Using Essential Oil Vapours to Protect Potato, Cabbage or Celery from *Pectobacterium Carotovotum*, may Result in Increased Virulence

Elad Landau

Dr. Roni Shapira's lab

Institute of Biochemistry, Food Science and Nutrition,
The Robert H. Smith Faculty of Agriculture, Food and Environment
The Hebrew University of Jerusalem

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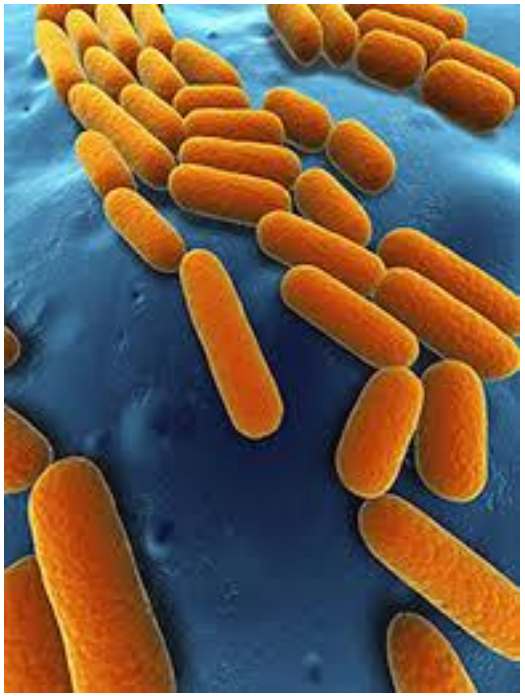
Bacteria are constantly exposed to sub-lethal stresses

Lack of nutrients

Temperature changes



Biocides

pH changes

Changes in osmolarity

Model systems for bacterial response to stress

Exposing bacteria to sub-lethal levels of antibiotics

- May result in development of resistant strains.
- Resistant strains appear at a faster rate then drug development.
- Sub-lethal antibiotics:
  - Signaling molecules.
  - Affect virulence.

- Little is known about the effect of sub-lethal exposure to non antibiotic antimicrobials.

Essential oils as model antimicrobials

- Secondary plant metabolites possessing antimicrobial activity.
- Volatile.
- Posses antimicrobial activity in the aqueous and gaseous phase.
- GRAS- generally regarded as safe.
- Mode of action is not yet fully understood.
- Effect of sub-lethal exposure has not been studied.

Research hypothesis

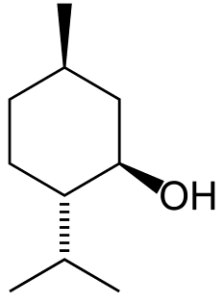
Exposing bacteria to sub-lethal levels of essential oil vapours will affect bacteria in a similar manner as exposure to sub-lethal levels of antibiotics.

Research questions

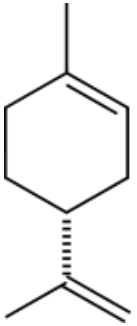
- How will sub-lethal exposure to essential oils affect bacterial virulence?
- What is the molecular mechanism underlying the process?

Menthol, Limonene, Carvacrol

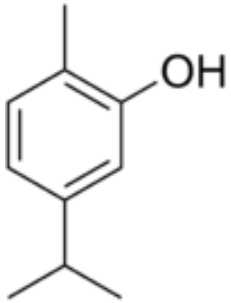
Menthol



Limonene

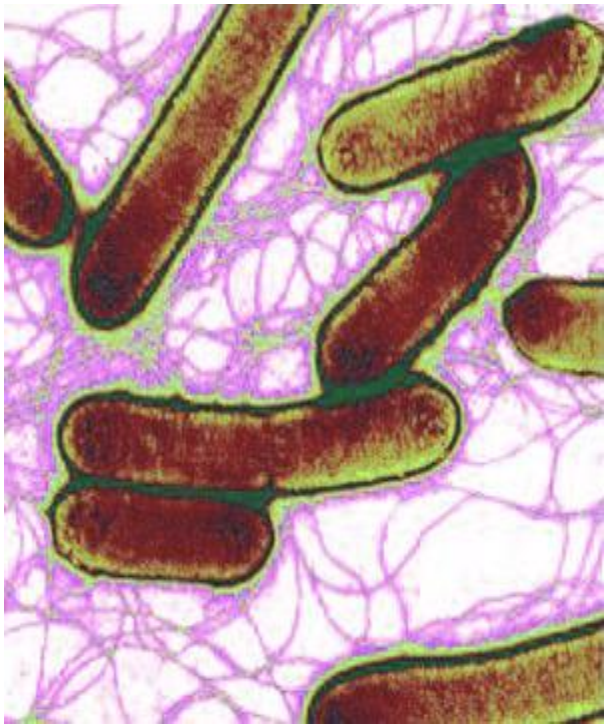


Carvacrol

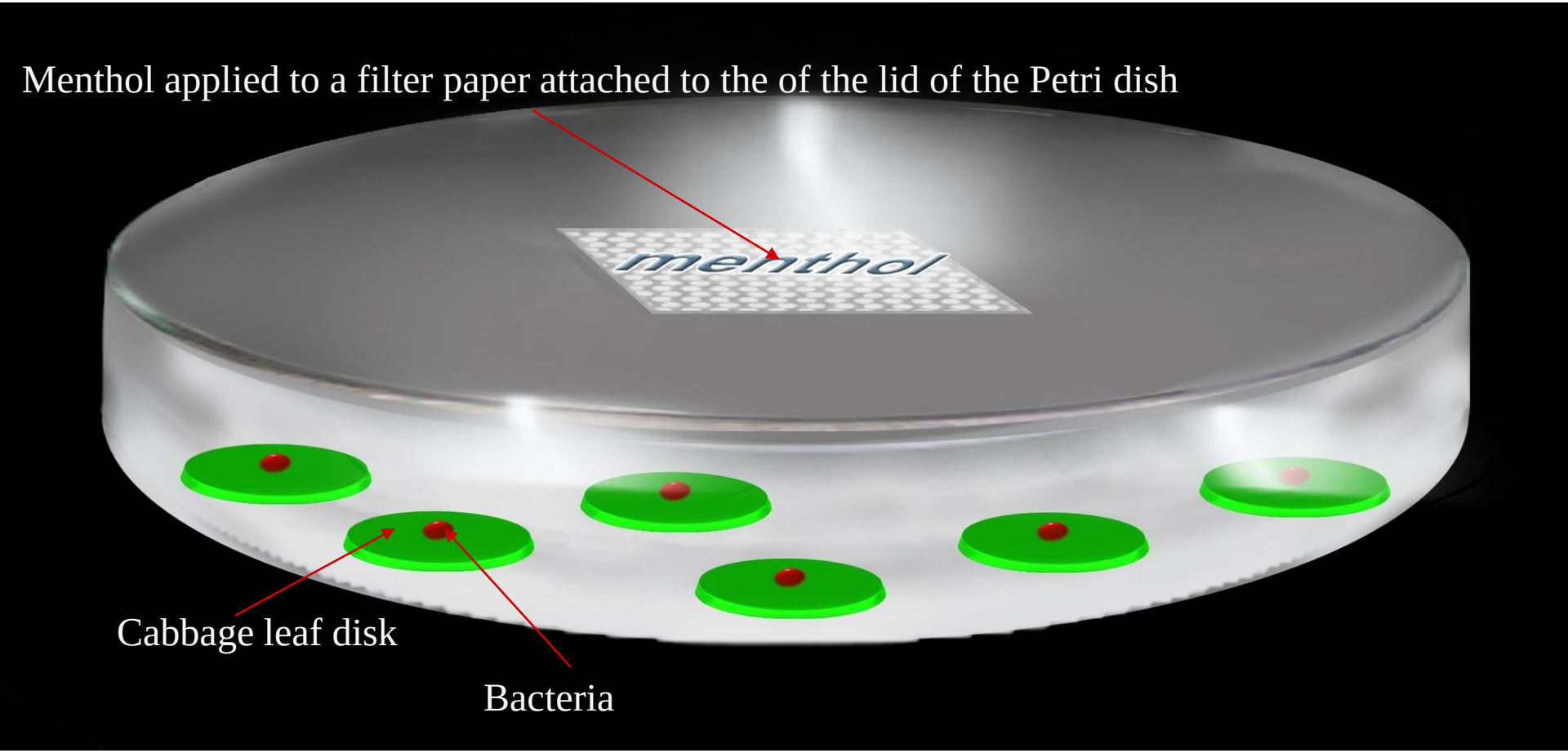


Pectobacterium carotovorum ssp. *Carotovorum (pcc)*

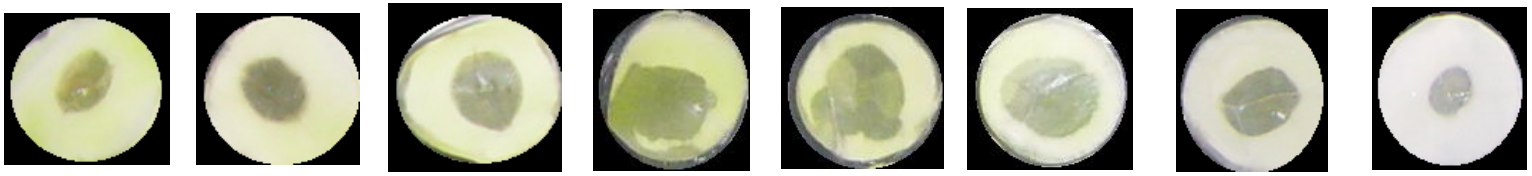
- Graham negative, rod.
- Plant pathogen, affecting potato, cabbage and a broad range of plants.
- Causes sort rot.
- Simple model for bacterial pathogenicity.



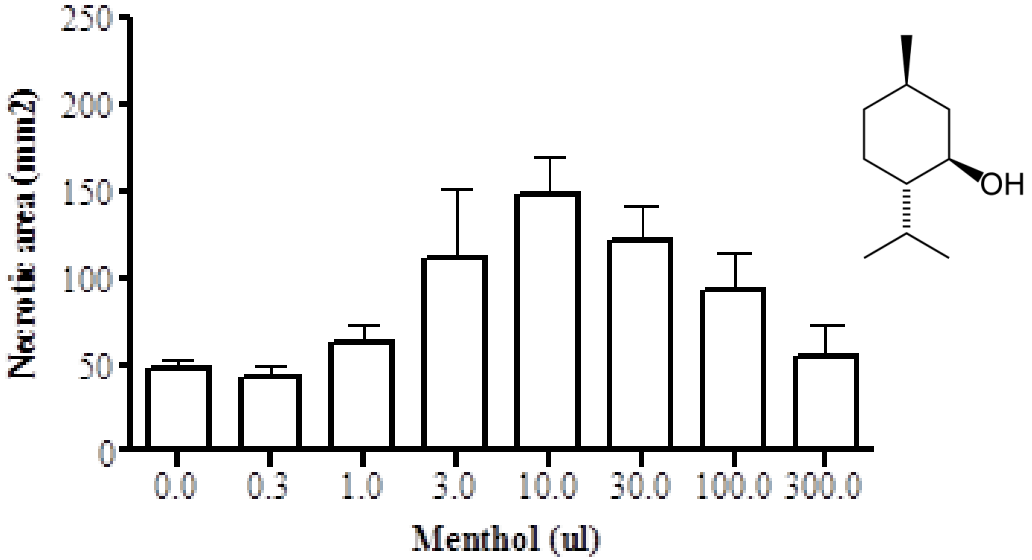
Model system



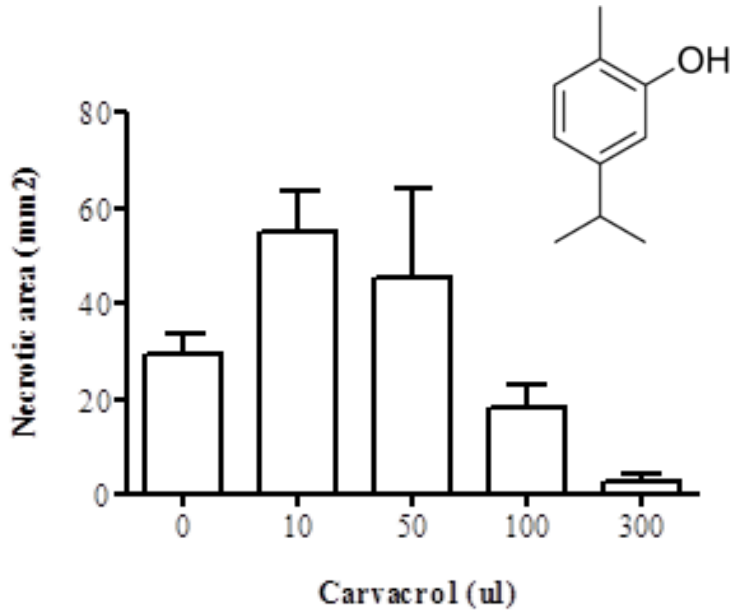
Exposing *P. carotovorum* to sub-lethal menthol vapors increases virulence

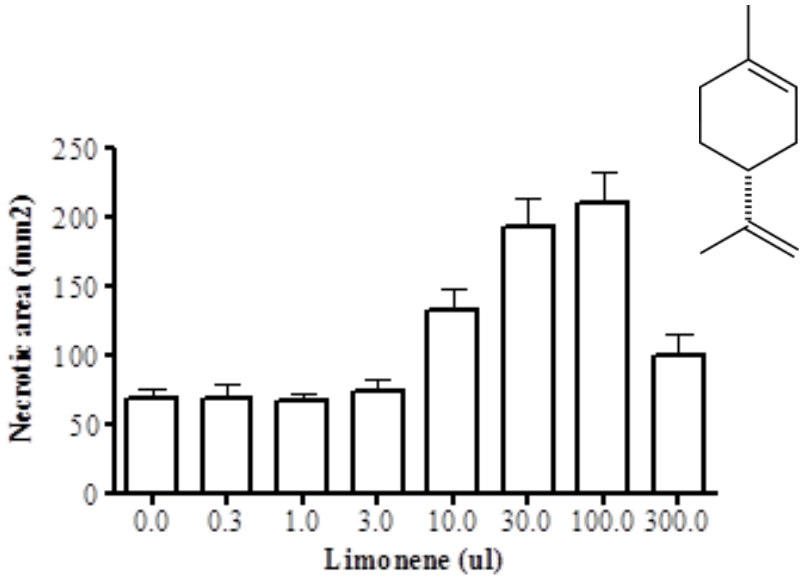


0ul 0.3ul 1ul 3ul 10ul 30ul 100ul 300ul

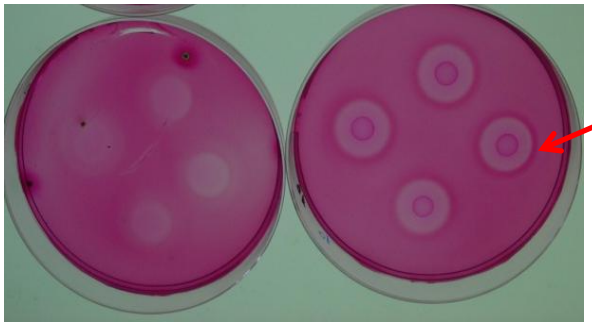


Exposure to sub-lethal levels of carvacrol or limonene increases bacterial virulence



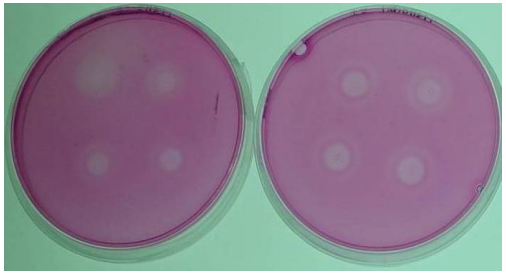


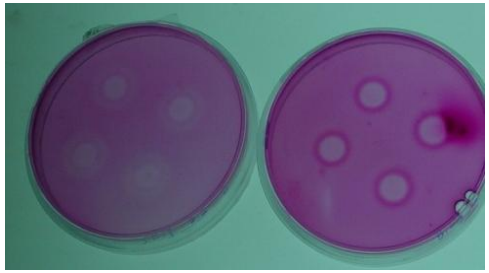
Exposing *P. carotovorum* to sub-lethal levels of essential oil vapours increases enzyme secretion



pectin methyl &esterase polygalacturoanse

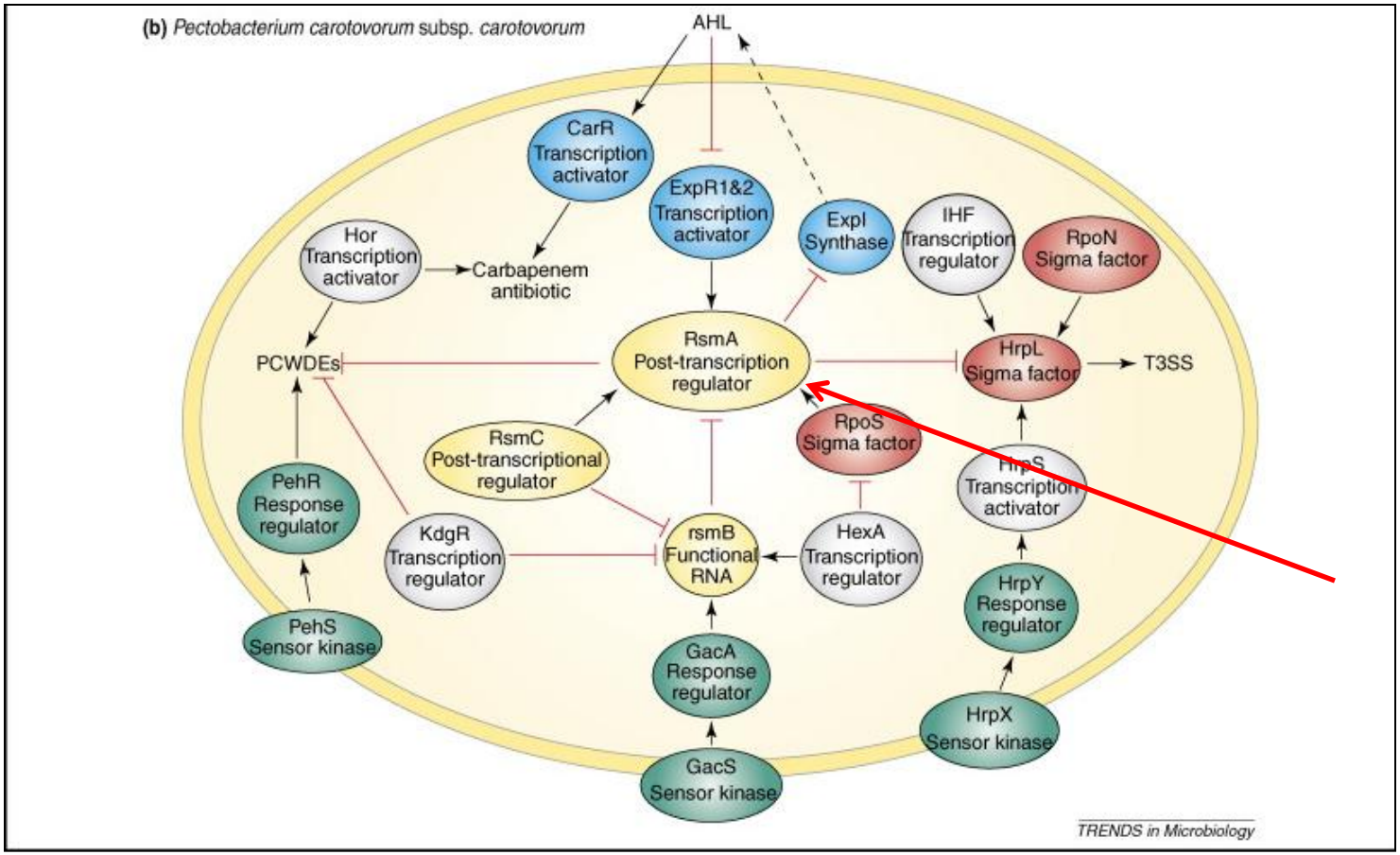
Menthol - +





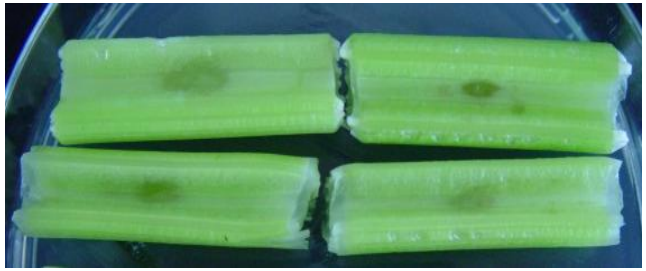
Limonene - + - + Carvacrol

RsmA

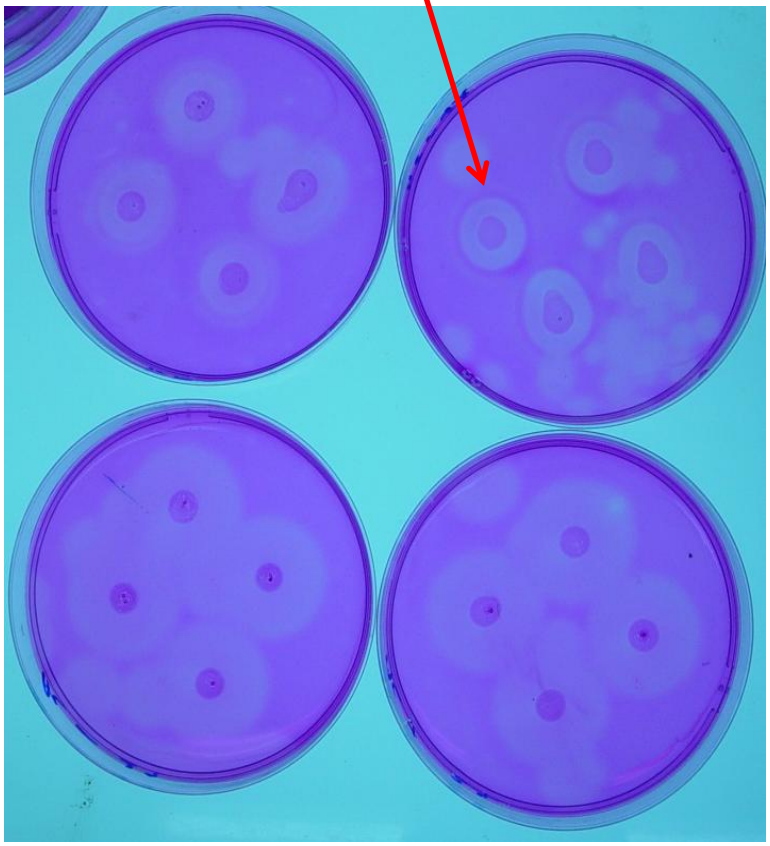


Mole BM et al. 2007

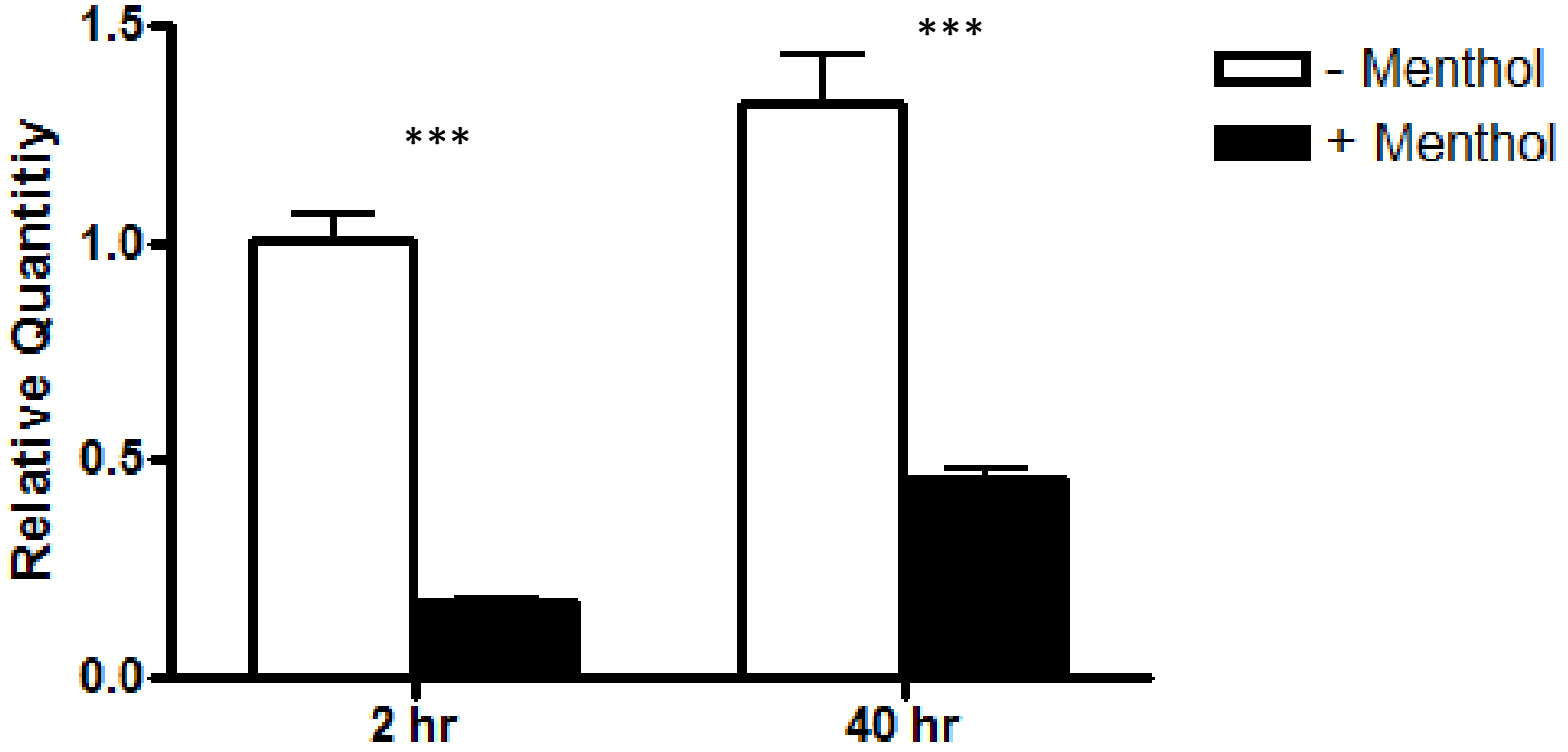
RsmA is involved in the increase in virulence caused by exposure to sub-lethal levels of essential oil vapours

	WT	ΔrsmA
-Menthol		
+Menthol		

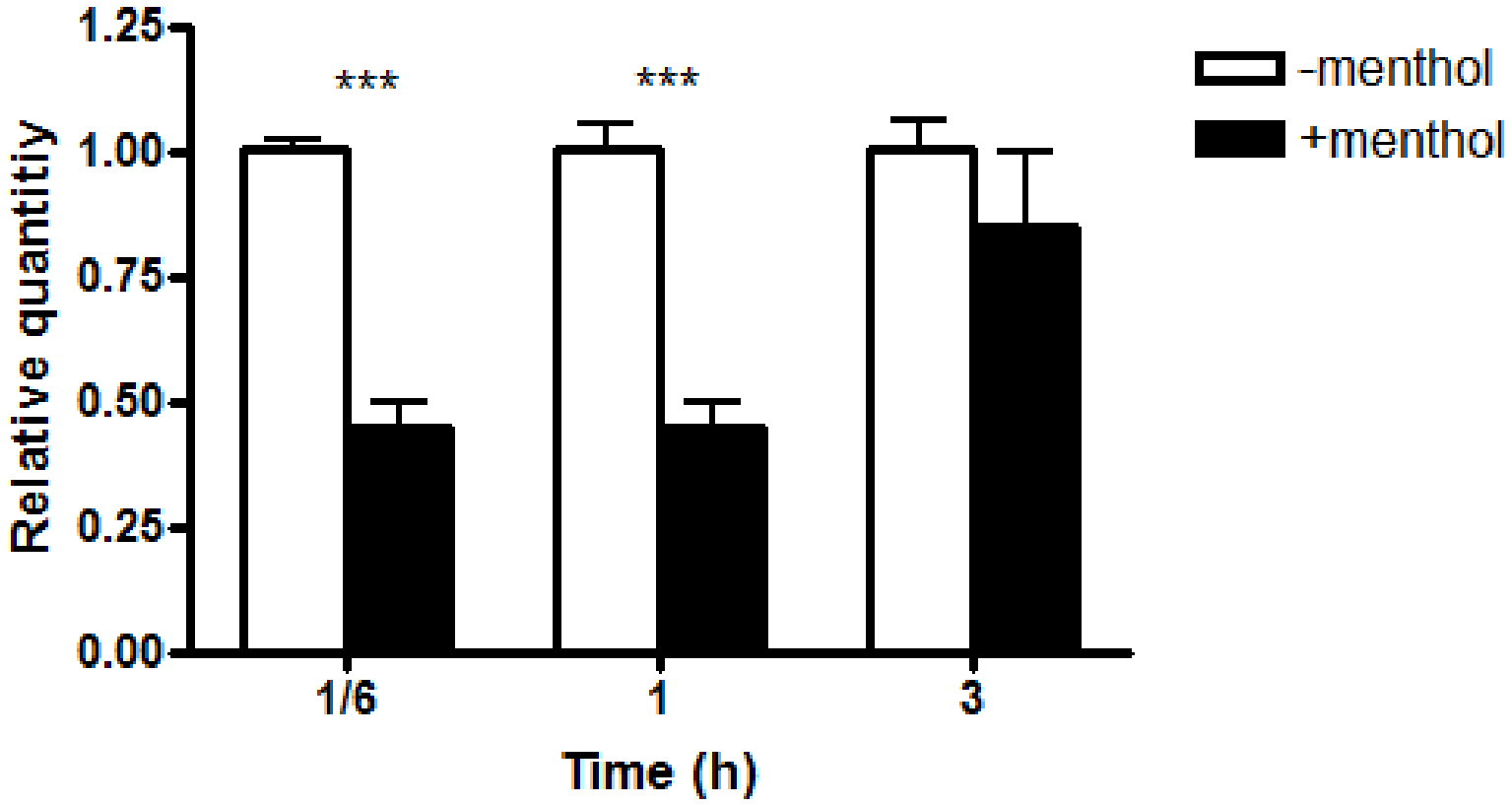
Rsma is involved in the increased enzyme secretion due to sub-lethal exposure to essential oil vapours



rsmA transcription declines after exposure to sub-lethal menthol levels on pectin agar

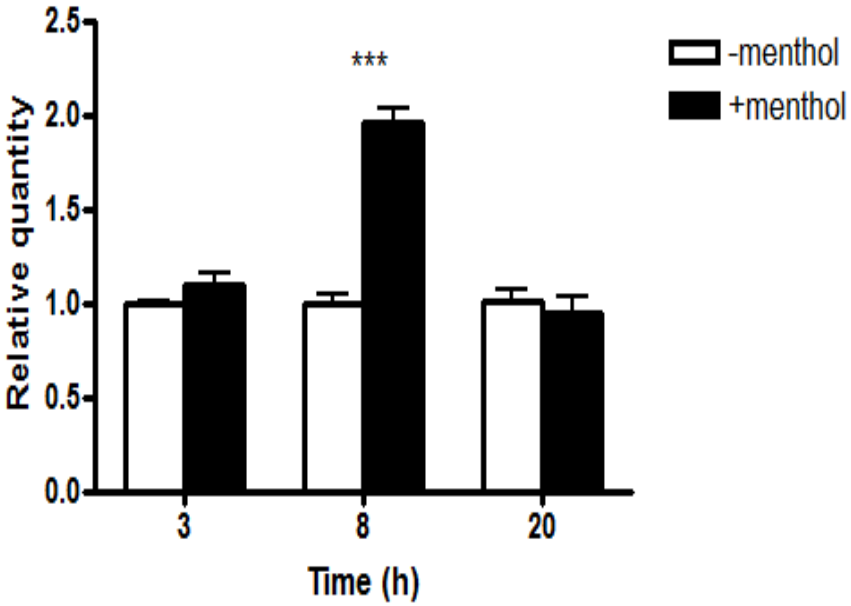


rsmA transcription declines after exposure to sub-lethal menthol levels on **cabbage**

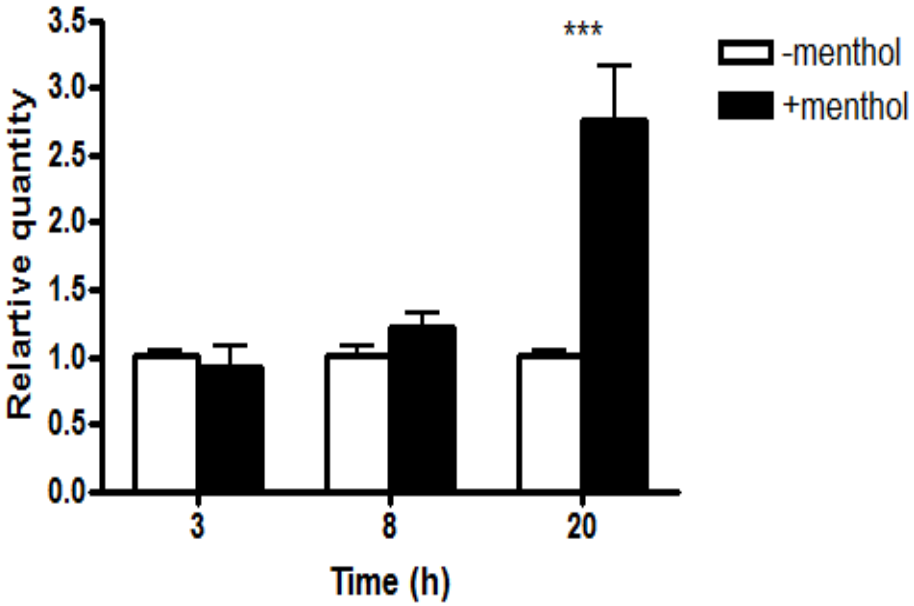


Enzyme transcription increases after to exposure to sub-lethal levels of essential oil vapours on **cabbage**

Polygalacturonase



Pectate lyase



Conclusions

- Exposing *P. carotovorum* cells to sub-lethal essential oil vapours causes an increase in virulence.
- The increased virulence is caused by an increase in enzyme secretion.
- The increase in enzyme secretion is due to a time dependant increase in their transcription and a reduction in *rsmA* transcription.
- **Probably the same phenomenon appear in potatoes**

Future prospects

- Identify the entire gene/ protein complex involved in transferring the signal generated by the presence of essential oils outside the cell into an increase in bacterial virulence.
  - DNA chips
  - 2D electrophoresys
  - Mutant libreries
- Identify the binding site/sensor for essential oils
  - Use of labeled essential oils
  - Affinity columns

Thanks

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