



# Elucidating the mechanism by which plant derived small molecules affect virulence determinants of *Pectobacterium*

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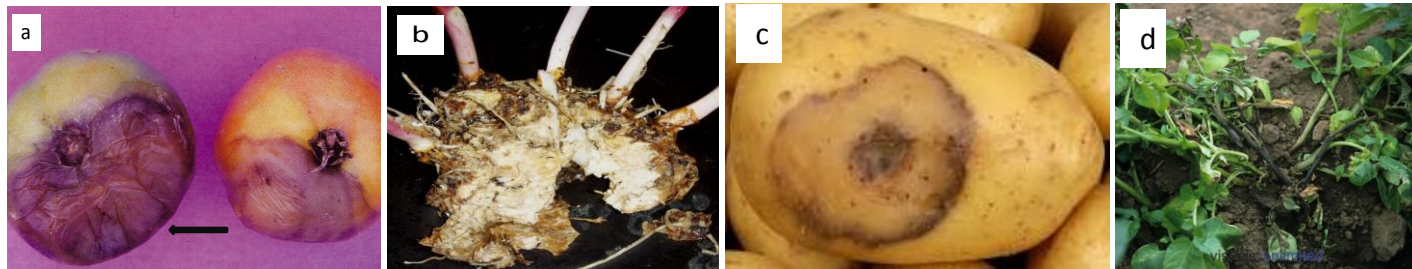
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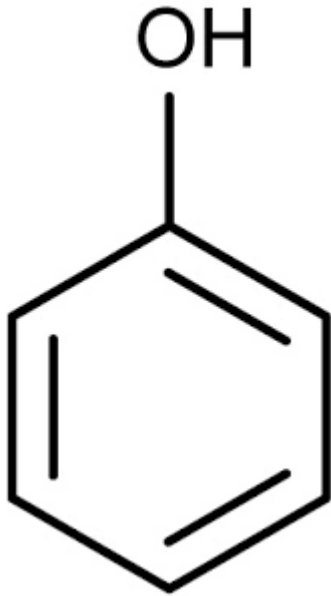
# Current status

*Pectobacterium carotovorum* ~ *Erwinia carotovorum*



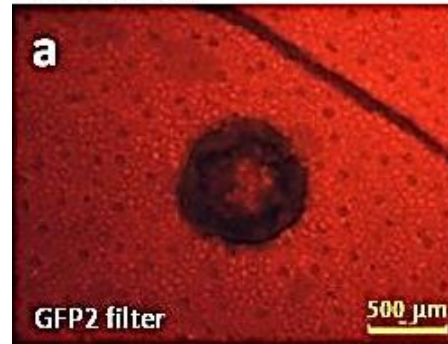
- ✓ **Host range:** 35% angiosperm & 50% monocot orders.
- ✓ No effective **control measures** (chemical, biological).
- ✓ Among the **'top-ten'** list of bacterial plant pathogens.

# Phenolic compounds

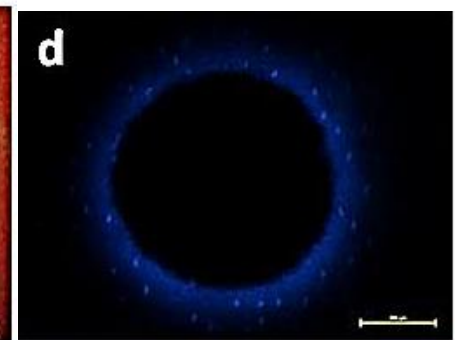
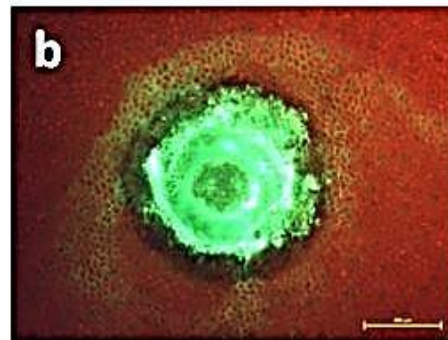


Phenol =  
Aromatic Hydrocarbon + OH

Control

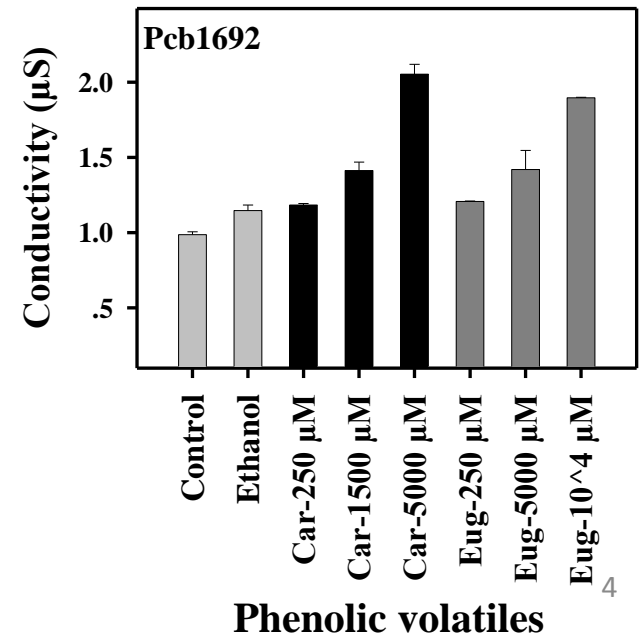
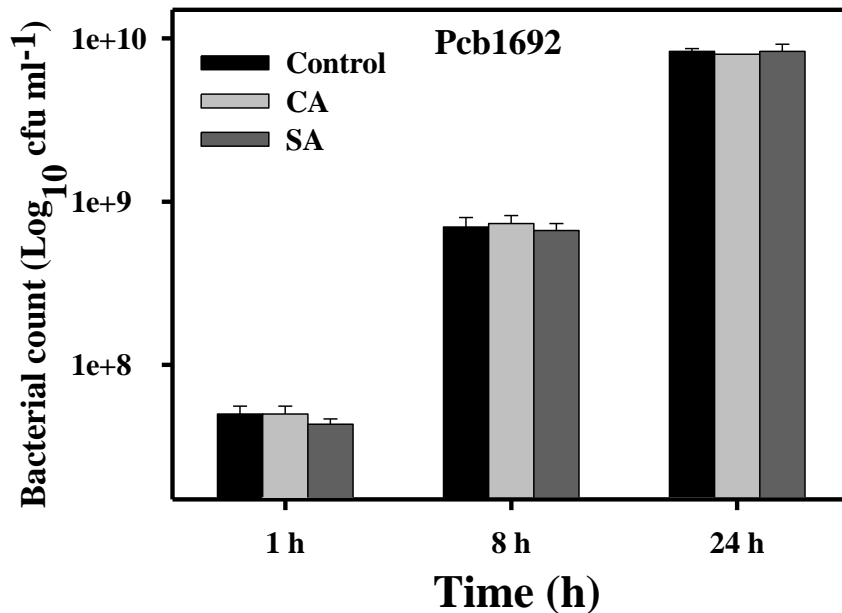
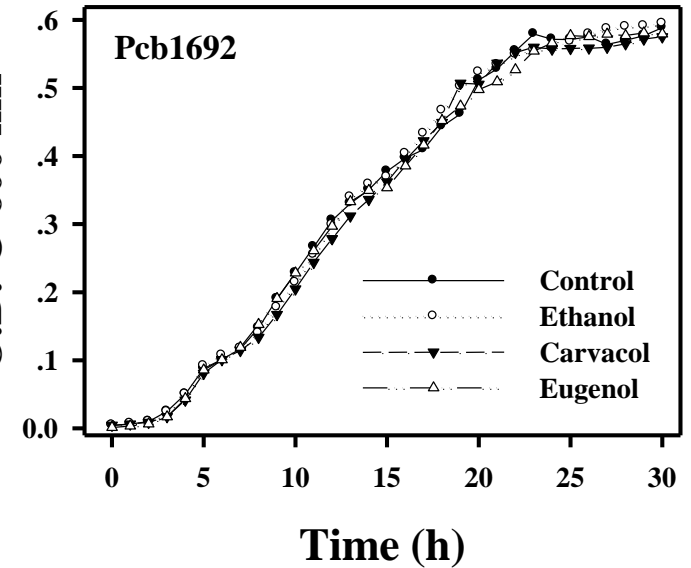
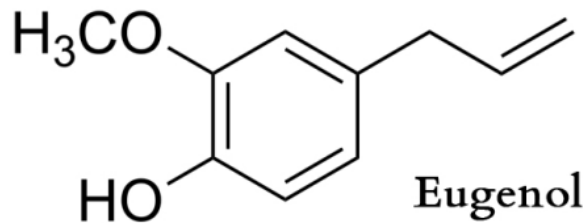
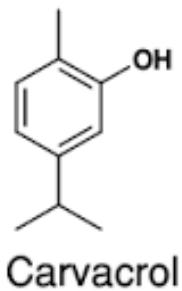
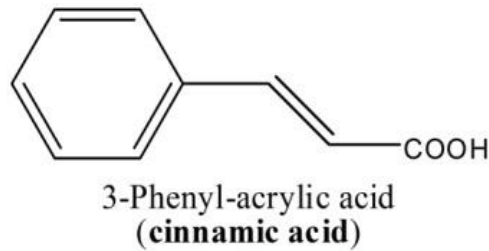
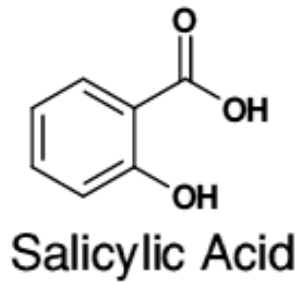


Infected



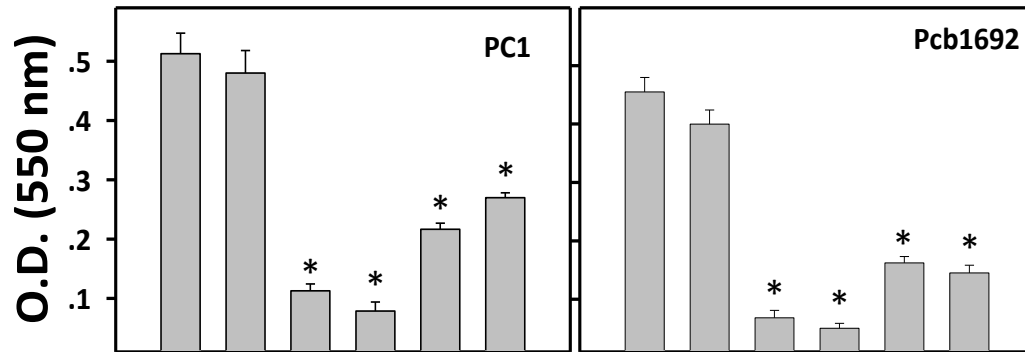
- ✓ Phenolic compounds increase in response to *Pectobacterium* infection.
- ✓ Thousands of phenolic compounds are known and have antimicrobial activity.

# Experiment standardization

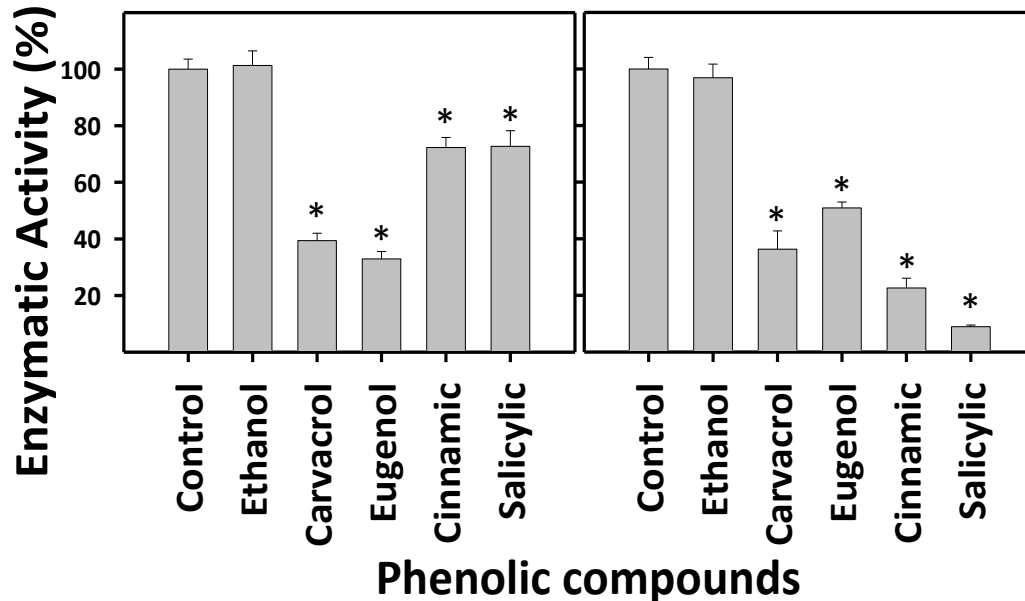


# Effect of phenolic compounds on virulence determinants

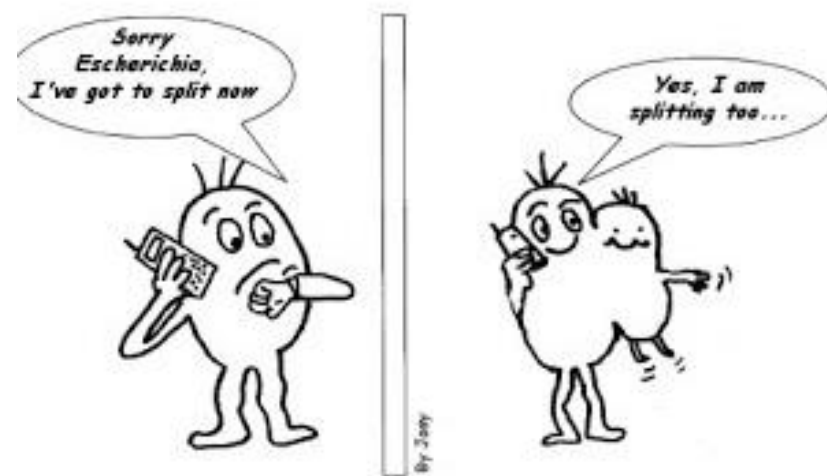
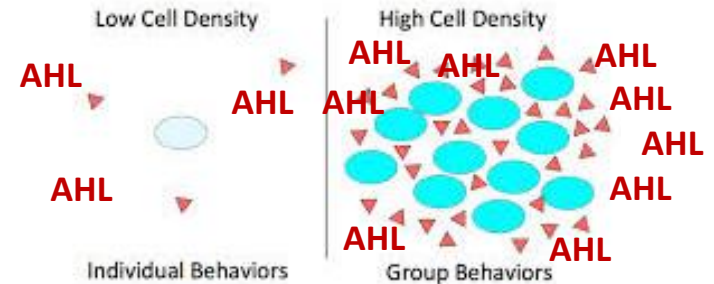
## A. Biofilm Formation



## B. Exoenzyme activity



## Quorum sensing (QS): Cell to cell communication system

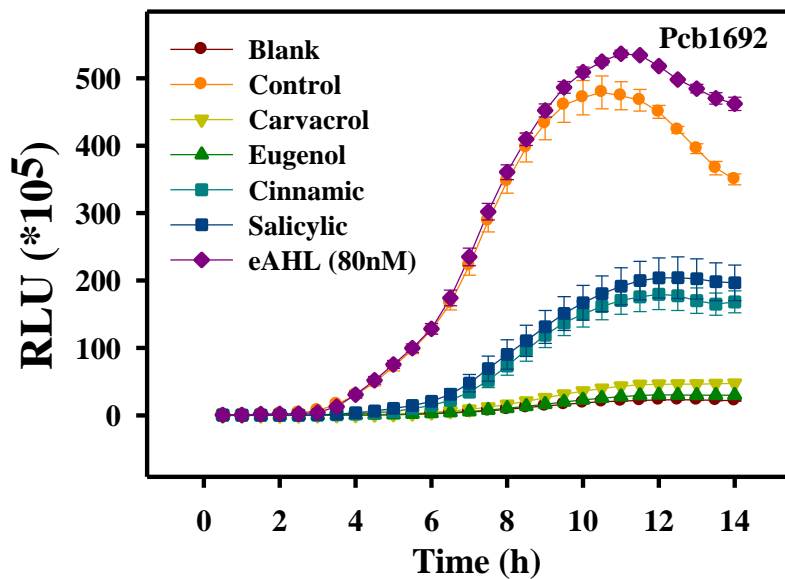
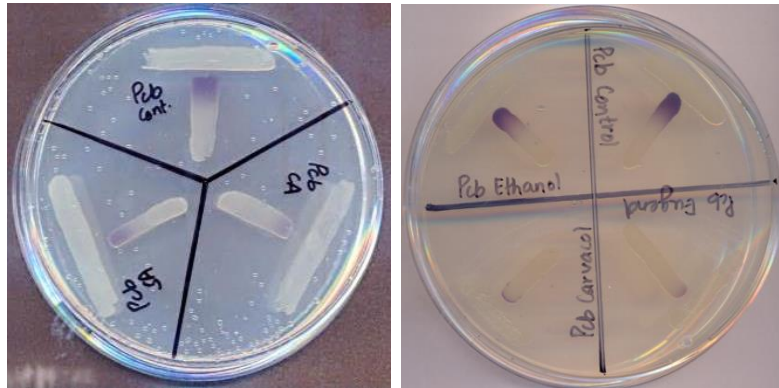


TYPICAL CELL PHONE CONVERSATION AMONG BACTERIA

Joshi et al (2015), *Research in Microbiology*.  
Joshi et al (2016), *Molecular Plant Pathology*.

# Effect of phenolic compounds on QS

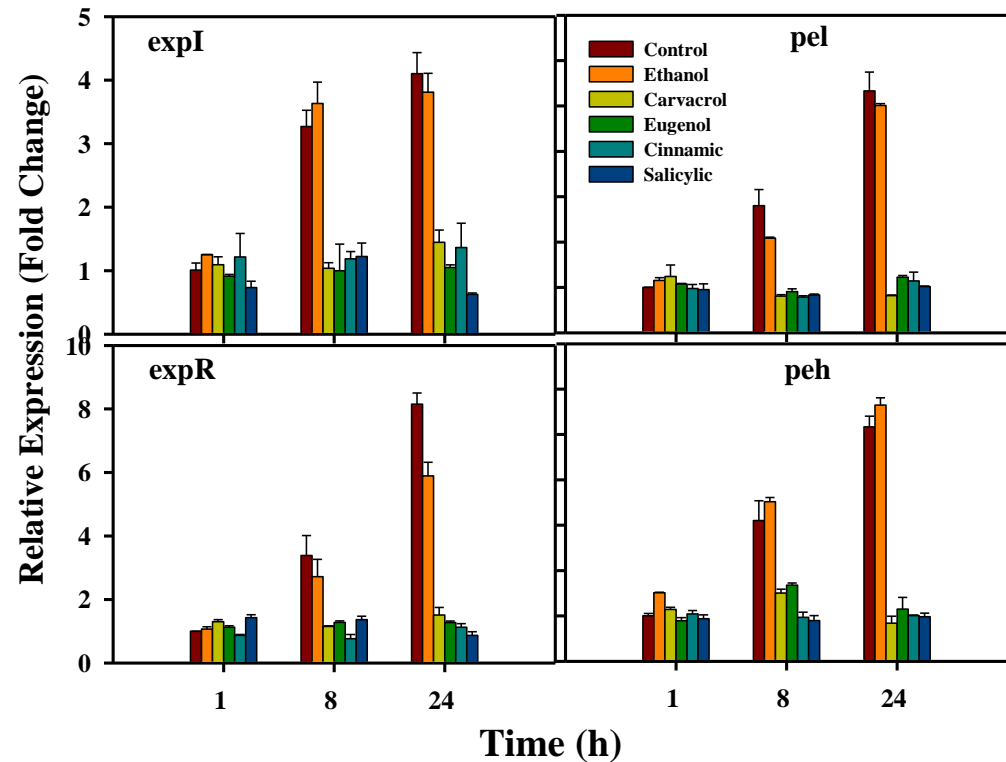
## QS reporter assay for AHL



## Gene Expression

### QS system genes

### Exoenzyme genes



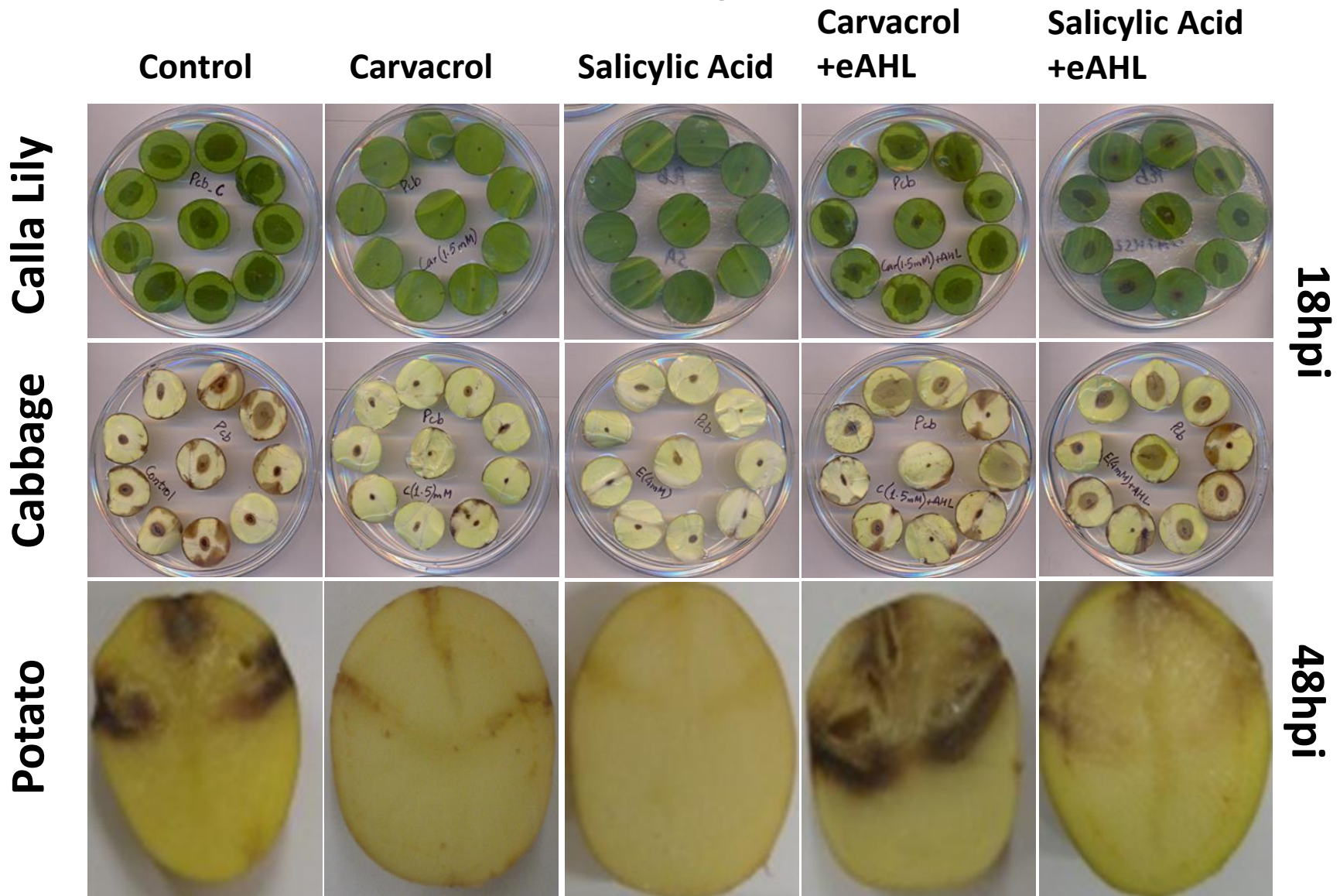
Joshi et al (2016), *Molecular Plant Pathology*

Joshi et al (2016), *Scientific Reports* (In Press)

**QS system is inhibited in the presence of studied phenolic compounds**



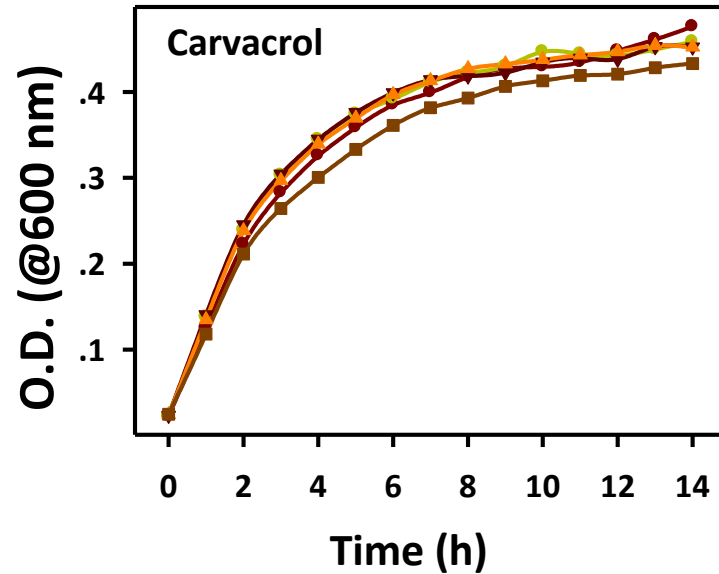
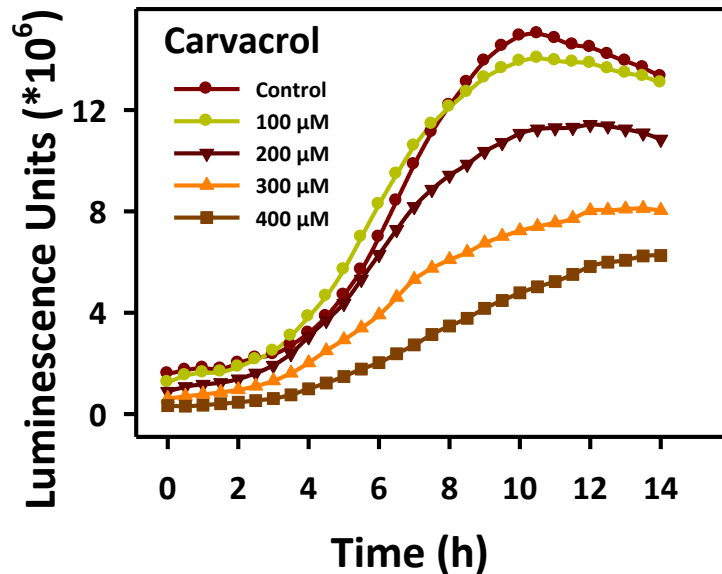
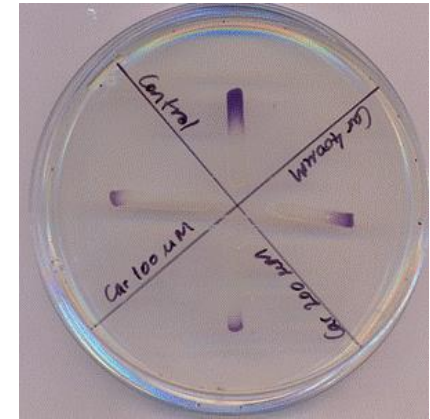
# Host Infection and Compensation



Infection is impaired in the presence of the tested phenolics and reversed on addition of exogenous (e) AHL.

# Effect of phenolic compounds on Expl and LuxR

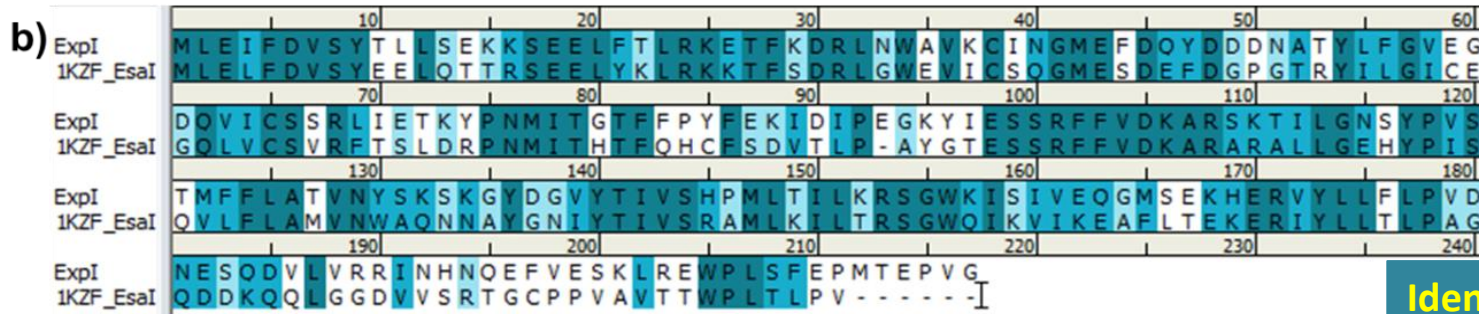
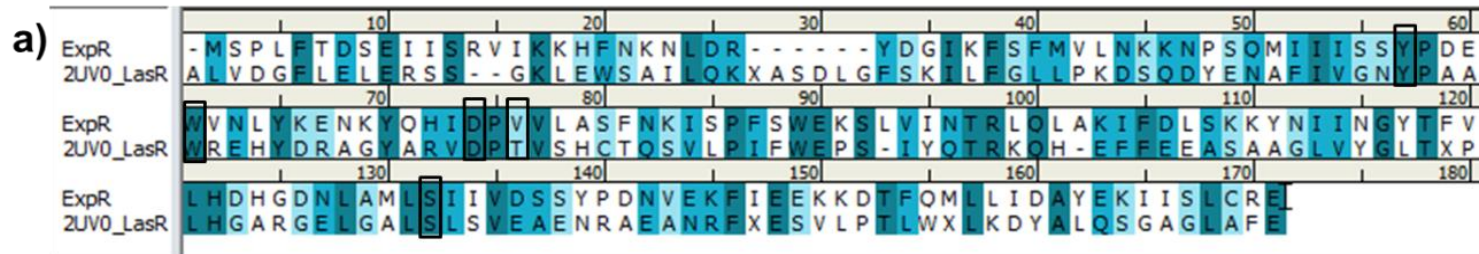
- ✓ Expl cloned to *E. coli* DH5 $\alpha$  (QS negative).
- ✓ Expl cloned to *E. coli* pSB401 (containing LuxR attached to bioluminescence vector).



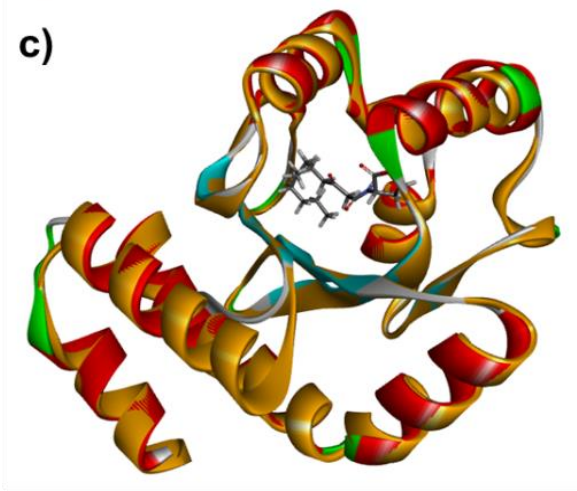
Tested phenolic compounds do not act upstream of Quorum Sensing system genes.



# Computational Modelling of ExpR and ExpI

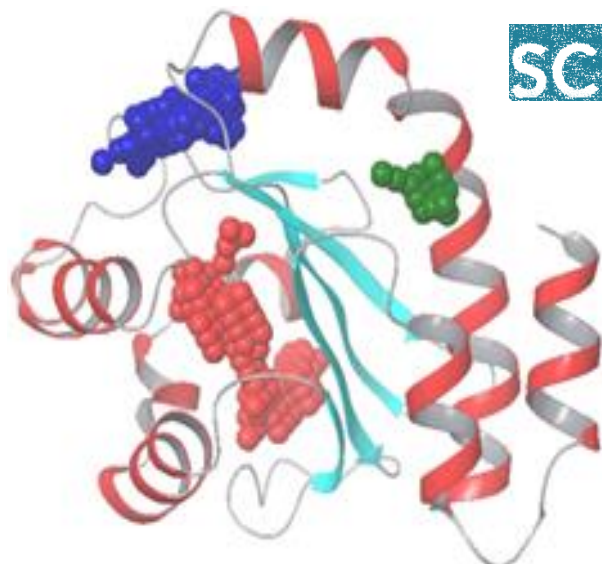


Identical  
Similar  
Non-similar



Homology models were generated using MODELLER program from Discovery Studio.

# SiteMap search for ExpR and Expl



SCHRODINGER



Glide-XP Score in ExpR

Ligand	Site 1	Site2	Site 3
C6-AHL	-7.2	-4.8	-2.6
Carvacrol	-6.7	-3.9	-2.5
Eugenol	-6.2	-3.0	-2.9
Furanone C-30	-4.6	-2.2	2.2

Glide-XP Score in Expl

Ligand	Site 1	Site2	Site 3	Site 4
J8-C8	-4.4	-2.4	-2.2	-1.6
Carvacrol	-6.0	-3.6	-4.1	-3.2
Eugenol	-6.2	-3.5	-2.8	-2.3

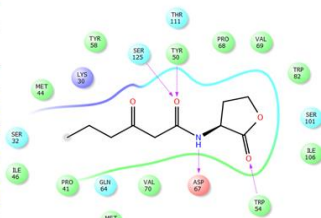
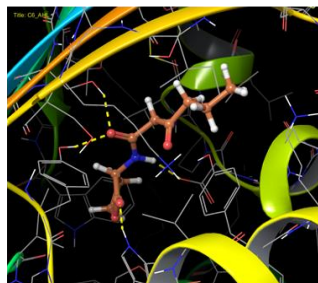
**Active sites for natural ligand in both proteins are preferred by all tested phenolic compounds**

# Molecular Docking

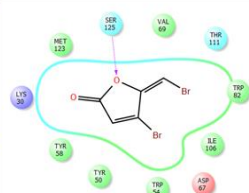


## A. ExpR (QS receptor)

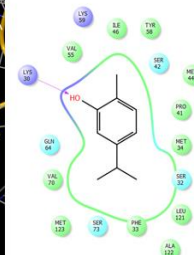
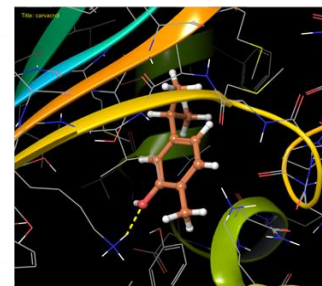
C6-AHL ( $G_{\text{score}} = -7.2$  kcal/mol)



I Furanone ( $G_{\text{score}} = -4.6$  kcal/mol)

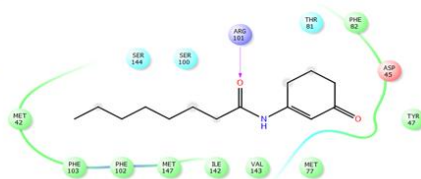
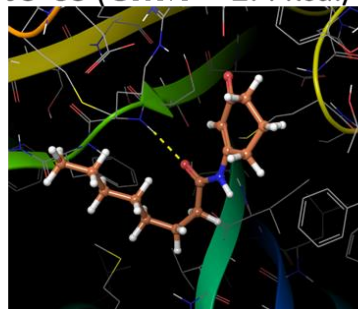


Carvacrol ( $G_{\text{score}} = -6.2$  kcal/mol)

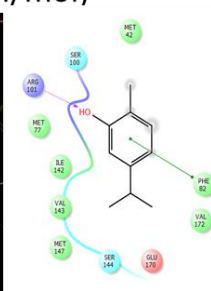
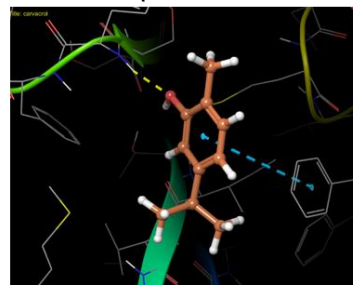


## B. Expl (QS synthase)

J8-C8 ( $G_{\text{score}} = -2.4$  kcal/mol)



Carvacrol ( $G_{\text{score}} = -3.6$  kcal/mol)



Docking poses of carvacrol with ExpR and Expl with Glide Score ( $G_{\text{score}}$ ) values

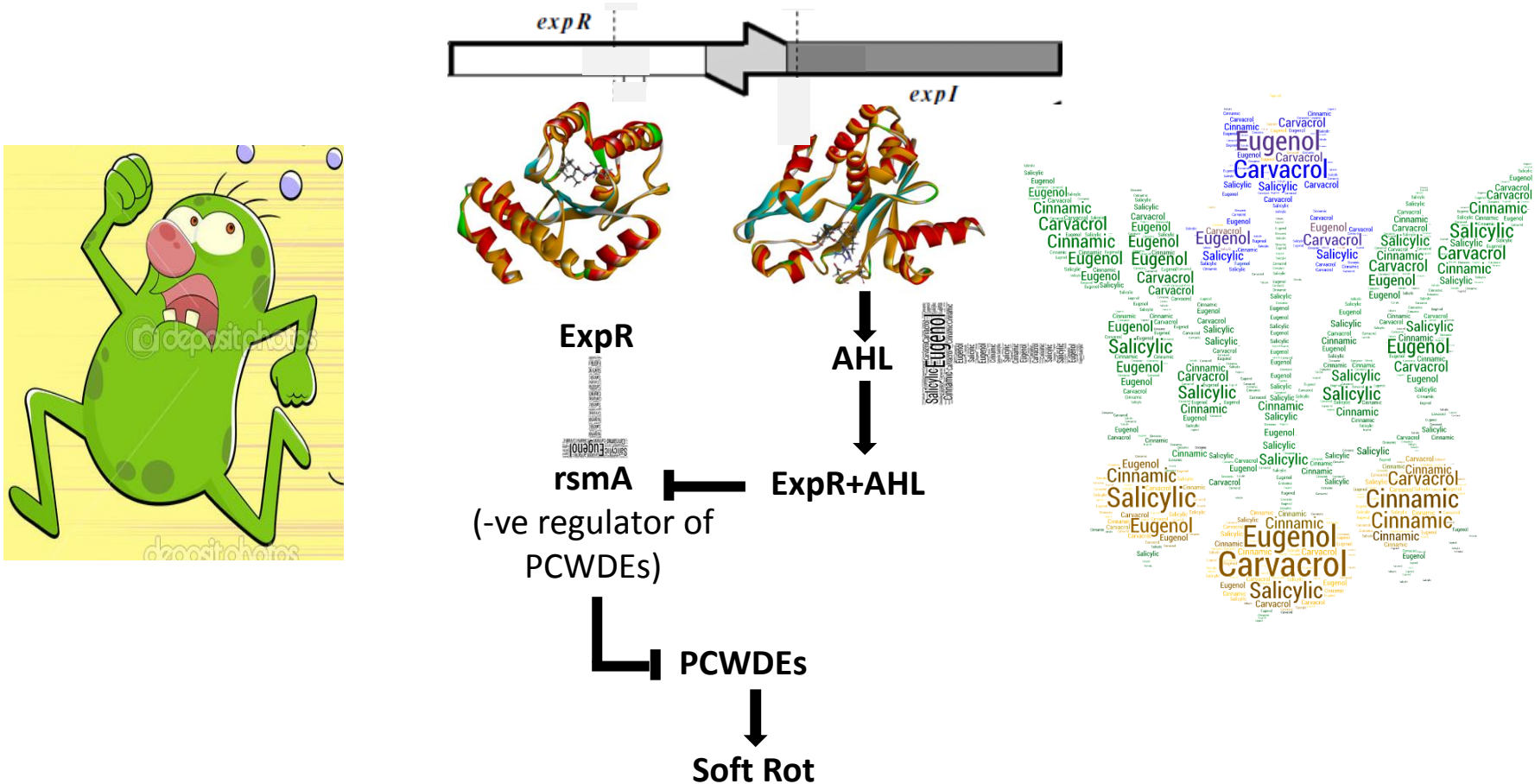
Joshi et al (2016), *Scientific Reports* (In Press)



# Summary

Phenolic compounds potentially block or interfere with Quorum sensing central protein (ExpI/ExpR), thus reducing virulence of *Pc*.

QS system of *Pectobacterium* spp.



Work in progress to show direct binding

# Take home Message:



## The tested phenolic compounds:



Inhibits the virulence traits of *Pectobacterium*.



Directly interfere the QS system in *Pectobacterium*.



Interfere with ExpI and block AHL production.



**Molecular Docking and Drug Discovery tools can also be applied in agriculture to design new drugs and control methods.**



# Acknowledgements:



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**Prof. Saul Burdman**



**Dr. Netaly Khazanov**



**Dr. Alex Lipsky**



Questions?

Thank  
You

Questions?

Questions?

Thank  
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Thank You

Questions?