of the veins of the leaves and the vascular tissue of the stem. As the disease progresses, wilting and necrosis throughout the plant occur [3]. Eleven races of *Xcc* have been recognised so far, defined based on their interaction with different *Brassica* cultivars following a gene-for-gene model [4][5][6]. Races 1 and 4 are the most pathogenic and widespread, accounting for 90% of the black rot in the world [4].

After the recognition of *Xcc*, the immune system of *Brassica* plants triggers various layers of defence, such as the synthesis of PR proteins and RLK receptors, the induction of defence hormones, an antioxidant response, and the synthesis of the secondary metabolites, glucosinolates (GSLs) [7]. GSLs are secondary metabolites found exclusively in Brassicaceae plants. Resistance to *Xcc* is related to the amount of GSLs in plants. The induction of the synthesis of GSLs confers a resistance to *Xcc* in *Brassica oleracea* [8]. A relationship was discovered [9] between the disease severity caused by *Xcc* and the GSL content in the Brassicaceae species cress (*Lepidium sativum*), salad rocket (*Eruca sativa*), and broccoli (*B. olerecea* L. var. *italica*). The modification of the content of specific GSLs affects the resistance to *Xcc*. After testing the genotypes of *B. oleracea* with a high and low content of the GSLs sinigrin, glucoiberin, and glucobrassicin, glucobrassicin clearly diminished the spread of *Xcc* in leaves; on the contrary, sinigrin and glucoiberin produced no clear effect [10].

GSLs are stored inactive in vacuoles. After tissue breakdown caused by injuries, pests, or necrotrophic pathogens, the glucosidases termed myrosinases convert GSLs into toxic products [11]. The toxicity significantly depends on the chemical structure. This ultimately relies on the structure of the original GSL and the presence of specific proteins in the host. Among the toxic products derived from GSLs, isothiocyanates (ITCs) are produced by default. The generation of nitriles and epithionitriles needs the presence of epithiospecific proteins, which modulate the activity of myrosinases [12]. GSL hydrolytic products (GHPs) have antimicrobial activities in vitro against plant pathogens [11][12][13]. Among them, ITCs are the most toxic, even at low concentrations [14]. Different evidence in vitro supports the antimicrobial activity of GHPs on *Xcc* growth [13][15][16]. The inhibition of growth is dependent on the concentration of GHP and on the race under study [13].

2. Transfer of Information in the Cell

FtsZ and ftsX were repressed by I3C. FtsZ is a key gene in the SOS-independent and -dependent DNA damage checkpoint [17]. FtsZ is the prokaryotic homolog of tubulin and is the first component of the cell division apparatus, which localises the division site. Once localised, ftsZ polymerises into the Z ring and provides a scaffold upon which ftsX and other components are recruited, forming the divisome. The inactivation of components of the divisome causes an arrest in cell division [18]. The SOS response is employed by bacteria upon DNA damage to ensure cell division is delayed, providing the cell with enough time for DNA repair. AITC overexpressed dnaE and I3C overexpressed dnaN, radA, and recX, which are related to DNA repair by homologous recombination (HR). Double-strand breaks can be repaired by HR, which requires the availability of an intact DNA template or by non-homologous end joining (NHEJ) if no intact template is available. Both GHPs induced ligD, which is part of NHEJ. DnaE, dnaN, radA, and recX are part of the SOS response;

therefore, both treatments delayed cell division to ensure the repair of DNA by activating the SOS response. The chaperone dnaK (Hsp70) was repressed by both GHPs. The downstream chaperonin of dnaK, groL2, was repressed by I3C. Proteins that interact with dnaK involve a broad range of cellular functions including recombination and repair, translation, and ribosomal structure in *E. coli* [19]. Additionally, ftsZ is as strong dnaK binder. Therefore, bacteria retard cell division to ensure a proper repair of DNA through dnaK inhibition. The treatment of *C. jejuni* with the ITC BITC induced dnaK and groL2, which can respond to the need for protein refolding [20]. Protein refolding can be achieved through the induction of chaperone htpG (hsp90) by AITC.

RpoA was repressed and lrp was induced by I3C. Both DEGs are related to transcription. RpoA codifies the subunit α of the RNA polymerase. Lrp is a leucine-responsive regulatory protein and is a global regulator affecting the expression of many genes and operons in *E. coli* including the majority of genes expressed upon entrance into the stationary phase [21]. The translation was downregulated by I3C in the GO analysis. InfB and fusA were repressed by I3C. InfB is the translation initiation factor IF-2 and fusA is the elongation factor G [22]. Lrp and fusA can be activated under a stringent response. A stringent response is modulated by the alarmone (p)ppGpp upon starvation and in response to imbalances in the outer membrane biogenesis [23]. I3C repressed gppA, which is involved in the hydrolysis of pppGpp to ppGpp. By inhibiting gppA, the ratio pppGpp/ppGpp increases, which can provoke a higher inhibition of replication elongation [24]. AITC induced dksa. It encodes a transcription factor that binds directly to the RNA polymerase and, together with (p)ppGpp, modulates the stringent response of bacteria [25]. The conjugation of ITC with amino acids may promote a stringent response by the depletion of free amino acids in fungi and bacteria [26]. The genes responsible for the synthesis of (p)ppGpp (spoT, relA) were not significantly different from the control in any of the treatments (data not shown). With our evidence, we cannot conclude that a stringent response was activated in *Xcc* upon treatment with AITC and I3C. The growth arrest caused by both GHPs seems to be related to the need of repair DNA. Following [27], AITC is mutagenic on *E. coli* DNA, and this capability is likely related to the formation of thiobarbituric acid reactive substances and ROS. Therefore, our results suggest that the genotoxicity of both GHPs is caused in part by damage to the DNA, similar to what occurs in bacteria pathogenic to humans. Other typical responses of bacteria to ITCs, such as the activation of the stringent response, could not be confirmed with our data.

References

1. Singh, S.; Dey, S.S.; Bhatia, R.; Batley, J.; Kumar, R. Molecular breeding for resistance to black rot [Xanthomonas campestris pv. campestris (Pammel) Dowson] in Brassicas: Recent advances. Euphytica 2018, 214, 196.
2. Rubel, M.H.; Abuyusuf, M.; Nath, U.K.; Robin, A.H.K.; Jung, H.J.; Kim, H.T.; Park, J.I.; Nou, I.S. Glucosinolate profile and glucosinolate biosynthesis and breakdown gene expression manifested by black rot disease infection in cabbage. Plants 2020, 9, 1121.
3. Taylor, J.D.; Conway, J.; Roberts, S.J.; Astley, D.; Vicente, J.G. Sources and origin of resistance to Xanthomonas campestris pv. campestris in Brassica genomes. Phytopathology 2002, 92, 105–111.
4. Vicente, J.G.; Conway, J.; Roberts, S.J.; Taylor, J.D. Identification and origin of Xanthomonas campestris pv. campestris races and related pathovars. Phytopathology 2001, 91, 492–499.
5. Fargier, E.; Manceau, C. Pathogenicity assays restrict the species Xanthomonas campestris into three pathovars and reveal nine races within X. campestris pv. campestris. Plant Pathol. 2007, 56, 805–818.
6. Cruz, J.; Tenreiro, R.; Cruz, L. Assessment of diversity of Xanthomonas campestris pathovars affecting cruciferous plants in Portugal and disclosure of two novel X. campestris pv. campestris races. J. Plant Pathol. 2017, 99, 403–414.
7. Tortosa, M.; Cartea, M.E.; Velasco, P.; Soengas, P.; Rodriguez, V.M. Calcium-signaling proteins mediate the plant transcriptomic response during a well-established Xanthomonas campestris pv. campestris Infection. Hortic. Res. 2019, 6, 1–10.
8. Sun, Q.; Zhang, E.; Liu, Y.; Xu, Z.; Hui, M.; Zhang, X.; Cai, M. Transcriptome analysis of two lines of Brassica oleracea in response to early infection with Xanthomonas campestris pv. campestris. Can. J. Plant Pathol. 2021, 43, 127–139.
9. Aires, A.; Dias, C.S.P.; Carvalho, R.; Oliveira, M.H.; Monteiro, A.A.; Simões, M.V.; Rosa, E.A.S.; Bennett, R.N.; Saavedra, M.J. Correlations between disease severity, glucosinolate profiles and total phenolics and Xanthomonas campestris pv. campestris inoculation of different Brassicaceae. Sci. Hortic. 2011, 129, 503–510.
10. Madloo, P.; Lema, M.; Francisco, M.; Soengas, P. Role of Major Glucosinolates in the defense of kale against Sclerotinia Sclerotiorum and Xanthomonas campestris pv. campestris. Phytopathology 2019, 109, 1246–1256.
11. Calmes, B.; N'Guyen, G.; Dumur, J.; Brisach, C.A.; Campion, C.; Lacomi, B.; PignÉ, S.; Dias, E.; Macherel, D.; Guillemette, T.; et al. Glucosinolate-derived isothiocyanates impact mitochondrial function in fungal cells and elicit an oxidative

stress response necessary for growth recovery. Front. Plant Sci. 2015, 6, 414.

12. Burow, M.; Losansky, A.; Müller, R.; Plock, A.; Kliebenstein, D.J.; Wittstock, U. The genetic basis of constitutive and her bivore-induced ESP-independent nitrile formation in Arabidopsis. Plant Physiol. 2009, 149, 561–574.
13. Sotelo, T.; Lema, M.; Soengas, P.; Cartea, M.E.; Velasco, P. In vitro activity of glucosinolates and their degradation prod ucts against Brassica-pathogenic bacteria and fungi. Appl. Environ. Microbiol. 2015, 81, 432–440.
14. Pastorczyk, M.; Bednarek, P. The function of glucosinolates and related metabolites in plant innate immunity. Adv. Bot. Res. 2016, 80, 171–198.
15. Aires, A.; Mota, V.R.; Saavedra, M.J.; Monteiro, A.A.; Simões, M.; Rosa, E.A.S.; Bennett, R.N. Initial in vitro evaluations of the antibacterial activities of glucosinolate enzymatic hydrolysis products against plant pathogenic bacteria. J. Appl. Microbiol. 2009, 106, 2096–2105.
16. Velasco, P.; Lema, M.; Francisco, M.; Soengas, P.; Elena Cartea, M. In vivo and in vitro effects of secondary metabolite s against Xanthomonas campestris pv. campestris. Molecules 2013, 18, 11131–11143.
17. Burby, P.E.; Simmons, L.A. Regulation of cell division in bacteria by monitoring genome integrity and DNA replication st atus. J. Bacteriol. 2020, 202, e00408-19.
18. Margolin, W. FtsZ and the division of prokaryotic cells and organelles. Nat. Rev. Mol. Cell Biol. 2005, 6, 862–871.
19. Calloni, G.; Chen, T.; Schermann, S.M.; Chang, H.C.; Genevaux, P.; Agostini, F.; Tartaglia, G.G.; Hayer-Hartl, M.; Hartl, F.U. DnaK functions as a central hub in the E. coli chaperone network. Cell Rep. 2012, 1, 251–264.
20. Dufour, V.; Stahl, M.; Rosenfeld, E.; Stintzi, A.; Baysse, C. Insights into the mode of action of benzyl isothiocyanate on Campylobacter Jejuni. Appl. Environ. Microbiol. 2013, 79, 6958–6968.
21. Calvo, J.M.; Matthews, R.G. The leucine-responsive regulatory protein, a global regulator of metabolism in Escherichia coli. Microbiol. Rev. 1994, 58, 466–490.
22. Caldas, T.; Laalami, S.; Richarme, G. Chaperone properties of bacterial elongation factor EF-G and initiation factor IF2. J. Biol. Chem. 2000, 275, 855–860.
23. Roghanian, M.; Semsey, S.; Løbner-Olesen, A.; Jalalvand, F. (P)PpGpp-mediated stress response induced by defects i n outer membrane biogenesis and ATP production promotes survival in Escherichia coli. Sci. Rep. 2019, 9, 1–11.
24. Denapoli, J.; Tehranchi, A.K.; Wang, J.D. Dose-dependent reduction of replication elongation rate by (p)PpGpp in Esch erichia coli and Bacillus subtilis. Mol. Microbiol. 2013, 88, 93–104.
25. Gourse, R.L.; Chen, A.Y.; Gopalkrishnan, S.; Sanchez-Vazquez, P.; Myers, A.; Ross, W. Transcriptional responses to p pGpp and DksA. Annu. Rev. Microbiol. 2018, 72, 163–184.
26. Dufour, V.; Stahl, M.; Baysse, C. The antibacterial properties of isothiocyanates. Microbiology 2015, 161, 229–243.
27. Kassie, F.; Knasmüller, S. Genotoxic effects of allyl isothiocyanate (AITC) and phenethyl isothiocyanate (PEITC). Che m. Biol. Interact. 2000, 127, 163–180.
