

1 Supplementary Data

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3 Horizontal Gene Transfer of Pectinases from Bacteria Preceded the Diversification of Stick  
4 and Leaf Insects

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25 Supplementary Data Legends

26

27 **Figure S1: Western blots confirming the successful expression of phasmid pectinases**  
28 **into Sf9 cells.** Marker (MW) used was PageRuler™ Plus Prestained Protein Ladder.

29

30 **Figure S2: TLC plates for individual enzyme activity on citrus pectin.** 10 $\mu$ l of desalted  
31 enzyme, water (negative control, -), or pectinase from *Aspergillus niger* (positive control, +)  
32 were incubated for 16 hours in microcentrifuge tubes with 1% w/v substrate in water, then  
33 spotted onto silica gel plates. Gels were stained with 0.2% (w/v) orcinol in 9:1  
34 methanol/sulfuric acid and developed with a heat gun. Bands indicate galacturonic acid tri-,  
35 di-, or mono-mers corresponding to the markers on the left of each row, with monomers at  
36 the top and larger oligomers on the bottom. Numbers represent each enzyme as in Table S2.  
37 AAS=*Aretaon asperrimus*, ga=galacturonic acid #-mers, PSC=*Peruphasma schultei*,  
38 RAR=*Ramulus artemis*, SSI=*Sipyloidea sipylus*.

39

40 **Figure S3: TLC plates for individual enzyme activity on polygalacturonic acid.** Same as  
41 Figure S2.

42

43 **Figure S4: TLC plates for individual enzyme activity on trigalacturonic acid.** Same as  
44 Figure S2.

45

46 **Figure S5: TLC plates for individual enzyme activity on digalacturonic acid.** Same as  
47 Figure S2.

48

49 **Figure S6: TLC plates for individual enzyme activity on xylogalacturonan.** Same as  
50 Figure S2, except bands can indicate galacturonic acid, galactose, or xylose tri-, di-, or mono-  
51 mers corresponding to the markers on the right. ga=galacturonic acid #-mers, gal=galactose,  
52 xy=xylose #-mers

53

54 **Figure S7: Agarose diffusion assays of the activity of individual pectinase enzymes**  
55 **against citrus pectin.** Enzymes were incubated overnight in wells made in gels of 0.1%  
56 substrate in 0.4% agarose (pH 5.0). Plates were stained with ruthenium red and enzymatic  
57 activity was detectable as clearings in the stained gel. We used pectinases from *Aspergillus*  
58 *niger* (Sigma) as positive control (+) and MilliQ water as negative control (-). Multiple wells  
59 with the same ID represent different Sf9 cell batches, as multiple transfection attempts were  
60 performed and tested together. Numbers correspond to the enzyme IDs in Table S1.

61

62 **Figure S8: Agarose diffusion assays of the activity of individual pectinase enzymes**  
63 **against polygalacturonic acid.** Same as Figure S7.

64

65 **Table S1: Pectinase genes from six phasmatodean species and the activities of those**  
66 **from four exemplar species.** If a new gene was identified from the transcriptome, its  
67 original sequence ID is given in brackets. Only if gene isolation was successful, primers are  
68 listed. The amino-acid sequences are from four conserved regions of the *E. carotovora*  
69 pectinase, with the original sequences being NTD, GDD, GHD, and RIK. The reads per  
70 kilobase of transcript per million mapped reads (RPKM) for the *P. schultei* enzymes are  
71 provided, with their rank for most highly expressed midgut transcript<sup>7</sup>. CP=citrus pectin,  
72 DGA=digalacturonic acid, PGA=polygalacturonic acid, TGA=trigalacturonic acid,  
73 XyG=xylogalacturonan.

74

75 **Table S2: Pectinase genes from the non-Phasmatodea used in Figure 4.** Accession

76 Numbers provided.

77

78 **Table S3: Polyneoptera transcriptomes from the 1KITE project.** Taxonomy is according

79 to the Phasmida Species File ([phasmida.speciesfile.org/](http://phasmida.speciesfile.org/)) with amendments from Bradler et

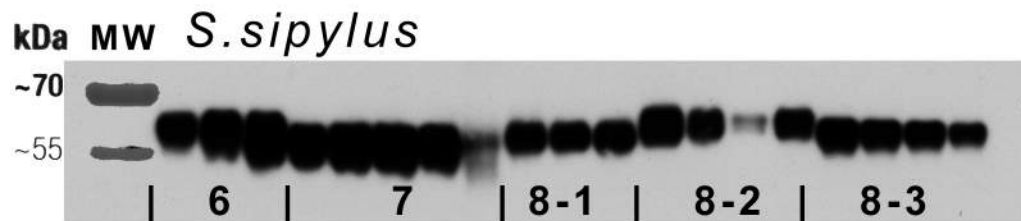
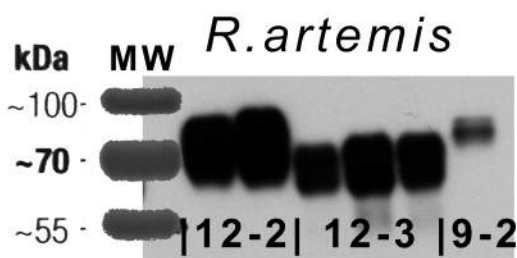
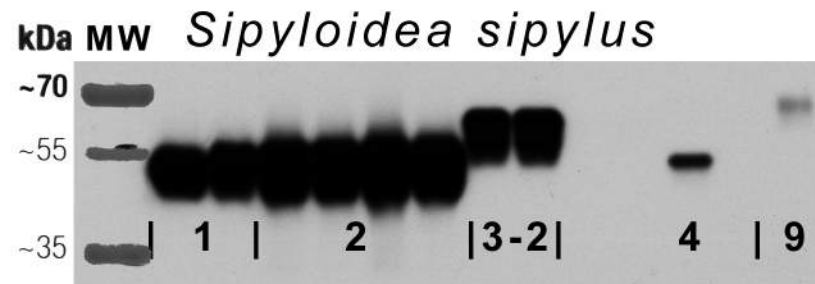
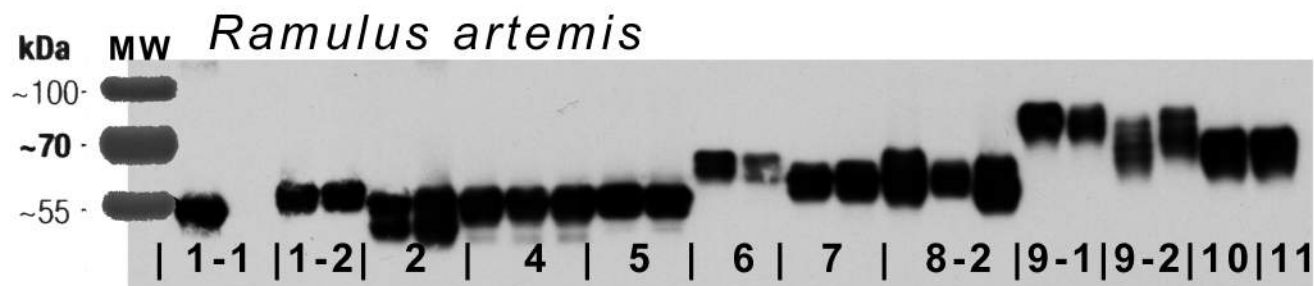
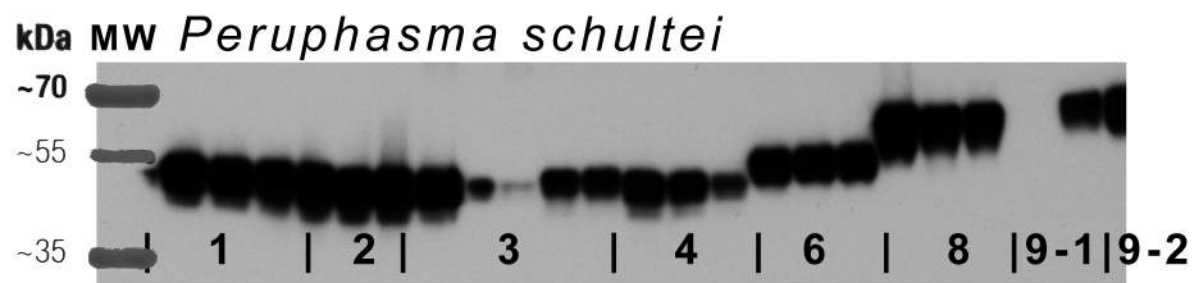
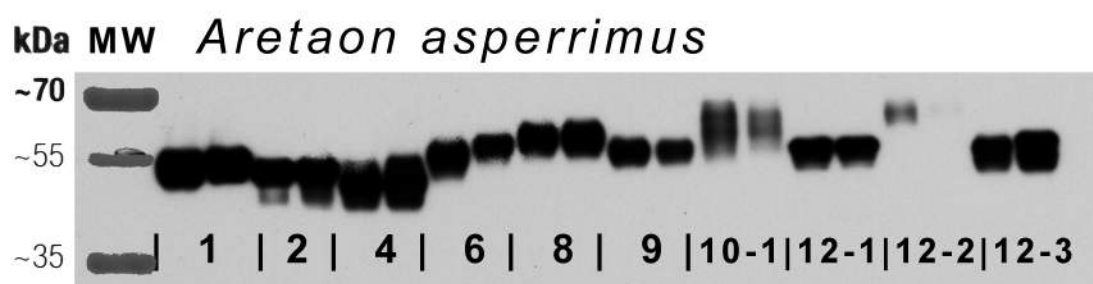
80 al.<sup>12,32</sup>. “Lib-Identified”: 1KITE library identification number. Listed is the tissue used to

81 generate the transcript libraries (whole animal for *Timema* and non-phasmatodean

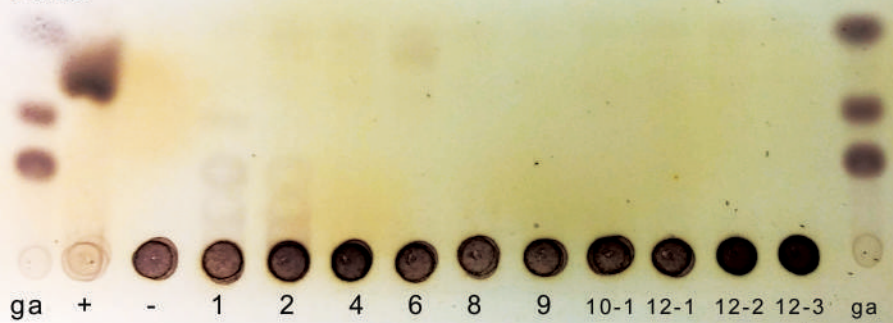
82 Polyneoptera, partial for the remaining species). Numbers of GH28 (pectinase) and GH9

83 (cellulase) transcripts were identified using tBlastn.

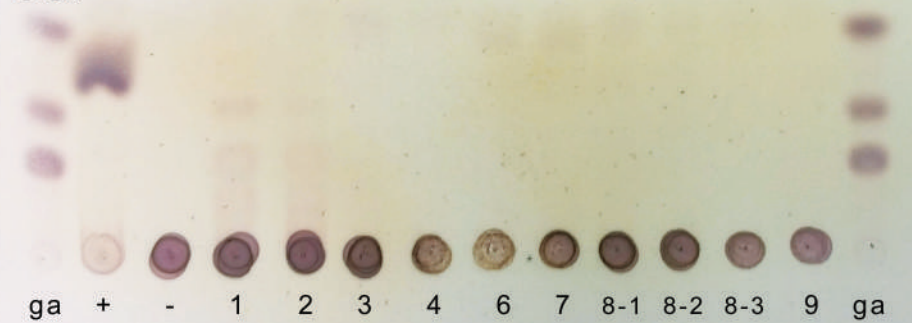
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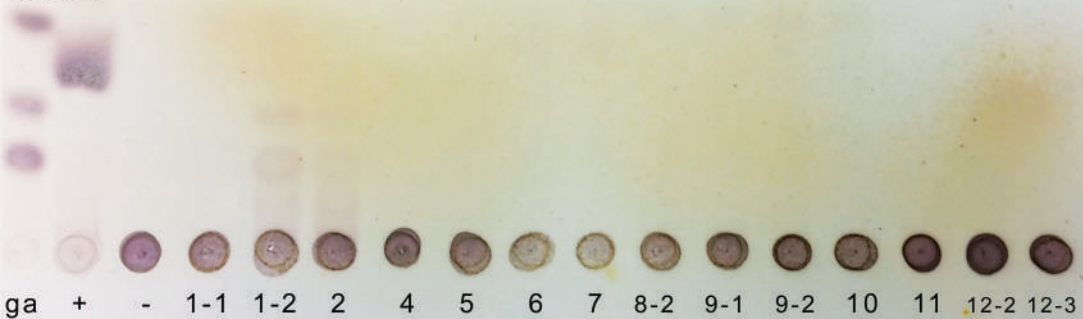
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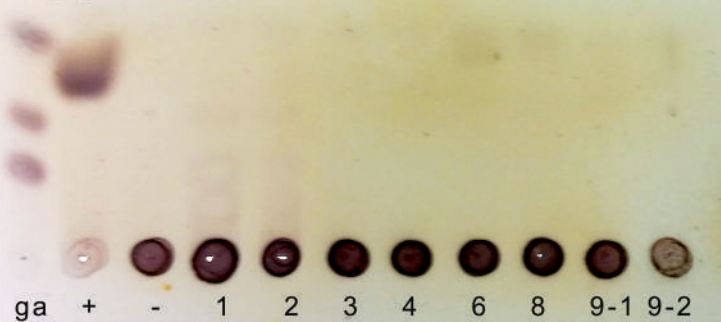
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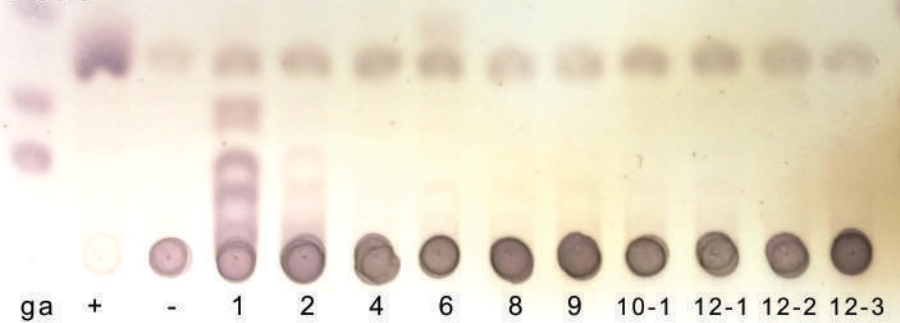
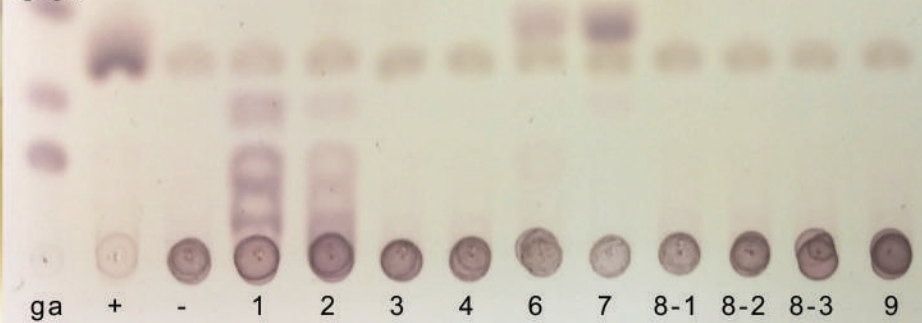
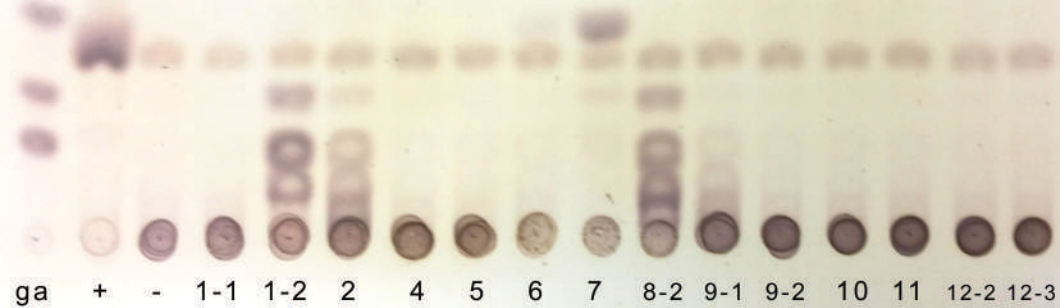
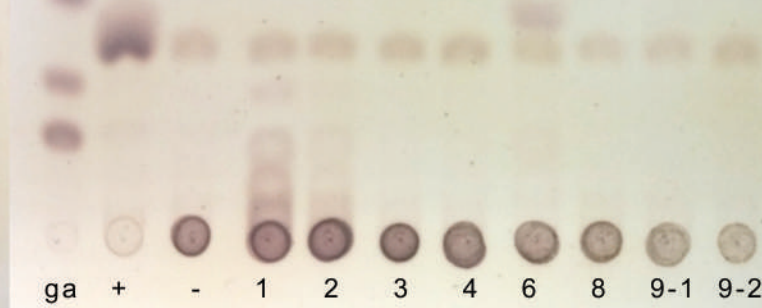


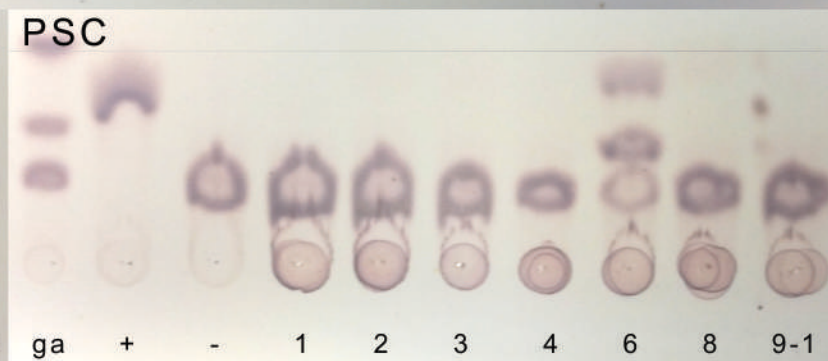
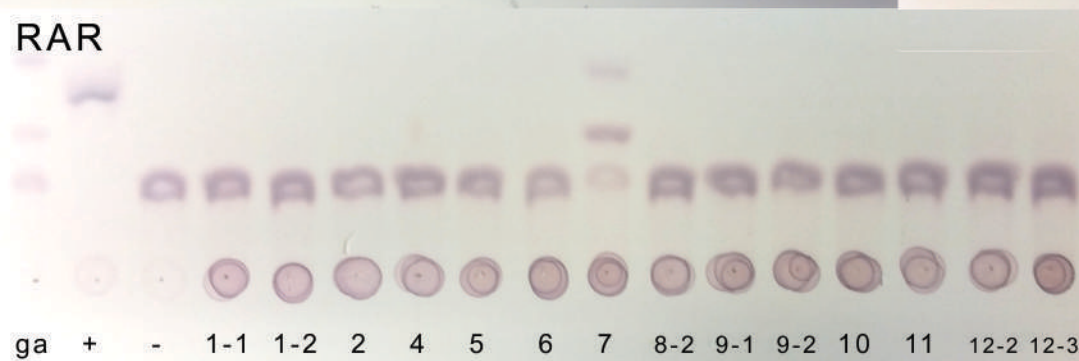
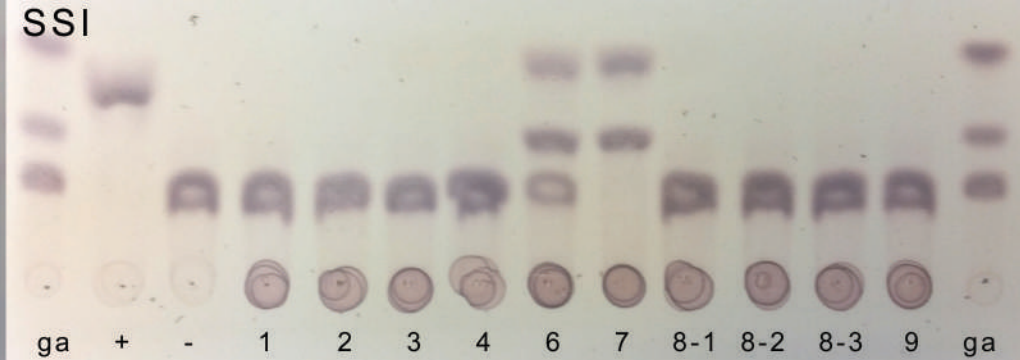
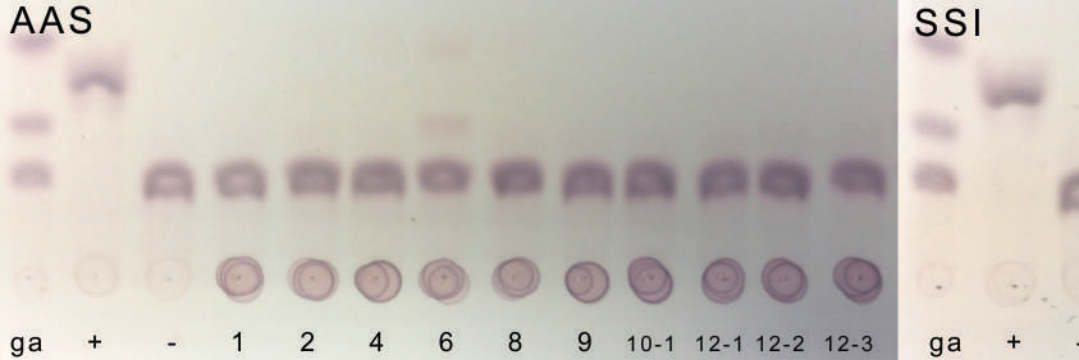
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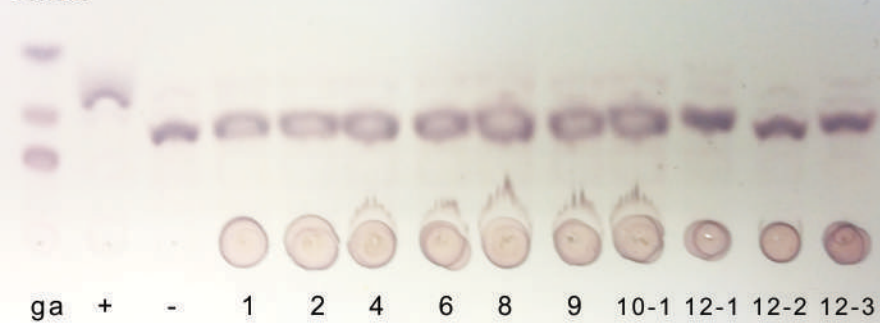
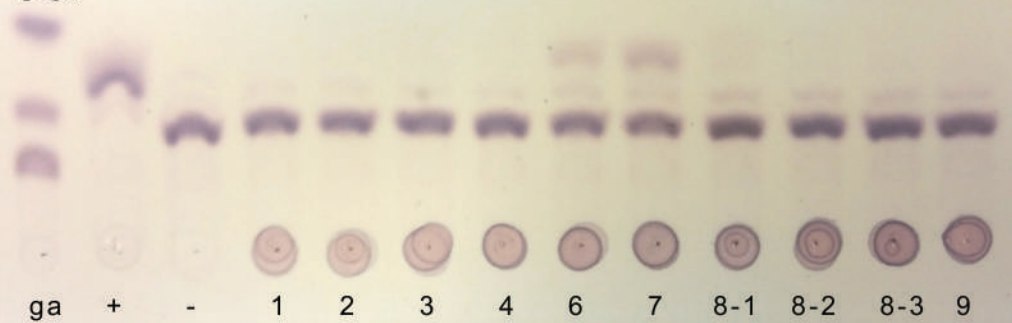
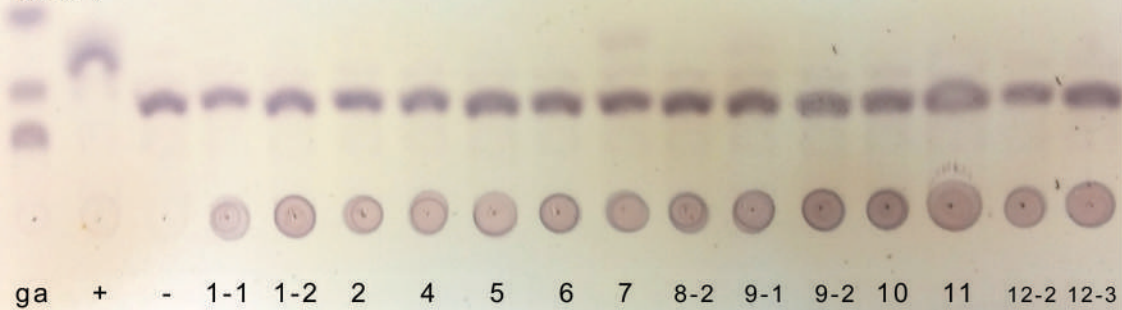
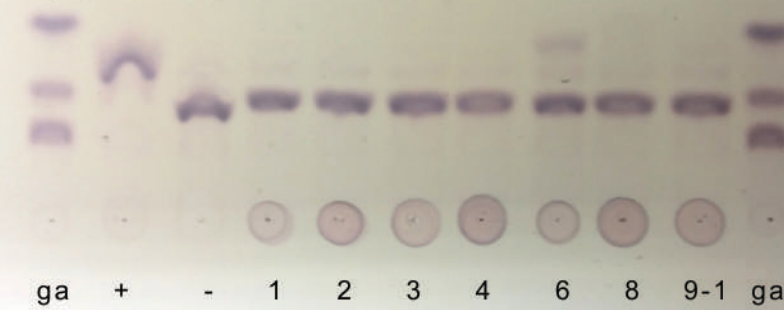
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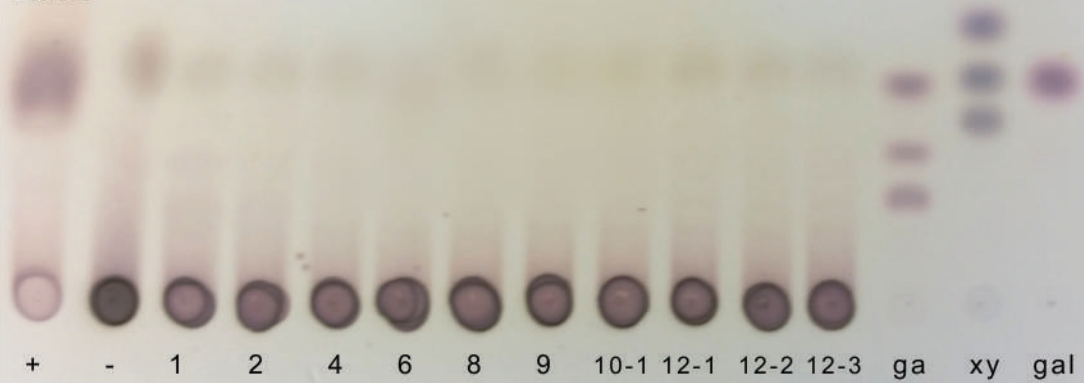
**AAS****SSI****RAR****PSC**



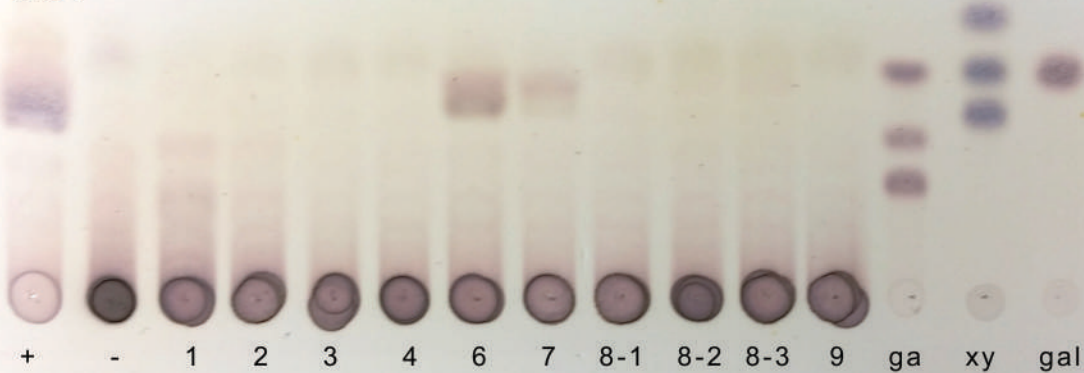


**AAS****SSI****RAR****PSC**

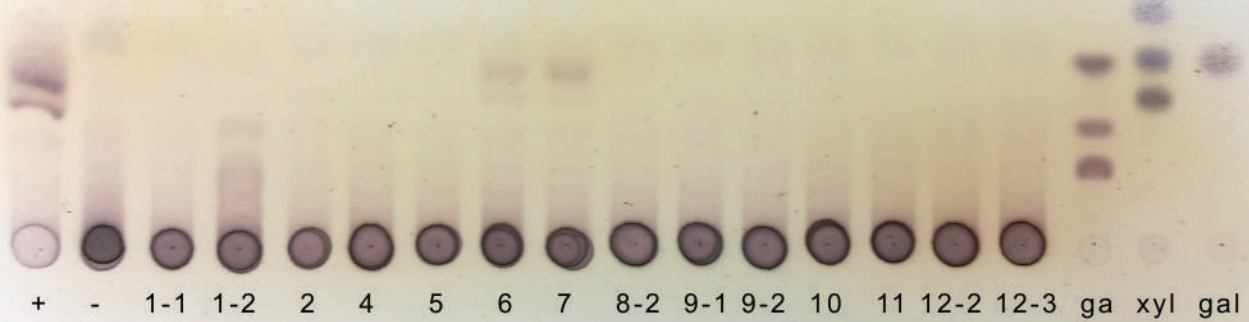
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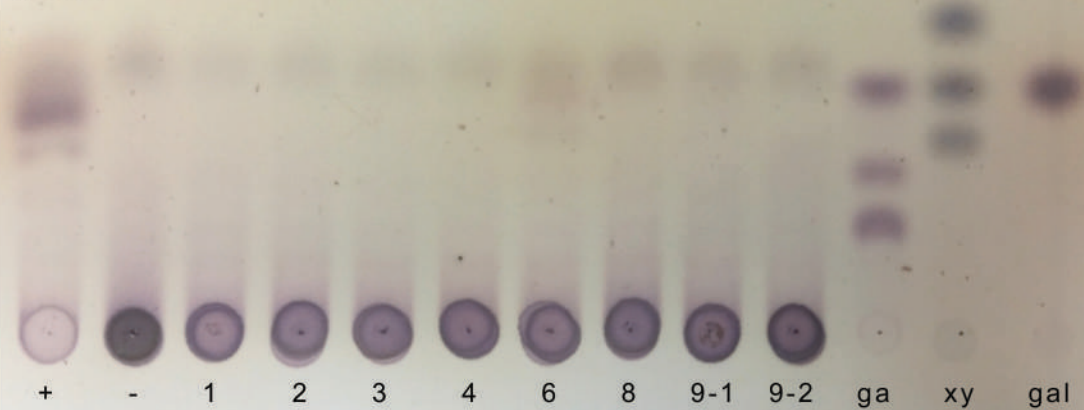
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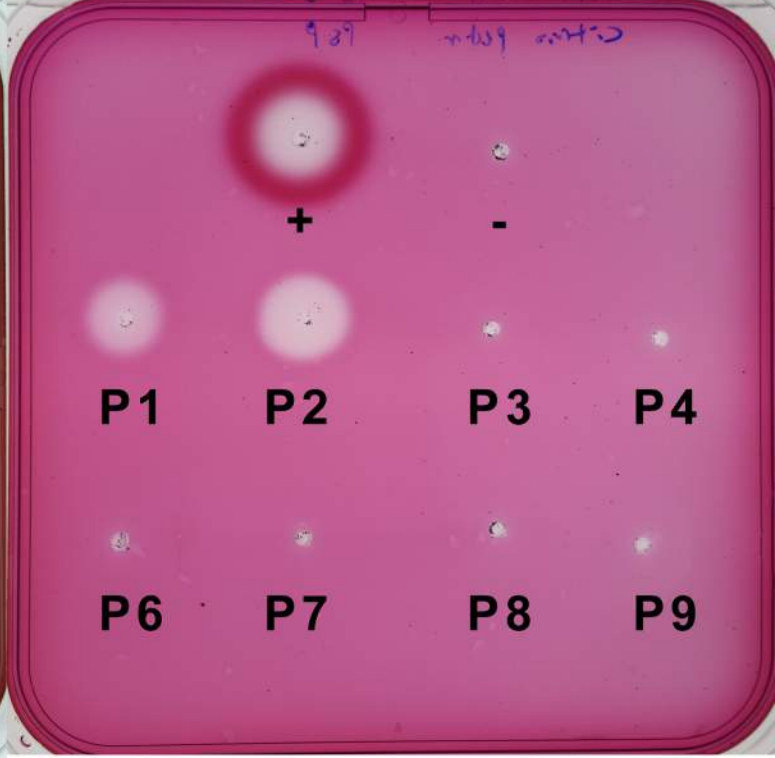
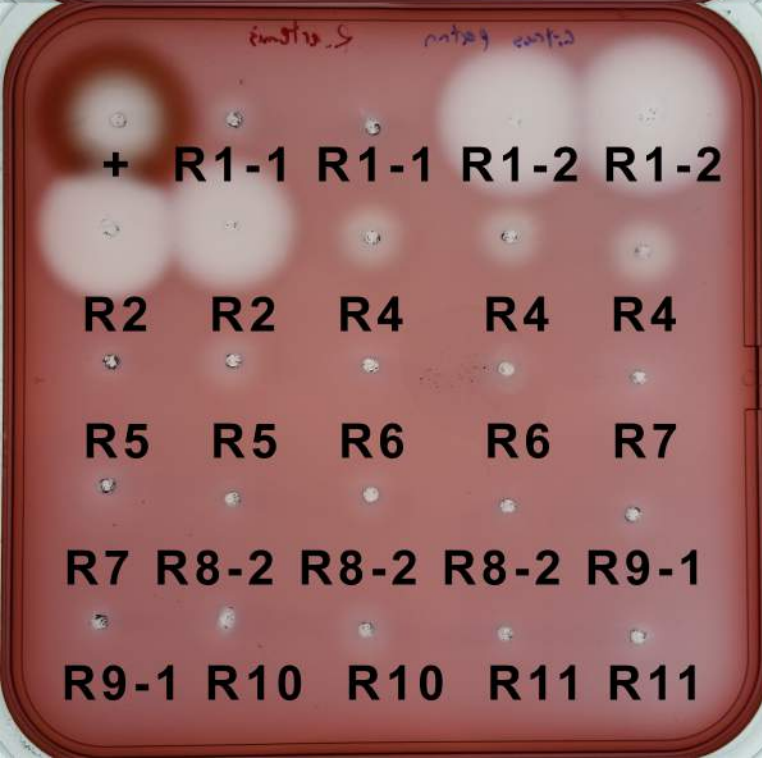
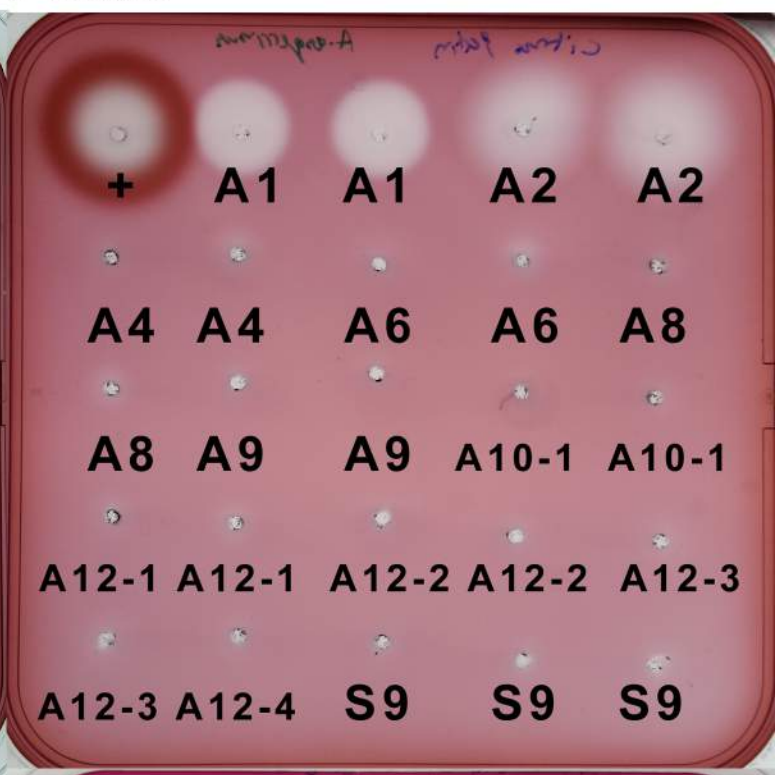
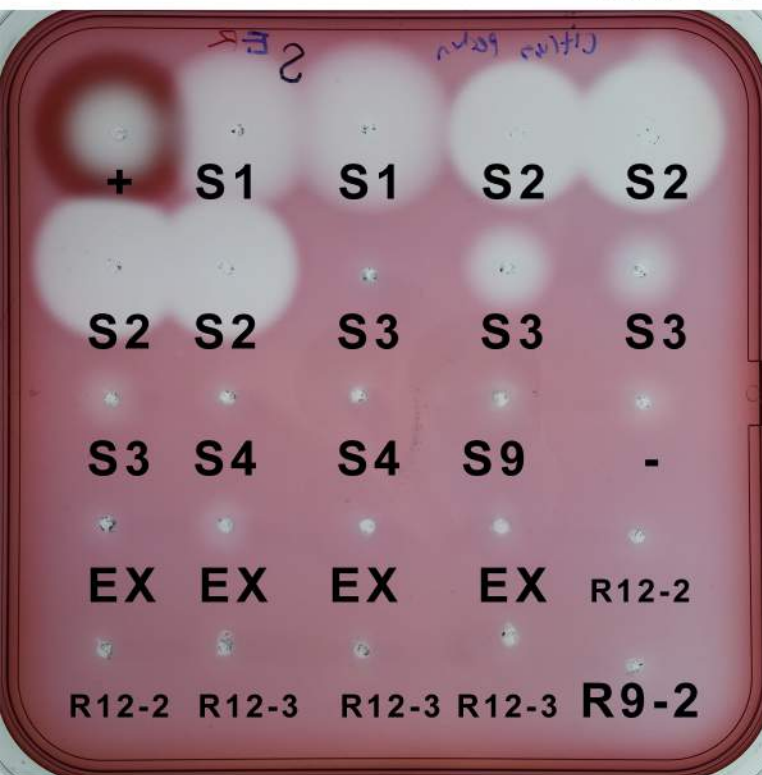
### RAR



### PSC



# Citrus Pectin





# Polygalacturonic Acid

