Virulent \textit{Rhodococcus fascians} Produce Unique Methylated Cytokinins

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Abstract: Some strains of \textit{Rhodococcus fascians} exist only as epiphytes on the plant surface whereas others can become endophytic and cause various abnormalities including the release of multiple buds and reduced root growth. The abnormalities reflect the action of cytokinin. The strains that can become endophytic harbour a linear plasmid that carries cytokinin biosynthesis, activation and destruction genes. However, both epiphytic and endophytic forms can release cytokinin into culture, affect cytokinin metabolism within inoculated plants and enhance the expression of sugar and amino acid transporters and cell wall invertases, but only the endophytic form markedly affects the morphology of the plant. A unique methylated cytokinin, dimethylated \(N^6-(\Delta^2\text{-isopentenyl})\text{adenine} (2\text{-MeiP})\), operating in a high sugar environment, is the likely causative factor of the severe morphological abnormalities observed when plants are inoculated with \textit{R. fascians} strains carrying the linear plasmid.

Keywords: apical dominance; cytokinin; methylated cytokinin; \textit{Rhodococcus fascians}; sugar will eventually be exported transporter; SWEET; amino acid transporter; sugar transporter; cell wall invertase

1. Introduction

Unique methylated cytokinins account for the morphological abnormalities induced by virulent strains of \textit{Rhodococcus fascians}. For many decades, cytokinins produced by \textit{R. fascians} have been implicated as the causative factors inducing shooty galls and reduced root growth, since application of cytokinin can mimic the disease symptoms [1]. Indeed, virulent strains harbour a linear plasmid that carries genes for cytokinin biosynthesis (\textit{fasD}), cytokinin activation (\textit{fasF}) and cytokinin destruction (\textit{fasE}), and avirulent strains lack such a plasmid [2–5]. Cytokinin biosynthesis usually involves the attachment of an isoprenoid side chain to a molecule of either AMP (bacteria) or ADP/ATP (plants) by an isopentenyl transferase (IPT). Additionally, cytokinins are also found associated with specific tRNA molecules, synthesised via tRNA-IPTs [6–8].

Both avirulent (epiphytic) as well as virulent strains of \textit{R. fascians} have been shown to extrude multiple different cytokinins into culture, most of which can, however, be derived from tRNA breakdown e.g., [9–11]. Likewise, multiple cytokinins can be extracted from plants inoculated with both virulent and avirulent strains e.g., [12–15]. Critically, the levels of individual cytokinins extracted from tissues inoculated with virulent strains have never been sufficiently elevated relative to mock-, or avirulent-inoculated plants to be convincing as the cause of the shooty galls.

In a well-cited publication, it was suggested that virulent strains of \textit{R. fascians} trick the plant into providing a compatible environment for the pathogen. The “Trick-with-the-Cytokinin-Mix” hypothesis is based on the accumulation of several cytokinins in tissue inoculated by a virulent strain. These cytokinins are more-or-less resistant to destruction by cytokinin oxidase/dehydrogenase (CKX) [16]. However, these data derive from a comparison between plants inoculated by a virulent strain and a mock-inoculated control, and lack a comparative analysis with avirulent-inoculated plants.
We have shown that both virulent and avirulent strains of *R. fascians* can produce the cytokinins implicated in the ‘Trick-with-the-Cytokinin-Mix’ hypothesis in planta [13–15]. Moreover, both a virulent and an avirulent strain caused increased expression of amino acid (AAP) and sugar (SWEET and SLIT) transporters and cell wall invertases (CWINV) [13,14]. A high sugar environment is required for release of apical dominance [17], so there is clearly something unique to the virulent strains that leads on to the initiation of the shooty galls and inhibition of root growth.

In 2015, Sakakibara’s lab identified two previously unknown methylated cytokinins, monomethylated \( \text{N}^6-(\Delta^2\text{-isopentenyl})\text{adenine (1-MeiP)} \) and dimethylated \( \text{N}^6-(\Delta^2\text{-isopentenyl})\text{adenine (2-MeiP)} \), in tobacco tissues of plants inoculated with a virulent *R. fascians* strain [18]. They showed expression of two methyl transferases (mt1, mt2) and fasD, all from the linear plasmid of a virulent strain, were sufficient to produce 2-MeiP in transgenic *E. coli* cultures. FasD utilised the dimethylated side chain to produce 2-MeiP, indicating that FasD is a dimethyl transferase rather than an isopentenyl transferase [18]. 2-MeiP is resistant to degradation by CKX and inhibited root growth [18]. Moreover, Vereecke’s lab had earlier reported that mt1 and mt2 mutants of *R. fascians* were non-pathogenic [19].

We recently showed that both mt1 and mt2, and fasD, are expressed in peas inoculated with a virulent strain but not in tissues inoculated with an avirulent strain or in controls. 1-MeiP and 2-MeiP were detected in pea tissues inoculated with the virulent strain [15].

We also showed that none of the cytokinins implicated in the ‘Trick-with-the-Cytokinin-Mix’ hypothesis correlated with virulence [15]. We suggested that 2-MeiP, a cytokinin produced uniquely by virulent *R. fascians* strains (ours and that used by Radhika et al., 2015) within the generally low level of cytokinins able to be produced by both virulent and avirulent strains, should be the subject of further analysis. As of now, assuming 2-MeiP is responsible for changes in the plant phenotype, it is the simpler explanation for the induction of the morphological abnormalities induced by virulent *R. fascians* and provides for a more readily testable hypothesis compared to the Trick-with-the-Cytokinin-Mix hypothesis. The interaction of 2-MeiP within a high sugar environment should be sufficient to initiate the substantive morphological differences seen between plants inoculated with virulent compared to avirulent strains of *R. fascians*, as depicted in Figure 1.

**Figure 1.** Model illustrating that the morphological abnormalities induced by virulent *Rhodococcus fascians* strains are caused by the production of a novel methylated cytokinin by *R. fascians* in a high sugar environment resulting in the release of apical dominance.
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**References**

1. Thimann, K.V.; Sachs, T. The role of cytokinins in the “fasciation” disease caused by Corynebacterium fascians. *Am. J. Bot.* 1996, 53, 731–739. [CrossRef]

2. Crespi, M.; Messens, E.; Caplan, A.B.; Van Montagu, M.; Desomer, M. Fasciation induction by the phytopathogen Rhodococcus fascians depends upon a linear plasmid encoding a cytokinin synthase gene. *EMBO J.* 1992, 11, 795–804. [CrossRef]

3. Stange, R.R.; Jefares, D.; Young, C.; Scott, D.B.; Eason, J.R.; Jameson, P.E. PCR amplification of the fas-1 gene for the detection of virulent strains of Rhodococcus fascians. *Plant Pathol.* 1996, 45, 407–417. [CrossRef]

4. Francis, I.; De Keyser, A.; De Backer, P.; Simon-Mateo, C.; Kalkus, J.; Pertry, I.; Ardiles-Diaz, W.; De Rycke, R.; Van de Putte, O.M.; El Jaziri, M.; et al. pFID188, the linear virulence plasmid of Rhodococcus fascians D188. *Mol. Plant Microbe Interact.* 2012, 25, 637–647. [CrossRef]

5. Savory, E.A.; Fuller, S.L.; Weisberg, A.J.; Thomas, W.J.; Gordon, M.I.; Creason, A.L.; Belcher, M.S.; Serdani, M.; Wiseman, M.S.; et al. Evolutionary transitions between beneficial and phytopathogenic Rhodococcus challenge disease management. *eLife* 2017, 6, e30925. [CrossRef]

6. Taller, B.J. Distribution, biosynthesis, and function of cytokinins in tRNA. In *Cytokinins: Chemistry, Activity, and Function*; Mok, D.W.S., Mok, M.C., Eds.; CRC Press: Boca Raton, FL, USA, 1994; pp. 101–112.

7. Miyawaki, K.; Tarkowski, P.; Matsumoto-Kitano, M.; Kato, T.; Sato, S.; Tarkowska, D.; Tabata, S.; Sandberg, G.; Kakimoto, T. Roles of Arabidopsis ATP/ADP isopentenyltransferases and tRNA isopentenyltransferases in cytokinin biosynthesis. *Proc. Natl. Acad. Sci. USA* 2006, 103, 16598–16603. [CrossRef] [PubMed]

8. Nishii, K.; Wright, F.; Chen, Y.Y.; Moller, M. Tangled history of a multigene family: The evolution of ISOPENTENYLTRANSFERASE genes. *PLoS ONE* 2018. [CrossRef] [PubMed]

9. Armstrong, D.J.; Scarbrough, E.; Skoog, F. Cytokinins in Corynebacterium fascians cultures: Isolation and identification of 6-(4-Hydroxy-3-methyl-cis-2-butenylamino)-2-methylthiopurine. *Plant Physiol.* 1976, 58, 749–752. [CrossRef] [PubMed]

10. Pertry, I.; Václavíková, K.; Gemrotová, M.; Spichal, L.; Galuszka, P.; Depuydt, S.; Temmerman, W.; Stes, E.; De Keyser, A.; Riefler, M.; et al. *Rhodococcus fascians* impacts plant development through the dynamic fas-mediated production of a cytokinin mix. *Mol. Plant Microbe Interact.* 2010, 23, 1164–1174. [CrossRef] [PubMed]

11. Tarkowski, P.; Václavíková, K.; Novák, O.; Pertry, I.; Hanuš, J.; Whenham, R.; Vereecke, D.; Šebela, M.; Strnad, M. Analysis of 2-methylthio-derivatives of isoprenoid cytokinins by liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* 2010, 680, 86–91. [CrossRef] [PubMed]

12. Gális, I.; Bilyeu, K.; Wood, G.; Jameson, P.E. Rhodococcus fascians: Shoot proliferation without elevated cytokinins? *Plant Growth Regul.* 2005, 46, 109–115. [CrossRef]

13. Dhandapani, P.; Song, J.; Novak, O.; Jameson, P.E. Infection by Rhodococcus fascians maintains cotyledons as a sink tissue for the pathogen. *Ann. Bot.* 2017, 119, 841–852. [CrossRef]

14. Dhandapani, P.; Song, J.; Novak, O.; Jameson, P.E. Both epiphytic and endophytic strains of Rhodococcus fascians influence transporter gene expression and cytokinins in infected Pisum sativum L. seedlings. *Plant Growth Regul.* 2018, 85, 231–242. [CrossRef]

15. Jameson, P.E.; Dhandapani, P.; Song, J.; Zatiloukal, M.; Strnad, M.; Remus-Emsermann, M.N.P.; Schlechter, R.O.; Novák, O. The cytokinin complex associated with Rhodococcus fascians: Which compounds are critical for virulence? *Front. Plant Sci.* 2019, 10, 674. [CrossRef] [PubMed]

16. Pertry, I.; Václavíková, K.; Depuydt, S.; Galuszka, P.; Spichal, L.; Temmerman, W.; Stes, E.; Schmulling, T.; Kakimoto, T.; Van Montagu, M.C.E.; et al. Identification of Rhodococcus fascians cytokinins and their modus operandi to reshape the plant. *Proc. Natl. Acad. Sci. USA* 2009, 10, 929–934. [CrossRef]

17. Barbier, F.F.; Lunn, J.E.; Beveridge, C.A. Ready, steady, go! A sugar hit starts the race to shoot branching. *Curr. Opin. Plant Biol.* 2015, 25, 39–45. [CrossRef]
18. Radhika, V.; Ueda, N.; Tsuboi, Y.; Kojima, M.; Kikuchi, J.; Kudo, K.; Sakakibara, H. Methylated cytokinins from the phytopathogen *Rhodococcus fascians* mimic plant hormone activity. *Plant Physiol.* **2015**, *169*, 1118–1126. [CrossRef]

19. Pertry, I. How the *Fas* Locus Contributes to *Rhodococcus Fascians* Cytokinin Production: An In-Depth Molecular and Biochemical Analysis. Ph.D. Thesis, Ghent University, Gent, Belgium, 2009. Available online: [http://hdl.handle.net/1854/LU-529624](http://hdl.handle.net/1854/LU-529624) (accessed on 25 April 2019).

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