
Article:
Wilkinson, S.W. orcid.org/0000-0002-4908-8766 and Ton, J. orcid.org/0000-0002-8512-2802 (2020) Methylation moulds microbiomes. Nature Plants, 6 (8). pp. 910-911.

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***Strapline*: Plant epigenetics and rhizosphere biology**

***Title*: Does methylation mould microbiomes?**

***Authors*: Samuel W Wilkinson and Jurriaan Ton**

***Standfirst*:**

Exudates released from plant roots can recruit beneficial micro-organisms which boost plant growth and immunity. In *Arabidopsis thaliana* and tomato, active DNA demethylation regulates the production of *myo*-inositol, a root exudate which recruits a specific plant-growth promoting rhizobacterium (PGPR).

Main Text:

Plant roots are associated with a rich and complex microbiome that plays an important role in plant growth and health. The composition of the root-associated microbiome is shaped by primary and secondary metabolites in root exudates. Specific micro-organisms with potentially beneficial activities are selected and/or recruited. For example, the root exudate L-malic acid recruits *Bacillus subtilis*, a bacterial species which can induce resistance against above ground pathogens[1]. Only recently has it become clear that plants can fine-tune their root-associated microbiome in accordance to their environmental and developmental needs, hence representing an important strategy by which plants adapt to changing environments. However, the identity of the underpinning chemical signals, as well as their regulation in the host plant, remains largely unknown.

In this issue of *Nature Plants*, Vílchez *et al*. describe a landmark discovery that sheds light on an important mechanism controlling plant-rhizobacteria interactions[2]. The team focused their study on the interaction between *Arabidopsis thaliana* and the plant-growth promoting rhizobacterium (PGPR) *Bacillus megaterium* strain YC4, which they had previously isolated from soil associated with the roots of the salt marsh grass *Spartina anglica*[3]. Emerging evidence from the literature suggests that DNA (de)methylation plays an important role in plant-microbe interactions. Vílchez and colleagues therefore examined the growth responses to YC4 bacteria in the Arabidopsis mutants *ros1dml2dml3 (rdd)* and *ros1,* which accumulate increased DNA methylation at/around transposable elements (TEs) due to reduced DNA demethylase activity. Both of these hyper-methylated mutants failed to show YC4-induced growth promotion and were impaired in YC4 colonisation. Subsequent comparison of the root exudation profiles revealed that the *rdd* mutant is strongly affected in exudation of *myo*-inositol, a carbocyclic sugar implicated in cellular signal transduction[4]. Experiments utilizing root coatings mimicking the natural slow-release of *myo*-inositol, as well as experiments with the *myo*-inositol biosynthesis mutant *vtc4-4*, confirmed that *myo*-inositol acts as a critical

semiochemical in the Arabidopsis-YC4 interaction. This notion was further supported by a transcriptome analysis of *myo*-inositol-treated YC4 bacteria. Genes involved in biofilm formation and chemotaxis, both of which are important for bacterial root colonization, were induced in YC4 in response to *myo*-inositol.

To examine the epigenetic basis of *myo*-inositol-dependent recruitment of YC4 bacteria, Vílchez *et al*. compared the transcriptomes (RNA-seq) and methylomes (BS-seq) of wild-type and *rdd* mutant plants. This analysis revealed a set of *myo*-inositol homeostasis genes showing increased expression in both *rdd* and *ros1*. Since ROS1 antagonizes DNA methylation established by the RNA-directed DNA methylation (RdDM) pathway[5], the authors subsequently confirmed that the introduction of an RdDM mutation (*nrpd1*) in the *ros1* mutant background restored the expression of *myo*-inositol homeostasis genes, YC4 root colonisation and YC4-dependent growth promotion. Hence, DNA (de)methylation is a critical regulatory factor in the *myo*-inositol-dependent interaction between Arabidopsis and YC4. Notably, this epigenetic regulation appears to be conserved as the authors finished their study by providing evidence for a similar phenomenon in tomato (*Solanum lycopersicum*).

The discovery by Vílchez *et al*. is significant as it links different disciplines in plant biology. The study not only uncovers a novel plant semiochemical controlling plant-PGPR interactions, but it also shows that this interaction is under epigenetic control by DNA methylation (Fig. 1). As such, the study opens new avenues for follow-up research. For instance, considering that ROS1/RdDM-dependent DNA methylation at TEs can decline under conditions of stress[6], it will be interesting to examine whether exposure to pests, pathogens or abiotic stress influences *myo*-inositol exudation. There is increasing evidence for the so-called 'cry-for-help hypothesis', wherein stressed plants alter their root exudate chemistry in order to select and/or recruit beneficial micro-organisms that help to resist stress[7]. It is plausible that a loss of methylation induced by stress may result in further heightened *myo*-inositol exudation rates and increased recruitment of beneficial bacteria, which can induce systemic resistance against existing and future challenges. However, in order to validate this hypothesis, it first needs to be established whether YC4 bacteria indeed induce stress resistance in Arabidopsis. While there is evidence that YC4 bacteria can enhance drought stress tolerance[3], it is not certain whether they induce resistance against pests and diseases. In fact, the transcriptome analysis conducted by Vílchez *et al.*, suggested that YC4 colonisation suppresses aspects of the plants immune system. Furthermore, since YC4 was isolated from salt marshes, it is unlikely that it naturally associates with Arabidopsis roots. Moreover, most experiments were carried out with tyndallized soil, in which the majority of the microbial population has been eradicated. Thus, there is a need for future studies that explore the wider impact of *myo*-inositol on the composition, activity and host effects of the root-associated microbiome. Specifically, there is an opportunity for these studies to focus on the non-sterile soils which naturally co-occur with the plant of interest.

Regions of increased DNA methylation were found in proximity to multiple *myo*-inositol homeostasis genes showing altered expression in the *rdd* mutant. This suggests that DNA methylation may regulate the expression of these genes in *cis*. However, when compared to the wild-type, the *rdd* mutant has differentially methylated regions across the

genome[8]. Moreover, not all *ros1*/*rdd*-repressed *myo*-inositol homeostasis genes had local regions of increased DNA methylation, indicating that *trans*-regulation has a part to play. Indeed, there is increasing evidence that DNA methylation at pericentromeric TEs can *trans*-regulate expression of defence-related genes across the Arabidopsis genome[9]. Long-range chromatin interactions, as well as small and long non-coding RNAs, have been suggested as possible mechanisms by which TE methylation regulates distal genes[10]. The use of chromosome conformation capture techniques and non-coding RNA sequencing could help to uncover the mechanisms by which TE DNA methylation regulates *myo*-inositol genes. Above all, it is clear that the study of Vílchez and colleagues will stimulate further research into the mechanisms by which plant epigenetic mechanisms control root exudation and microbiome assembly.

1. Rudrappa, T. Czymmek, K.J., Paré, P.W. & Bais, H.P. Root-secreted Malic acid recruits beneficial soil bacteria. *Plant Physiology* **148**, 1547-1556 (2008).
2. Vílchez, J.I. et al. DNA demethylases are required for *myo*-inositol-mediated mutualism between plants and beneficial rhizobacteria. *Nature Plants* **X**,XXX (2020).
3. Vilchez, J.I. et al. Genome sequence of *Bacillus megaterium* strain YC4-R4, a plant growth-promoting rhizobacterium isolated from a high-salinity environment. *Genome announcements* **6**, e00527-18 (2018)
4. Gillaspy, G.E. The cellular language of *myo*-inositol signaling. *New Phytologist* **192**, 823–839 (2011)
5. Tang, K., Lang, Z., Zhang, H. & Zhu, J-K. The DNA demethylase ROS1 targets genomic regions with distinct chromatin modification. *Nature Plants* **2**, 16169 (2016)
6. Zhang, H., Lang, Z. & Zhu, J-K. Dynamics and function of DNA methylation in plants. *Nature Reviews Molecular Cell Biology* **19**, 489-506 (2018)
7. Rolfe, S.A., Griffiths, J. & Ton, J. Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. *Current Opinion in Microbiology* **49**, 73-82 (2019)
8. Qian, W. et al. A histone acetyltransferase regulates active DNA demethylation in Arabidopsis. *Science* **336**, 1445-1448 (2012)
9. Furci, L. et al. Identification and characterisation of hypomethylated DNA loci controlling quantitative resistance in Arabidopsis. *eLife* **8**, e40655 (2019)
10. Wilkinson, S.W. et al. Surviving in a Hostile World: Plant Strategies to Resist Pests and Diseases. *Annual Review of Phytopathology* **57**, 505–529 (2019)

Author Information

Affiliations: Plant Production and Protection Centre, Department of Animal and Plant Sciences, The University of Sheffield, S10 2TN, UK

Corresponding Author: Jurriaan Ton: j.ton@sheffield.ac.uk

ORCID: Samuel W Wilkinson - 0000-0002-4908-8766; Jurriaan Ton - 0000-0002-8512-2802

Figure:

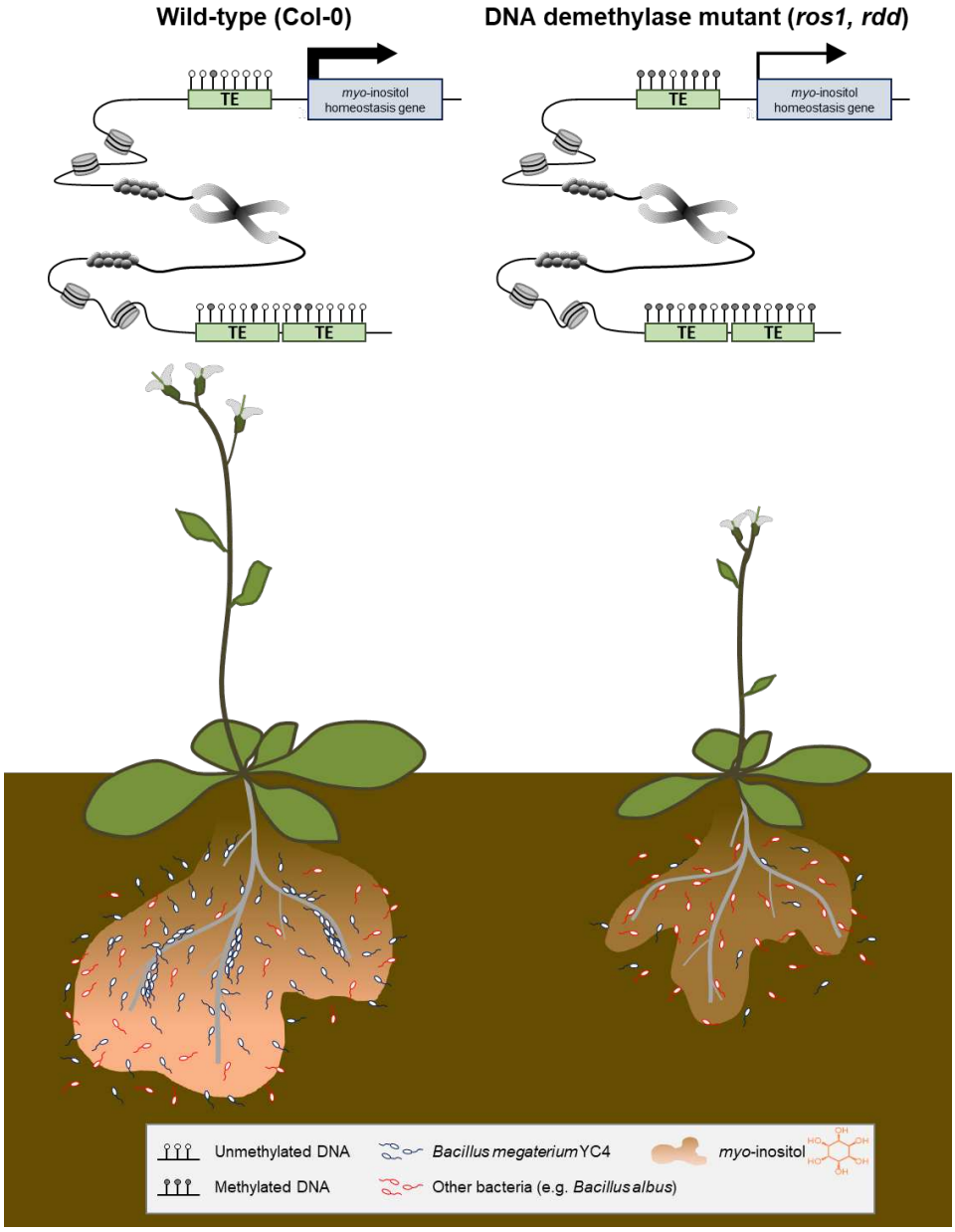

Fig. 1 DNA methylation represses root exudation of *myo*-inositol, which recruits the plant growth promoting rhizobacterium (PGPR) *Bacillus megaterium* YC4. DNA methylation of transposable elements (TEs) is regulated by DNA demethylases, such as ROS1, DML2 and DML3. The study by Vilchez *et al.* demonstrated that increased TE methylation in the hyper-methylated *ros1* and *ros1dml2dml3* (*rdd*) mutants of *Arabidopsis thaliana* affects expression of *myo*-inositol homeostasis genes via yet unknown *cis*- and/or *trans*-acting mechanisms. Consequently, these mutants show reduced root exudation of *myo*-inositol in comparison to wild-type plants (Col-0) and are less effective in recruiting plant growth-promoting *B. megaterium* YC4. Hence, DNA methylation at TEs controls plant-PGPR interactions, indicating that epigenetic mechanisms may emerge as important regulators of the assembly and activity of the root-associated microbiome.

***Author contributions*:** J.T. and S.W wrote the article; J.T. and S.W. designed the figure.

Competing interest statement: the authors declare no competing interests as defined by Nature Research, or other interests that might be perceived to influence the results and/or discussion reported in this paper.