

Metabolomic Characterisation of Discriminatory Metabolites Involved in Halo Blight Disease in Oat Cultivars Caused by *Pseudomonas syringae* pv. *coronafaciens*

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Supplementary material – All relevant material referred to, but not included in the main text are included in the following supplementary section. All experimental raw data is available upon request from Prof I.A. Dubery at the Department of Biochemistry, University of Johannesburg.

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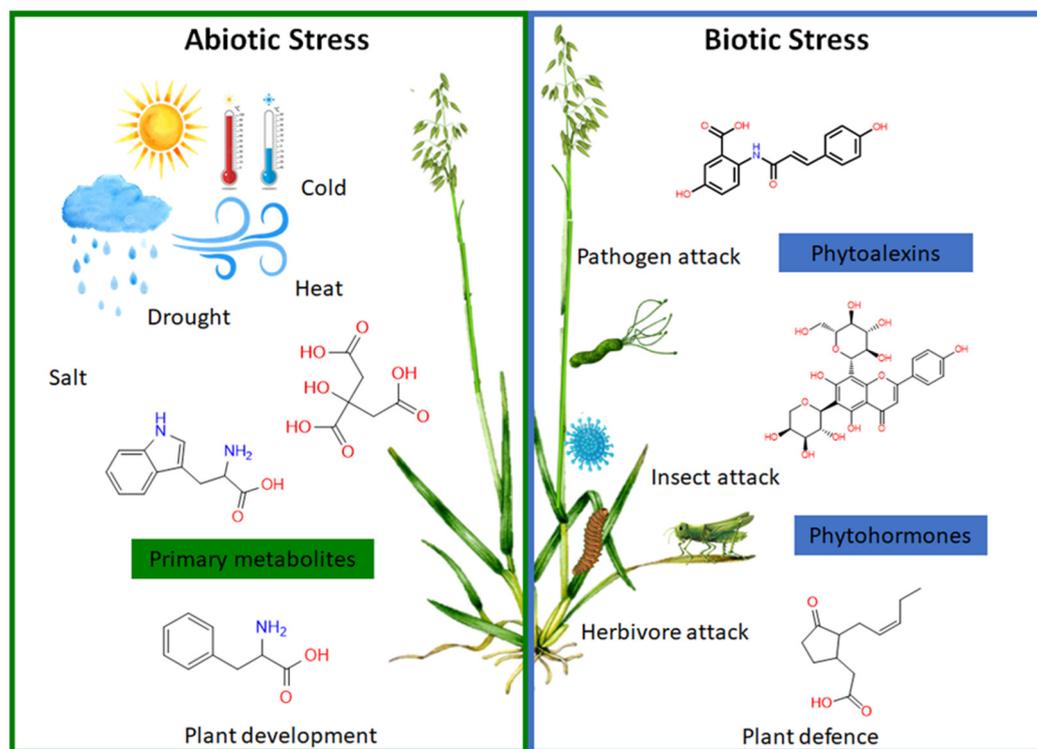


Figure S1. Key metabolic components that underlie plant responses to abiotic and biotic stressors. Plant metabolomics is a powerful tool for studying the metabolic mechanisms that trigger and embody plant stress responses to environmental (left) and/or pathogenic (right) threats.

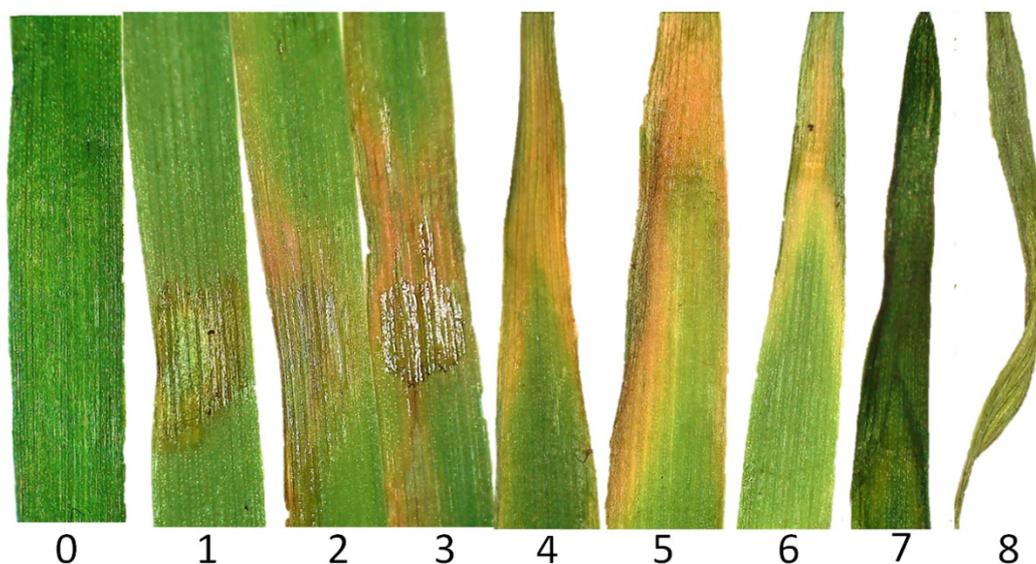


Figure S2. Halo blight disease severity index on *Avena sativa* L. inoculated with *Pseudomonas syringae* pv. *coronafaciens*. Example images represent visual observation of disease severity using a 0 - 8 scale, where 0 = no disease symptoms, 1-3 = slight disease symptoms, 3-6 = moderate disease symptoms and 6-8 = severe symptoms of yellowing and wilting.

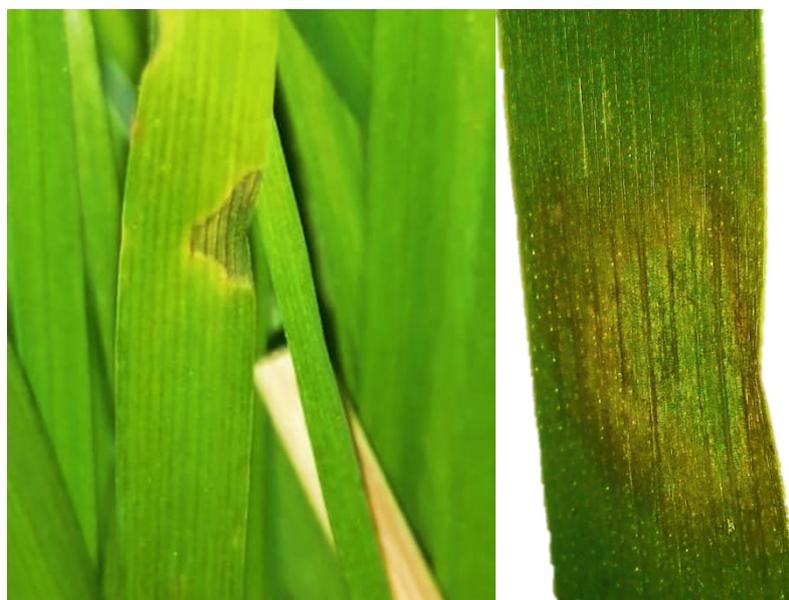


Figure S3. Typical symptom development of halo blight on 'Dunnart' leaves in response to *Ps-c* infection. Characteristic yellow halo formation around infected leaf tissue, typically associated with halo blight disease.

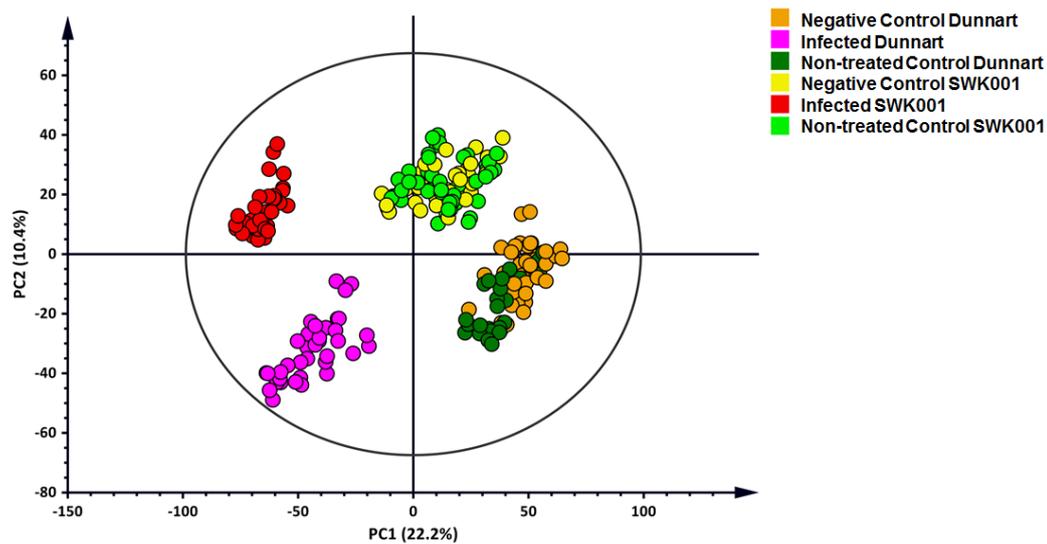


Figure S4. Principal component analysis (PCA) of the ESI(-) data illustrating the two infected oat cultivars and the respective control groups. The PCA scores plot of all the samples showing the infected and control groups (non-treated vehicle- and negative control) for ‘Dunnart’ and ‘SWK001’. The non-treated and negative controls clustered together for both cultivars, meaning that there were no significant differences among the controls, i.e. the underlying metabolic profiles are similar compared to the infected groups.

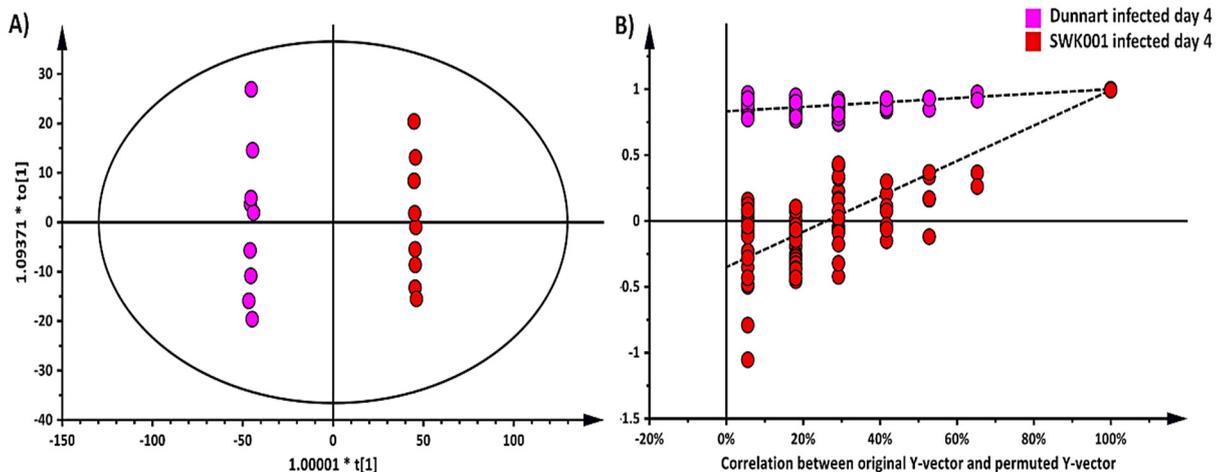


Figure S5. An orthogonal projection to latent structures discriminant analysis (OPLS-DA) model of the two infected cultivars, ‘Dunnart’ and ‘SWK001’. **(A)** OPLS-DA scores plot illustrating the relationship between the two cultivars at 4 d.p.i. based on their leaf-extracted metabolic profiles obtained in ESI (-) mode ($R^2=0.999$, $Q^2=0.996$, CV-ANOVA p -value= 1.06365×10^{-14}). **(B)** Permutation test plot ($n = 100$) for the OPLS-DA model **(A)** was used to validate the predictive capability.

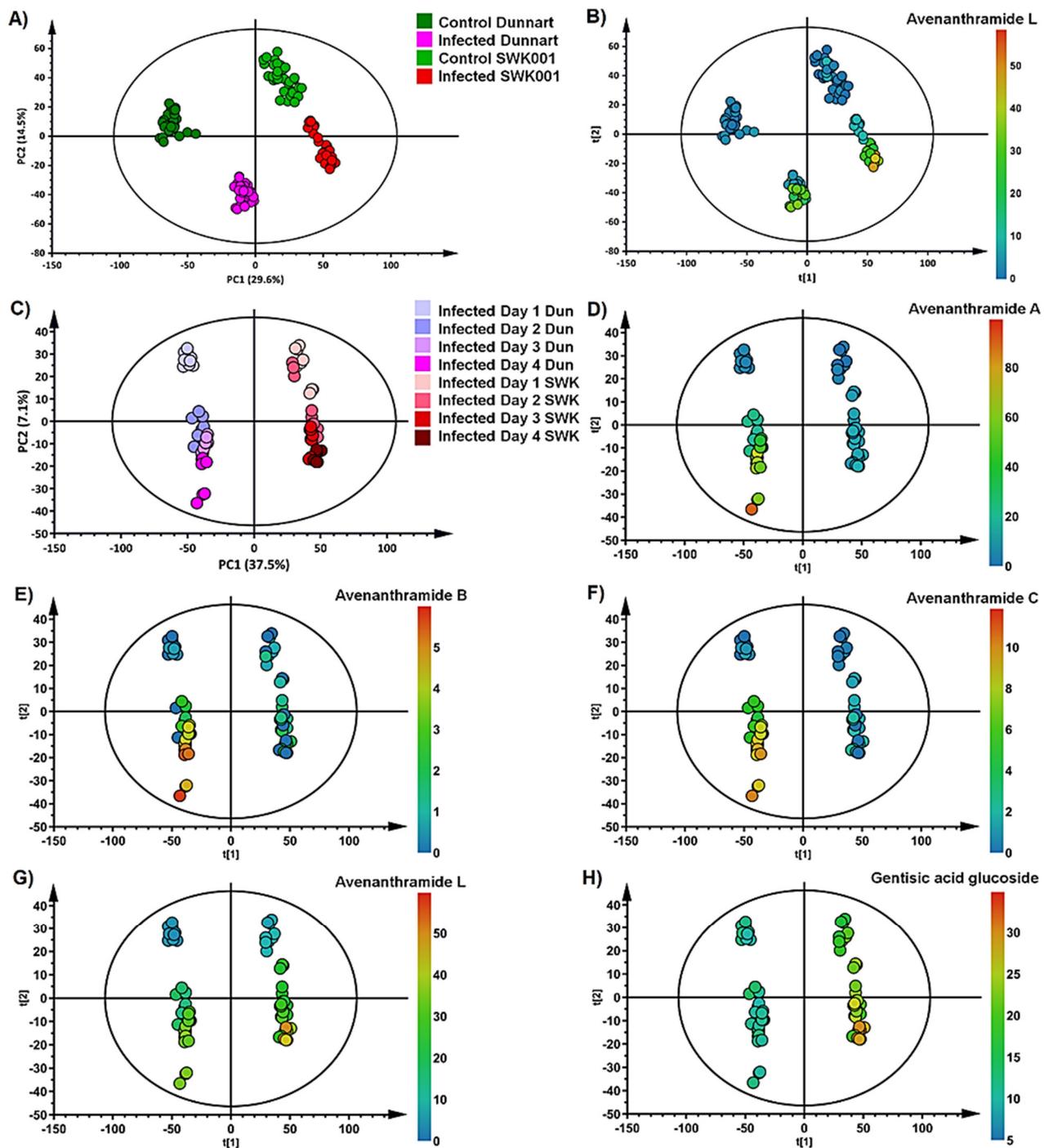


Figure S6. Colour coded PCA score plots showing the presence and increasing abundance of discriminatory ions in the respective treated cultivars (ESI- mode), 'Dunnart' & 'SWK001', (red- high abundance, blue- low abundance). **(A)** PCA scores plot of both cultivars showing control and infected groups, **(B)** Avenanthramide L (absent in control groups and present in infected groups), **(C)** PCA scores plot showing the infected groups for both cultivars from 1 d.p.i. to 4 d.p.i. **(D)** Avenanthramide A, **(E)** Avenanthramide B, **(F)** Avenanthramide C, **(G)** Avenanthramide L and **(H)** Gentisic acid glucoside.

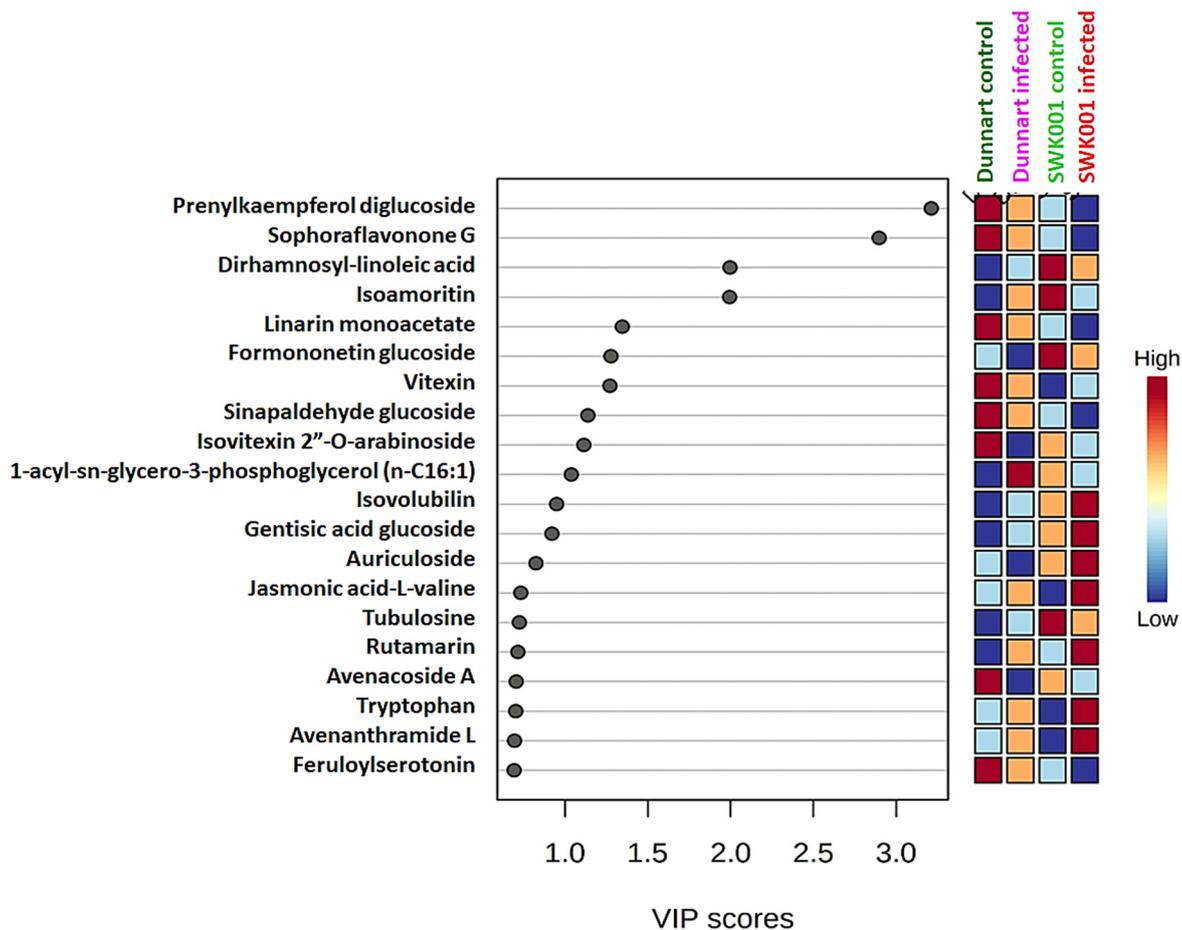
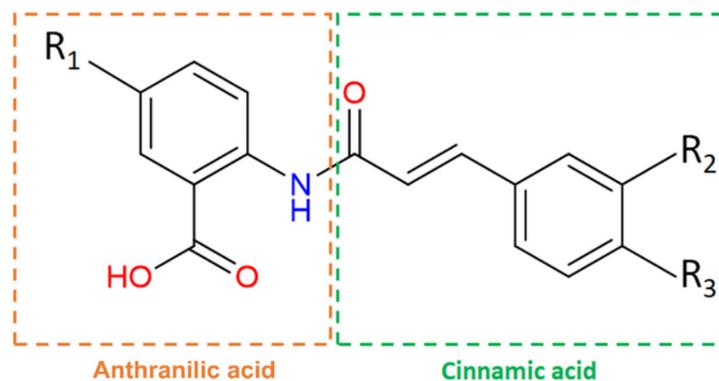


Figure S7. Variable importance in projection (VIP) scores generated using MetaboAnalyst software. Indicated are the top 15 discriminating ions in oat leaves from the respective cultivars 'Dunnart' & 'SWK001' for their treated and control groups. Metabolites with a VIP score ≥ 0.5 were considered to be significant in the discrimination between the cultivars.



Compound	R ₁	R ₂	R ₃	Anthranilic acid	Cinnamic acid
Avenanthramide A	OH	H	OH	5-hydroxyanthranilic acid	<i>p</i> -coumaric acid
Avenanthramide B	OH	OCH ₃	OH	5-hydroxyanthranilic acid	ferrulic acid
Avenanthramide C	OH	OH	OH	5-hydroxyanthranilic acid	caffeic acid

Figure S8. Avenanthramide structures illustrating the core structure and various functional groups unique to each compound.

***Pseudomonas syringae* pv. *coronafaciens* 16S rRNA sequence (Inqaba)**

GTCACCTAATGCGTTAGCTGCGCCACTAAGAGCTCAAGGCTCCCAACGGCTAGTTGACAT
 CGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCT
 CAGTGT CAGTATCAGTCCAGGTGGTCGCCCTTCGCCACTGGTGTTCCTTCTATATCTACG
 CATTTACCGCTACACAGGAAATTCACCACCCTCTACCATACTCTAGCTTGCCAGTTTT
 GGATGCAGTCCCAGGTTGAGCCCGGGGATTTACATYCAACTTAACAAACCACCTACGC
 GCGCTTTACGCCAGTAATCCGATTAACGCTTGACCCTCTGTATTACCGCGGCTGCTG
 GCACAGAGTTAGCCGGTGCTTATTCTGTCCGTAACGTCAAACAATCACGTATTAGGTAA
 CTGCCCTTCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTACACACGCGGC
 ATGGCTGGATCAGGCTTTCGCCATTGTCCAATATCCCCACTGCTGCCTCCCGTAGGAG
 TCTGGACCGTGTCTCAGTTCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGT
 CGCCTTGGTGAGCCATTACCTACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCG
 CAAGGCCCGAAGGTCCCCTGCTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTC
 CGAGCGTTATCCCCACTACCAGGCAGATTCTAGGCATTACTACCCGTCCGCCGCTCG
 CCACCAGGTACAAGTACCCGTGCTGCCGCTCGACTTGCATGTGTAGGCTGCCGCCAGC
 GTTCAATCTGAGCCATGATCAAACWCTGAAG

Figure S9. 16S rRNA sequence of *Pseudomonas syringae* pv. *coronafaciens*. The sequence exhibits 99.19 % identity with that of *P. coronafaciens* X-1, causing halo blight disease in oats [19].