

# Optimized enzymes for controlling phytopathogens

David Daudé (PhD, HDR) – CEO and CSO of Gene&GreenTK

Gene  
&  
GreenTK



# Phytopharmaceutical issues

**Nonspecific**

**Persistence**

**Resistance**

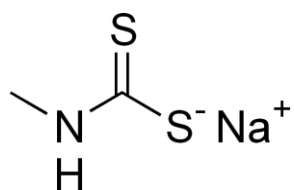
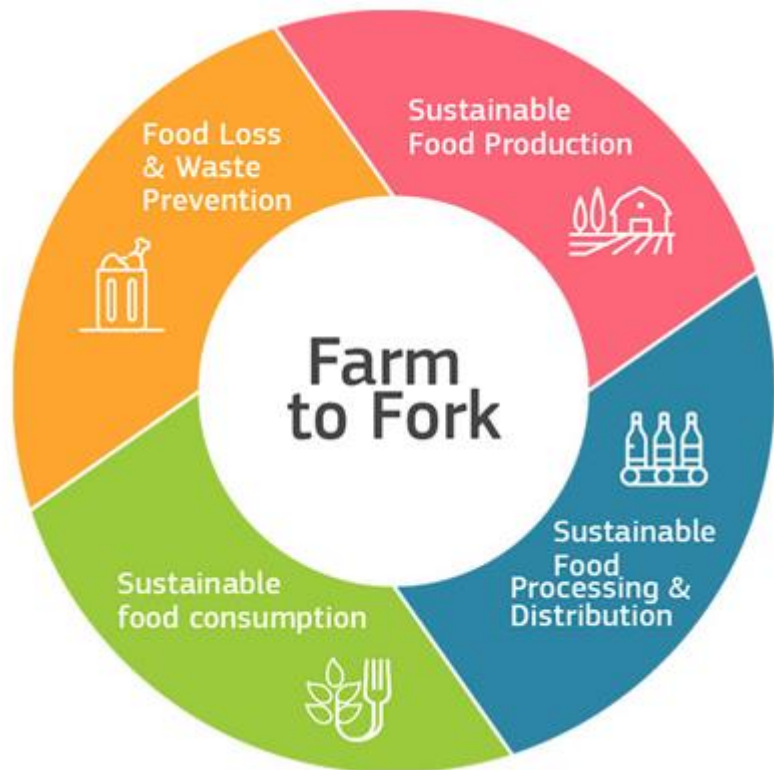
**Limited efficacy**



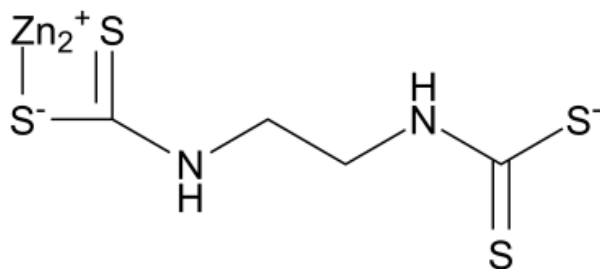
# Phytopharmaceutical issues



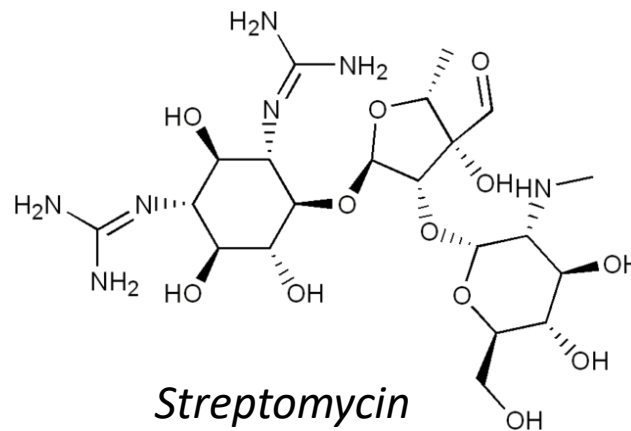
# Phytopharmaceutical issues



Metam sodium  
**SUSPENDED**



Mancozeb  
**SUSPENDED**

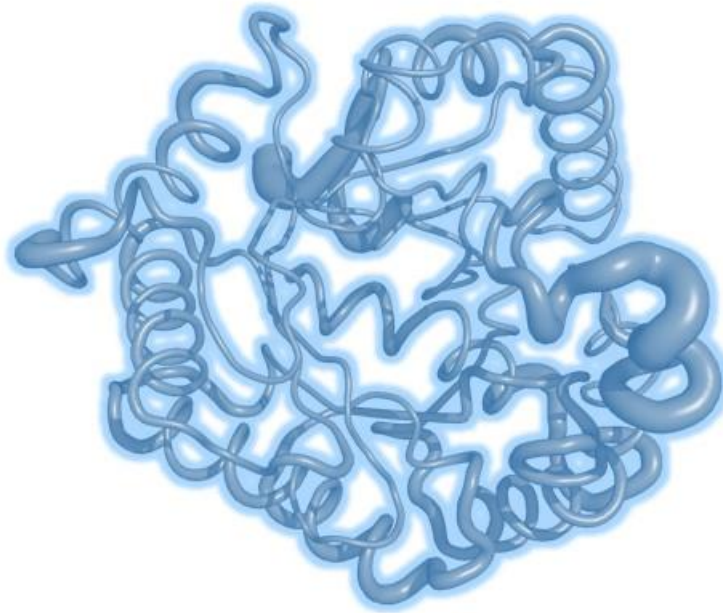


Streptomycin  
**NOT AUTHORIZED (EU)**  
**AUTHORIZED (Nth Am)**



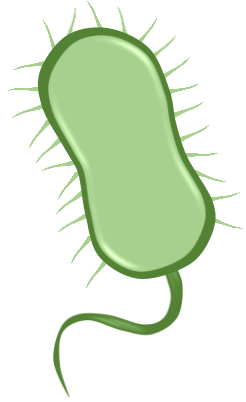
Copper derivatives  
**CANDIDATE TO SUBSTITUTION**

# *Urgent need for sustainable antimicrobials*

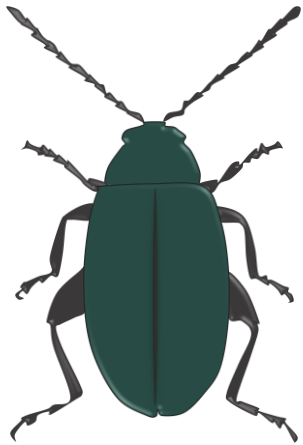


**ENZYMES**

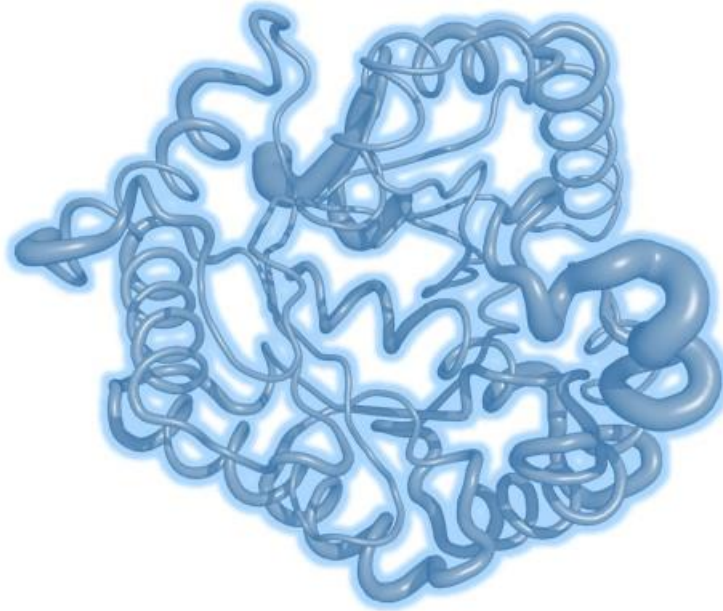
# Towards new biopesticides



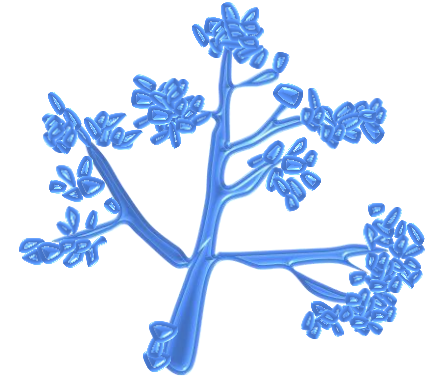
*Bacteria*



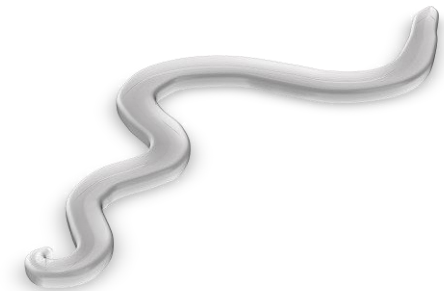
*Insects*



**ENZYMES**

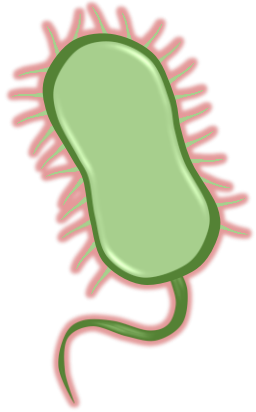


*Fungi*

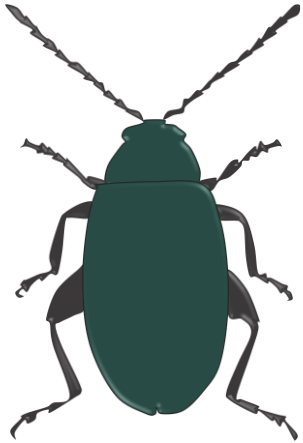


*Nematodes*

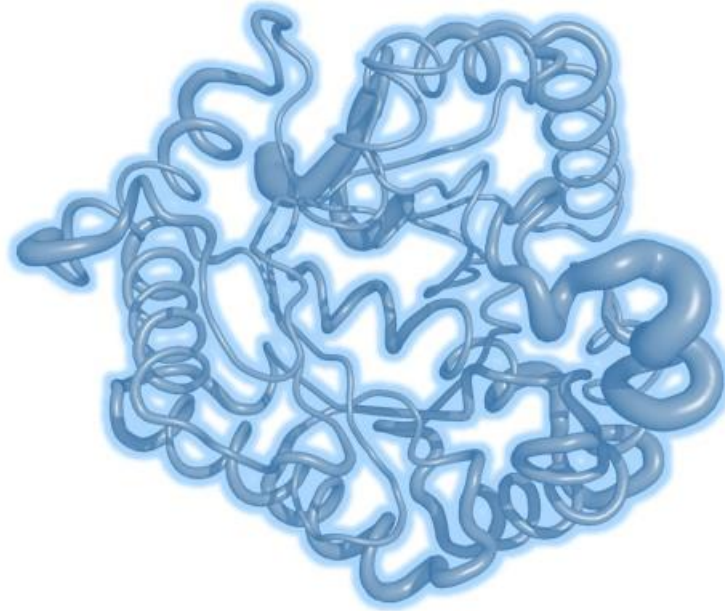
# Towards new biopesticides



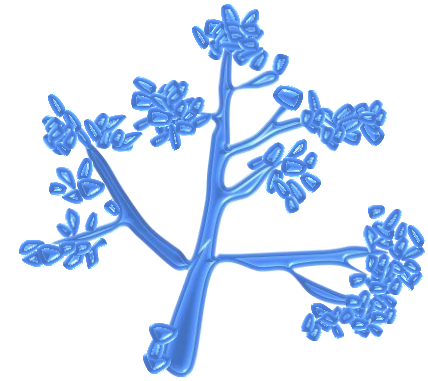
*Bacteria*



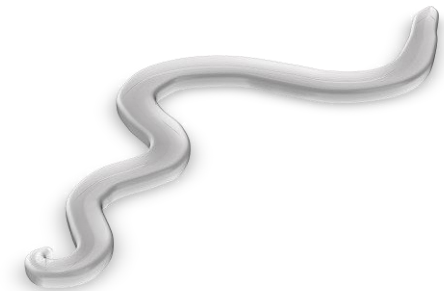
*Insects*



**ENZYMES**

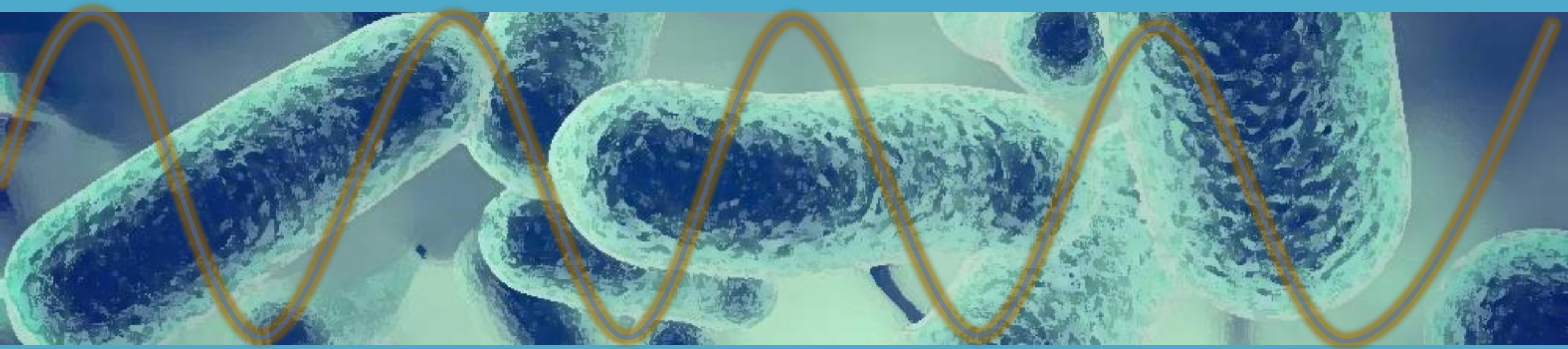


*Fungi*



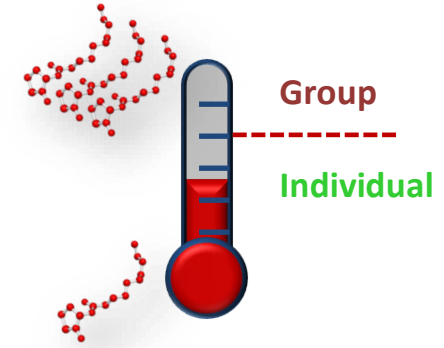
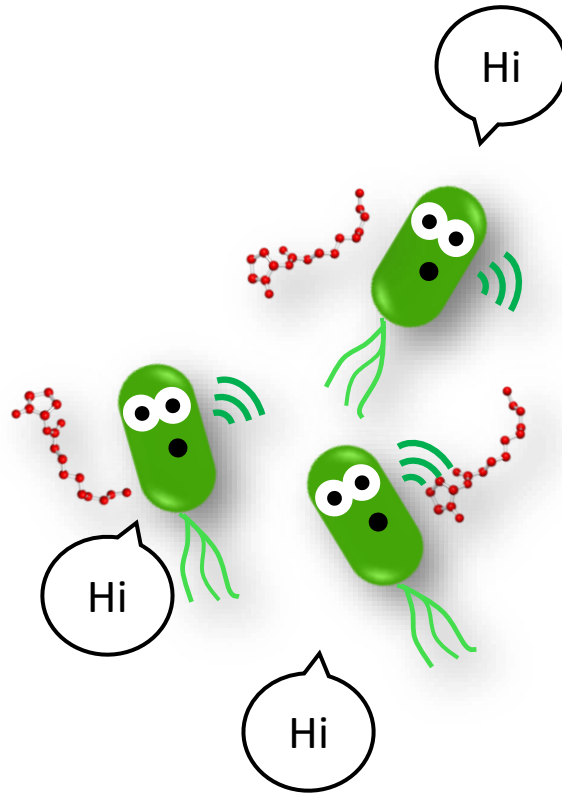
*Nematodes*

# What is *Quorum Sensing* ?

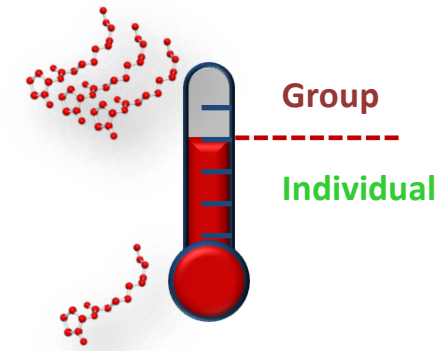
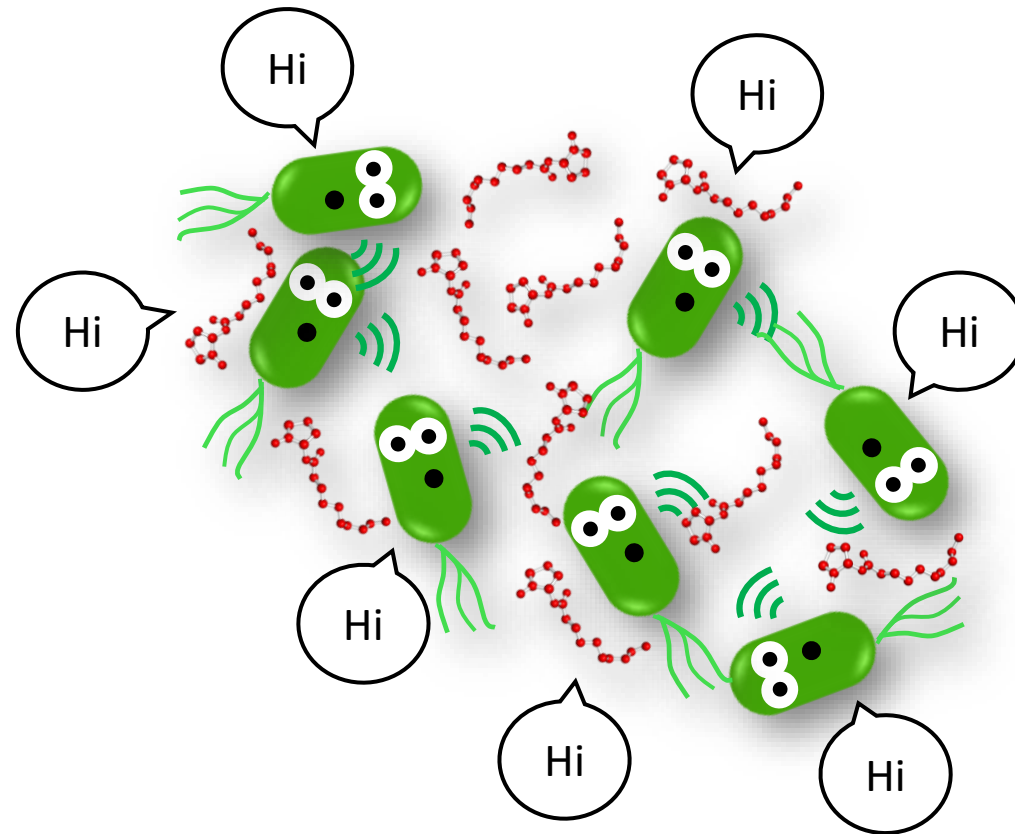




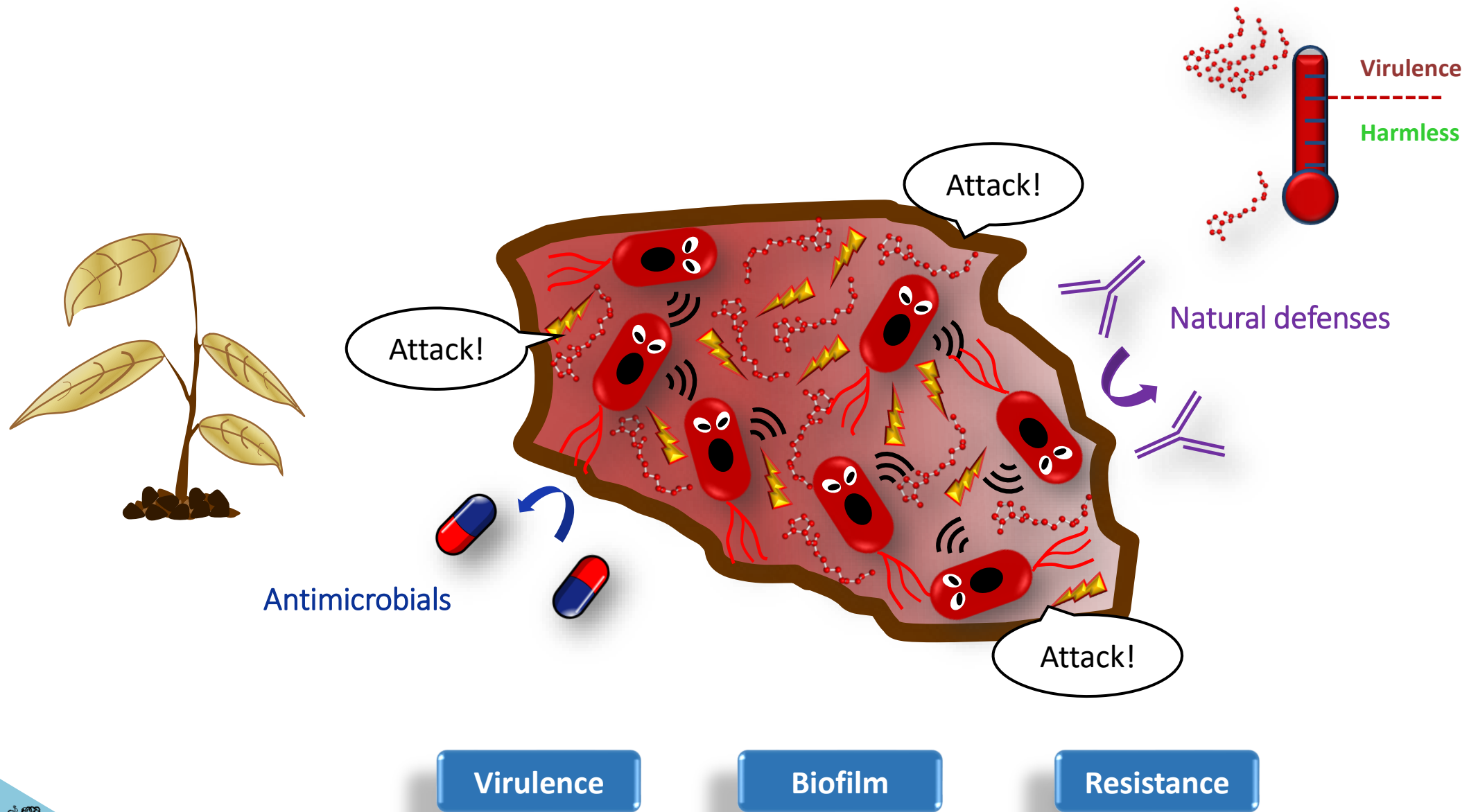
# Targeting bacterial phytopathogens



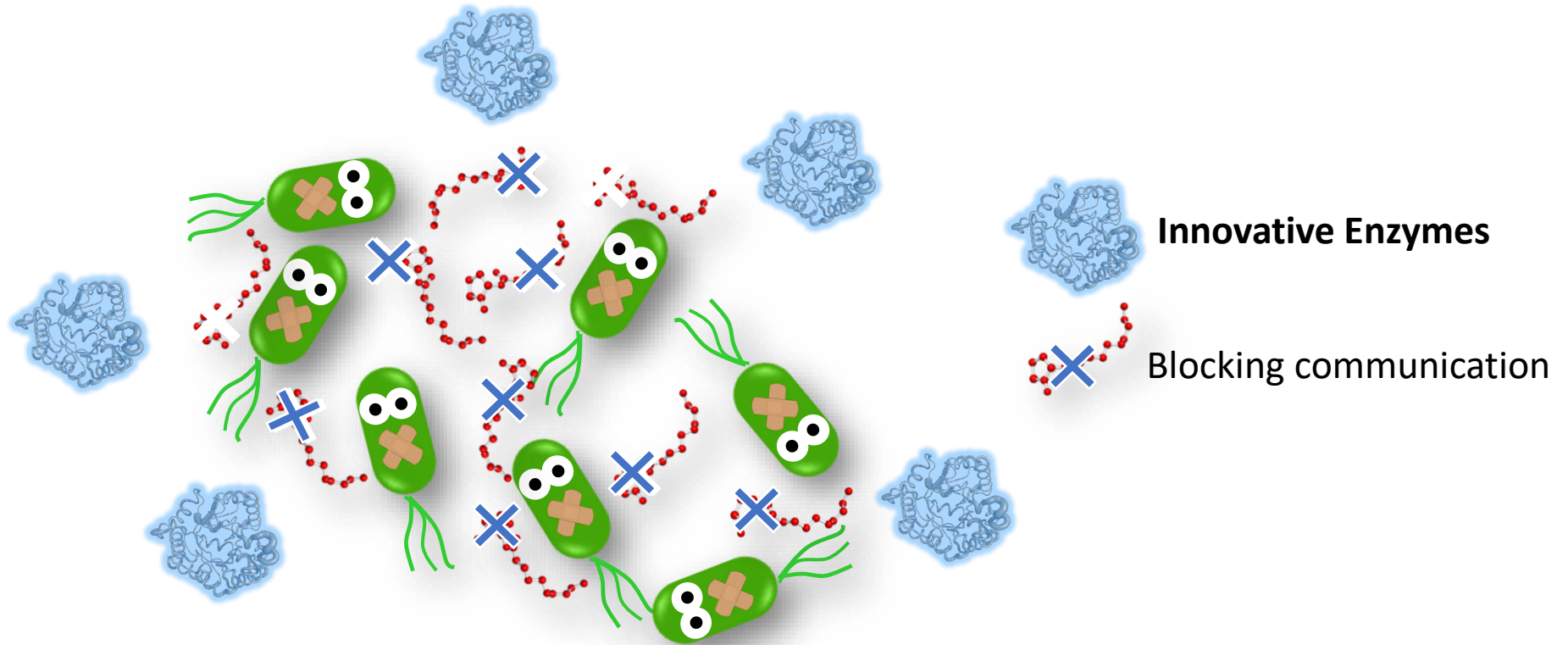
# Targeting bacterial phytopathogens



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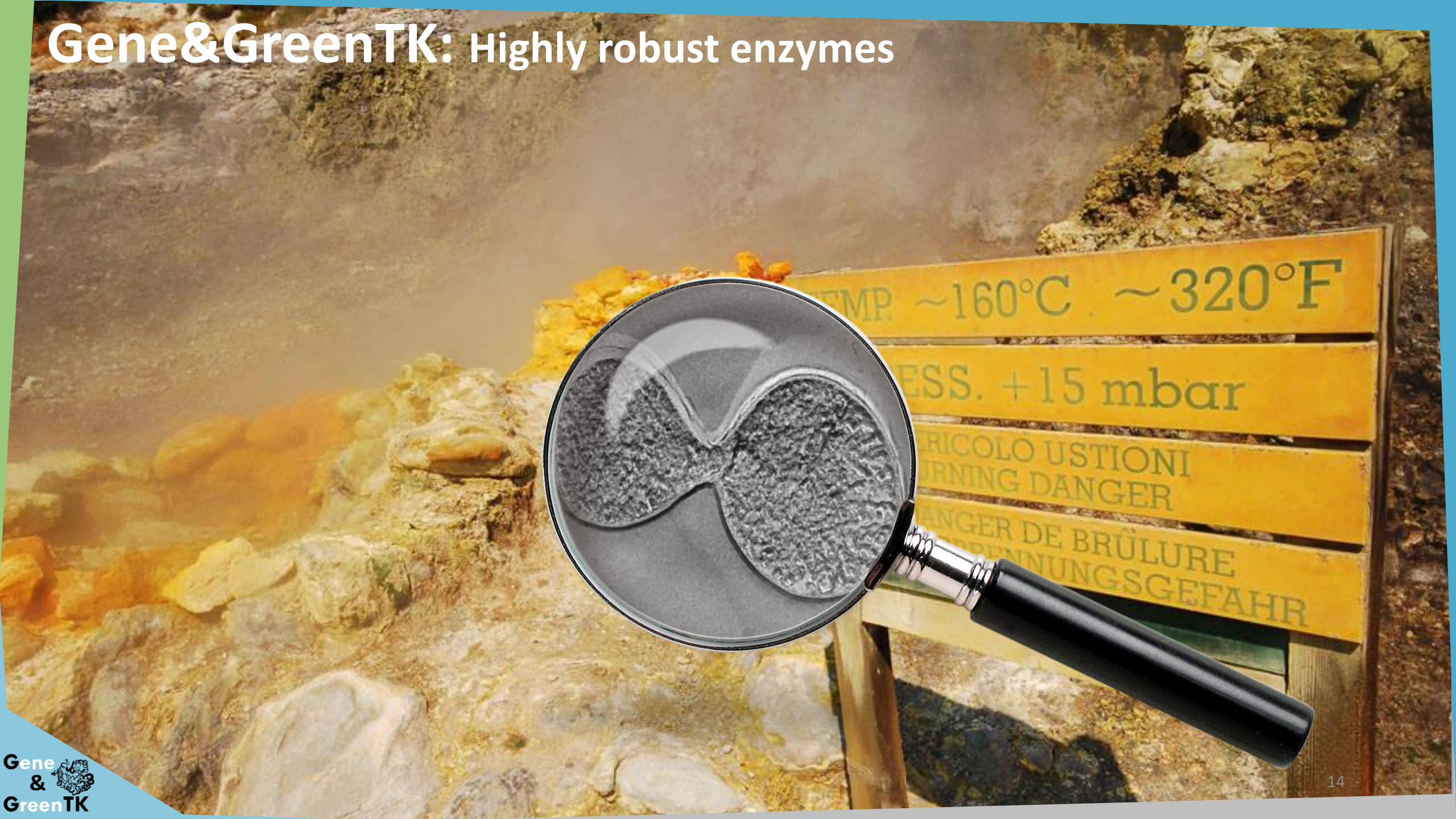
# Targeting bacterial phytopathogens



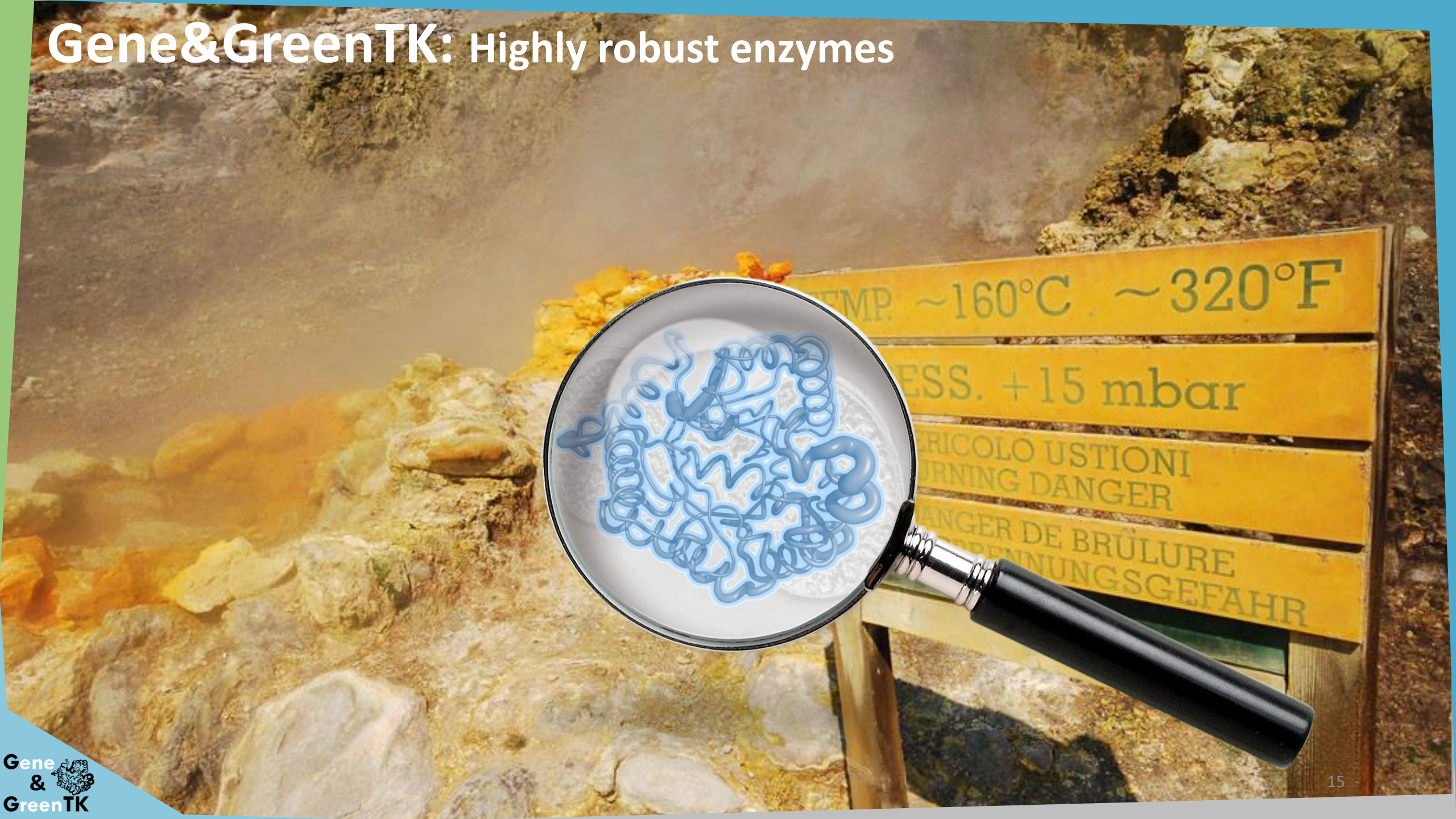
# Gene&GreenTK: Highly robust enzymes



# Gene&GreenTK: Highly robust enzymes



# Gene&GreenTK: Highly robust enzymes



# Our approach: enzymes from extreme environments

***Hyperthermostable***



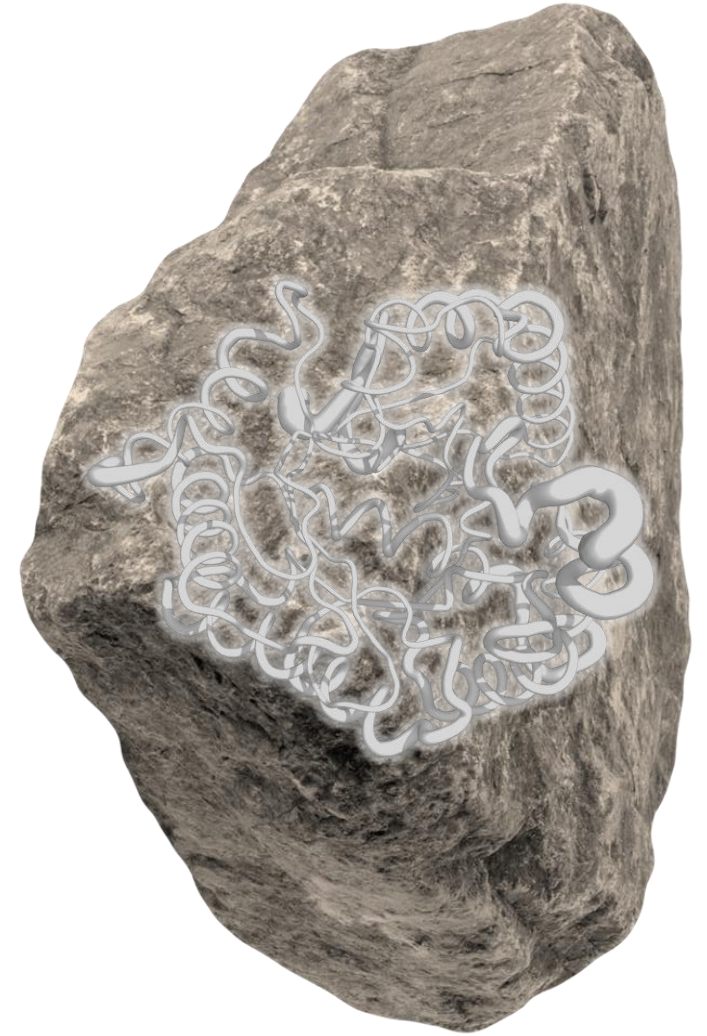
***Up to 100°C***

***Sub-zero temperatures***



***Down to -18°C***

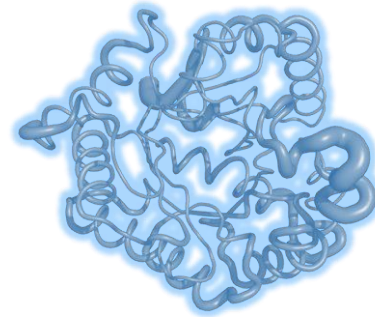
***Robustness***



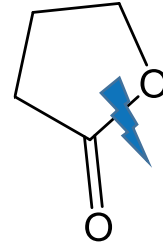
***Solvents, Proteases, Detergents***



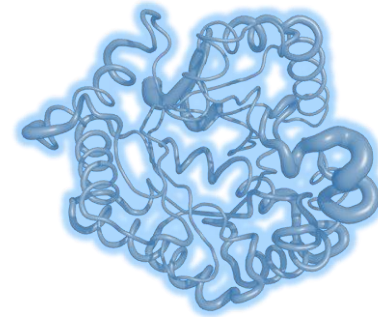
# Lactonase activity



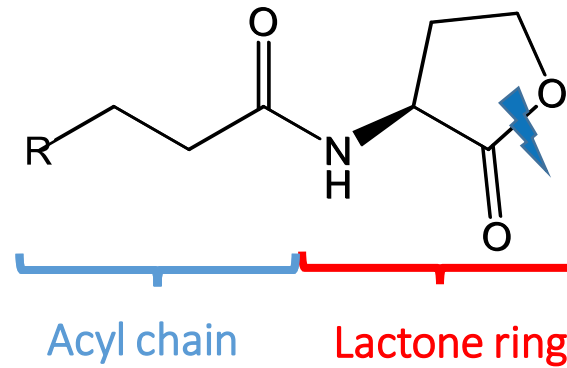
Lactonase



# Lactonase activity

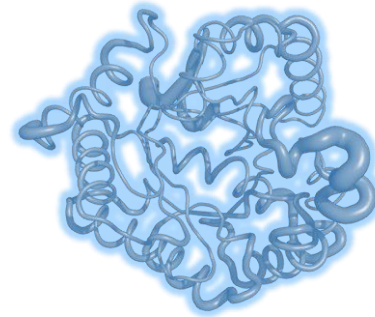


Lactonase

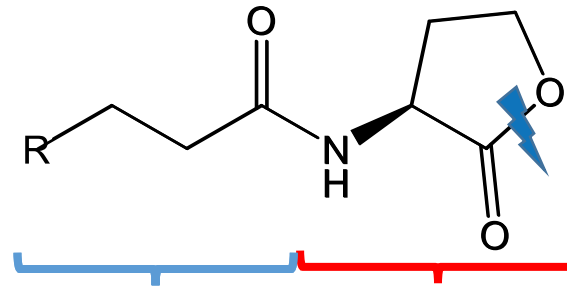


Acyl-Homoserine Lactones (AHL)

# Lactonase activity



Lactonase



Acyl-Homoserine Lactones (AHL)

Acyl chain

Lactone ring



**Bacterial canker**

*Pseudomonas syringae*



**Apple fireblight**

*Erwinia amylovora*



**Softrot, black leg**

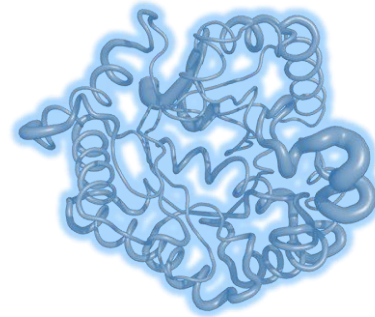
*Dickeya sp.*  
*Pectobacterium sp.*



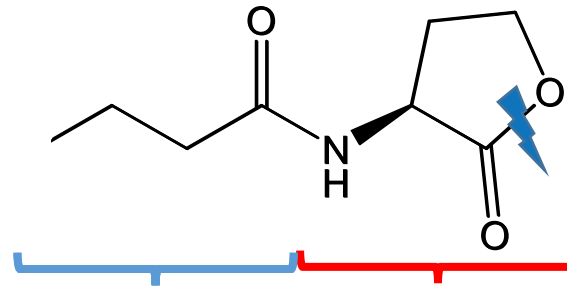
**Hairy root**

*Agrobacterium rhizogenes*

# Lactonase activity



Lactonase



Short chain

Lactone ring

Acyl-Homoserine Lactones (AHL)



**Bacterial canker**

*Pseudomonas syringae*



**Apple fireblight**

*Erwinia amylovora*



**Softrot, black leg**

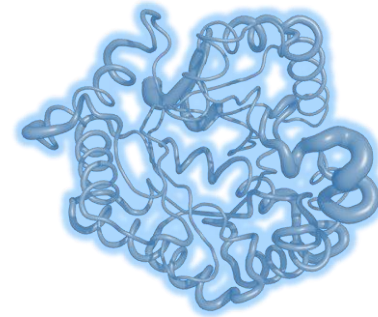
*Dickeya sp.*  
*Pectobacterium sp.*



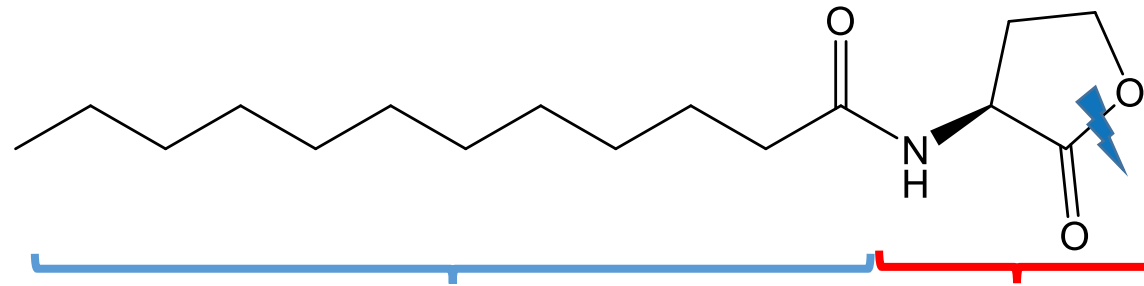
**Hairy root**

*Agrobacterium rhizogenes*

# Lactonase activity



Lactonase



Acyl-Homoserine Lactones (AHL)

Long chain

Lactone ring



**Bacterial canker**

*Pseudomonas syringae*



**Apple fireblight**

*Erwinia amylovora*



**Softrot, black leg**

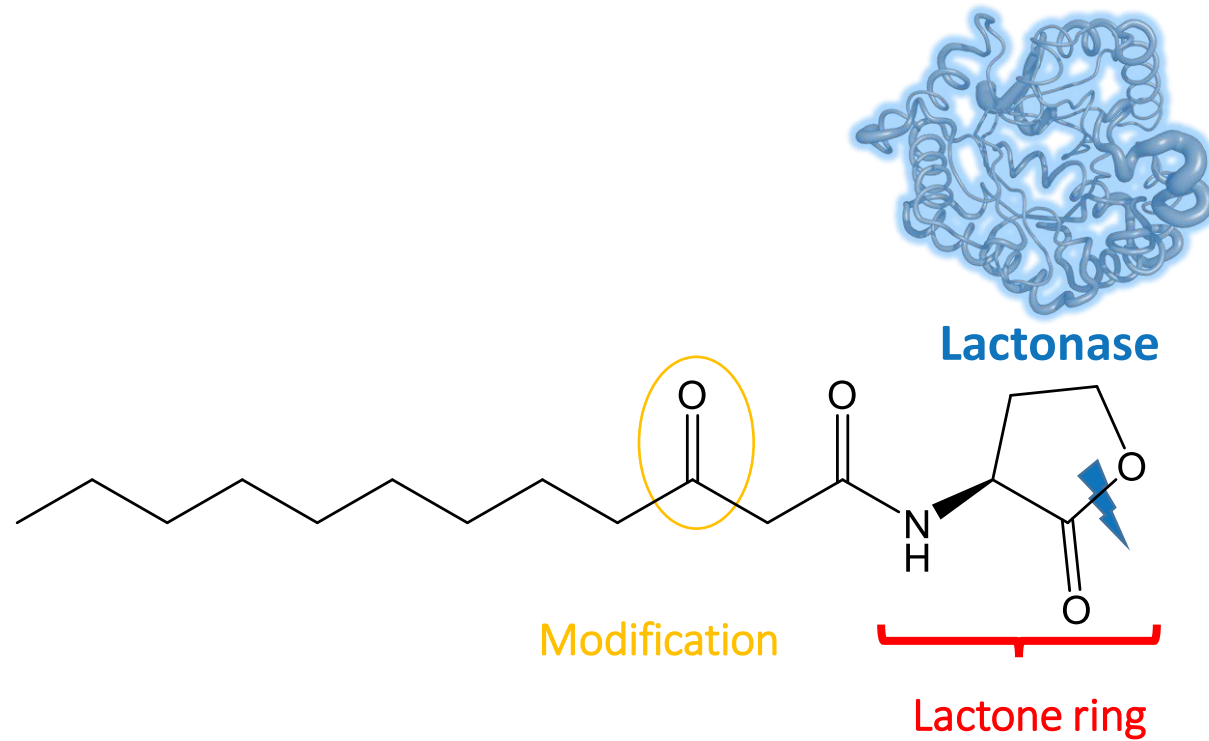
*Dickeya sp.*  
*Pectobacterium sp.*



**Hairy root**

*Agrobacterium rhizogenes*

# Lactonase activity



**Bacterial canker**

*Pseudomonas syringae*



**Apple fireblight**

*Erwinia amylovora*



**Softrot, black leg**

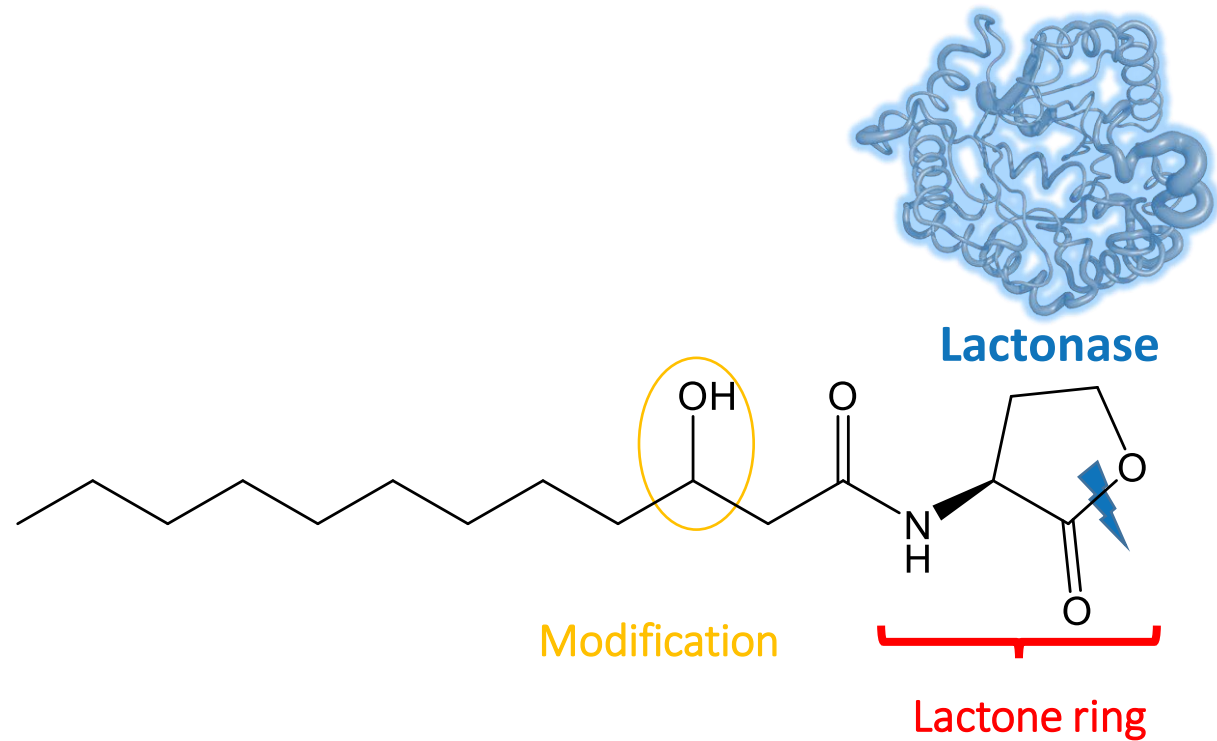
*Dickeya sp.*  
*Pectobacterium sp.*



**Hairy root**

*Agrobacterium rhizogenes*

# Lactonase activity



**Bacterial canker**

*Pseudomonas syringae*



**Apple fireblight**

*Erwinia amylovora*



**Softrot, black leg**

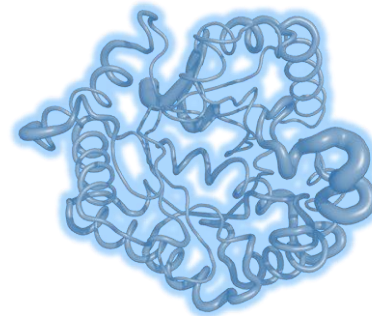
*Dickeya sp.*  
*Pectobacterium sp.*



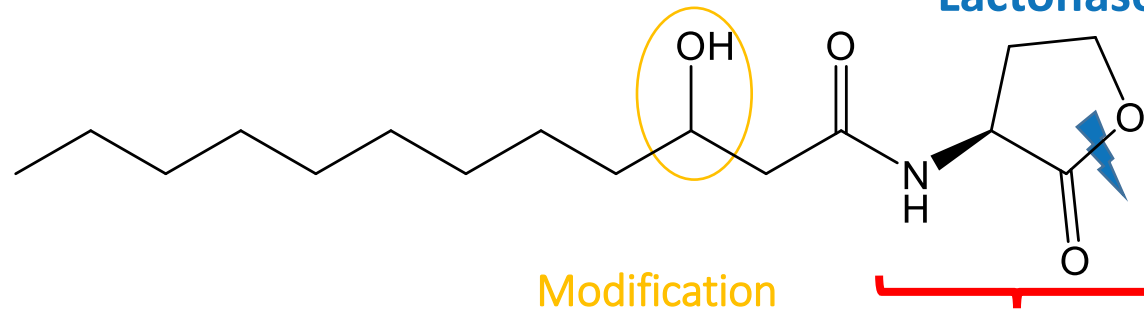
**Hairy root**

*Agrobacterium rhizogenes*

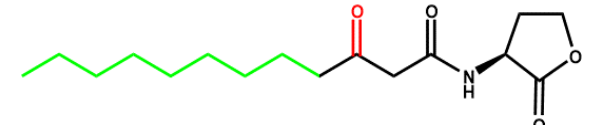
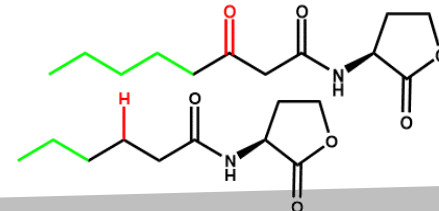
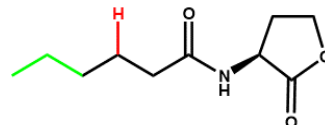
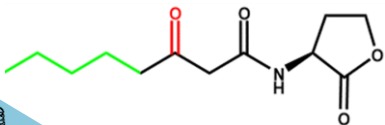
# Lactonase activity



Lactonase

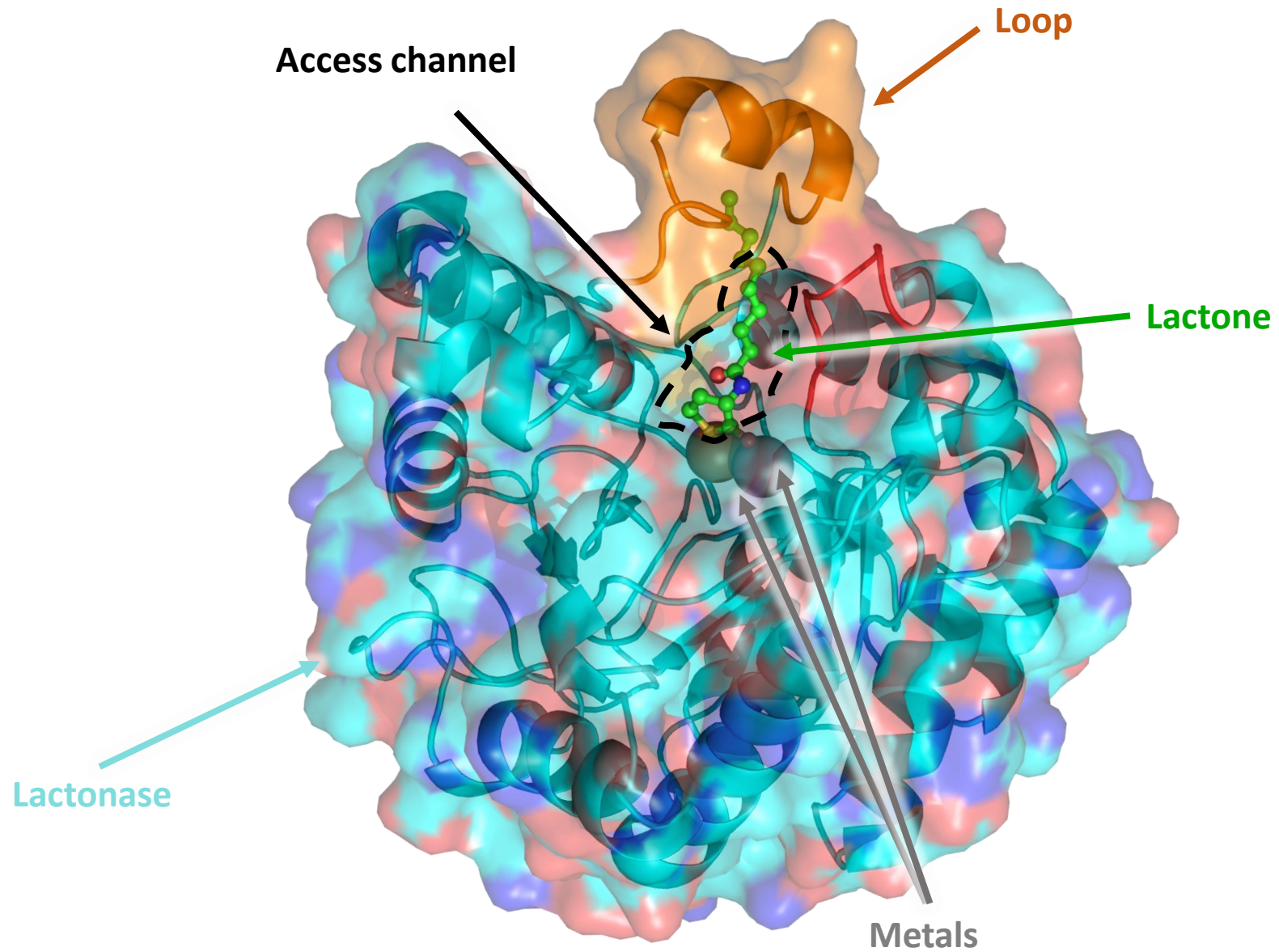


Acyl-Homoserine Lactones (AHL)

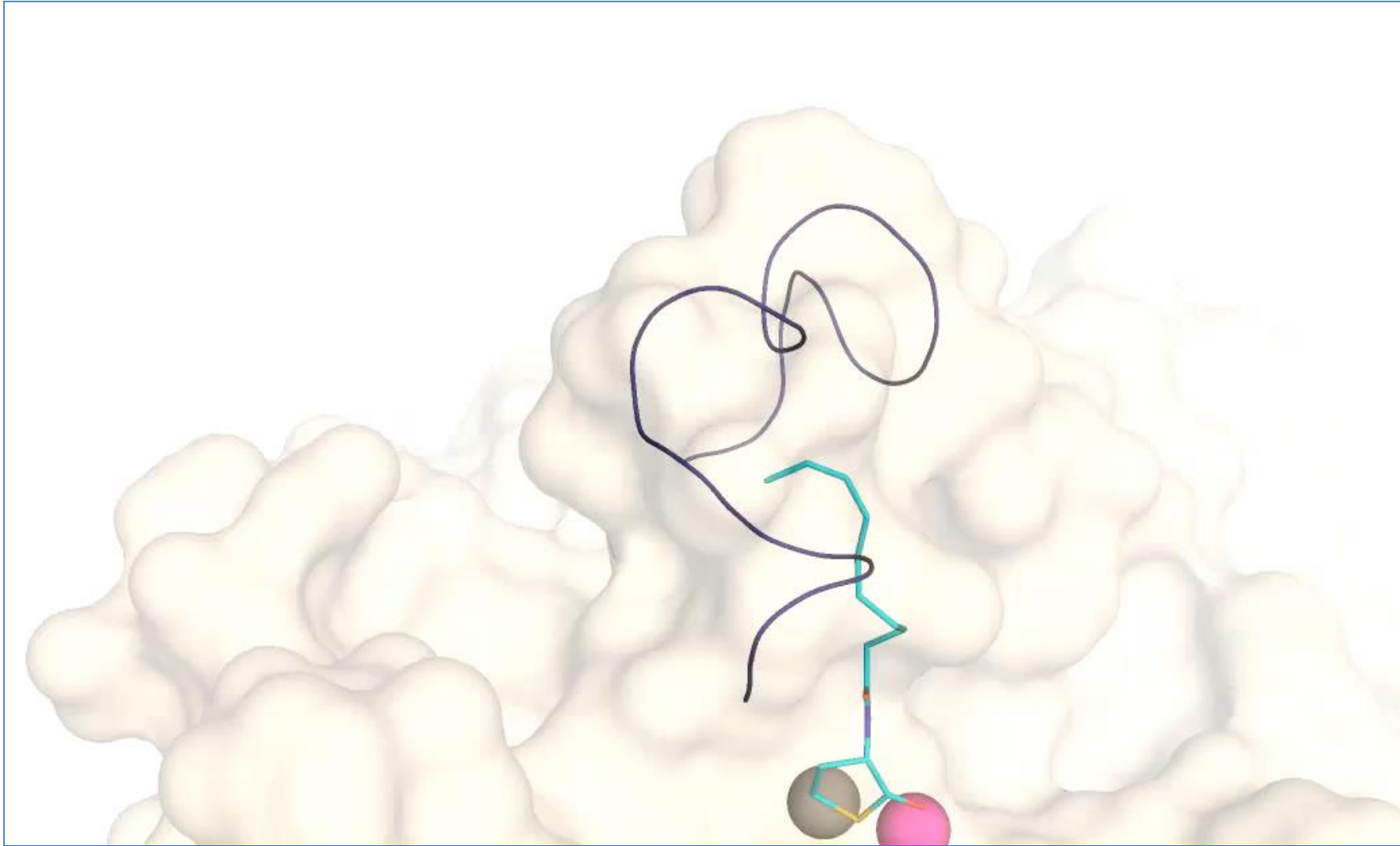




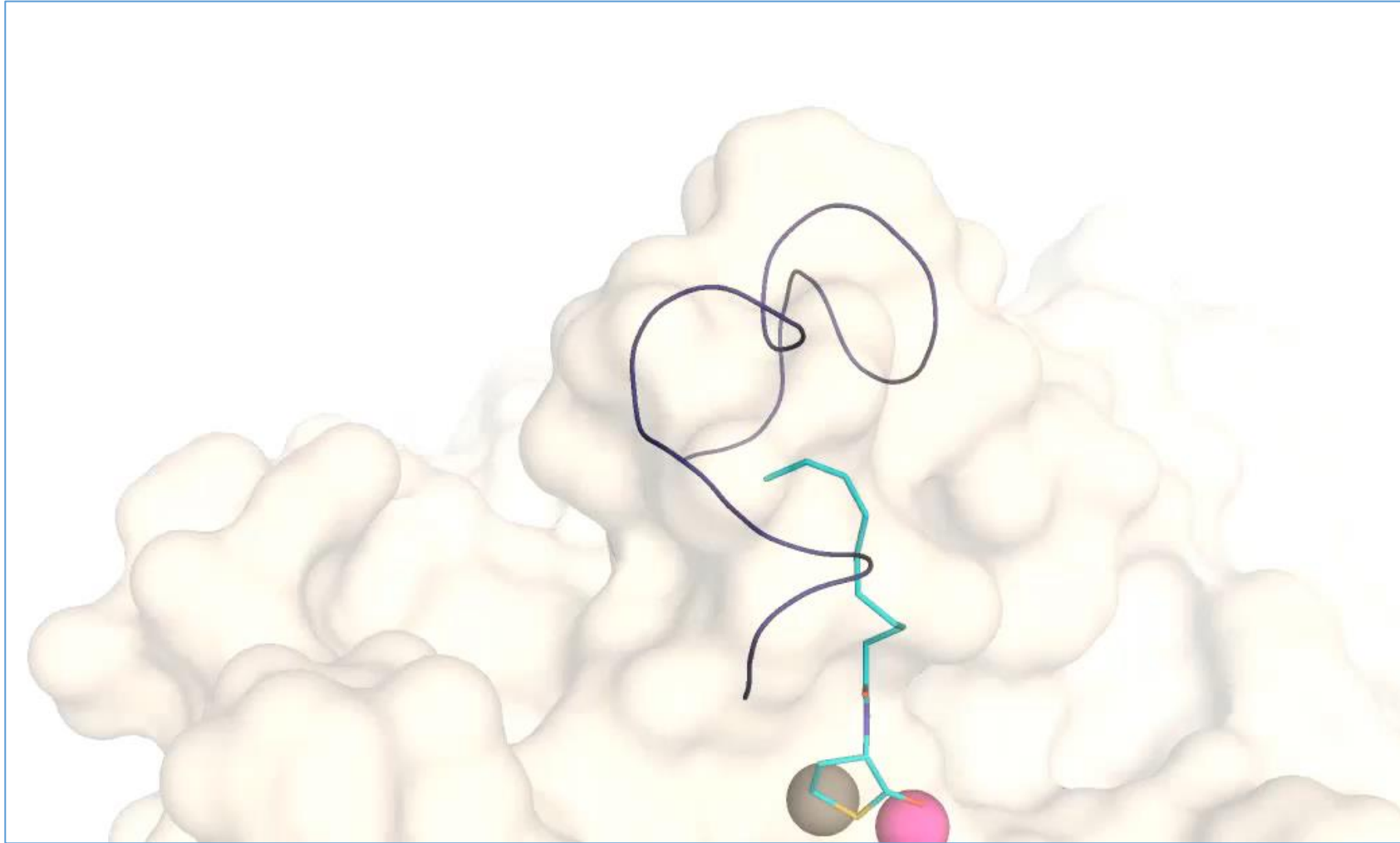
# Lactonase engineering towards various AHL



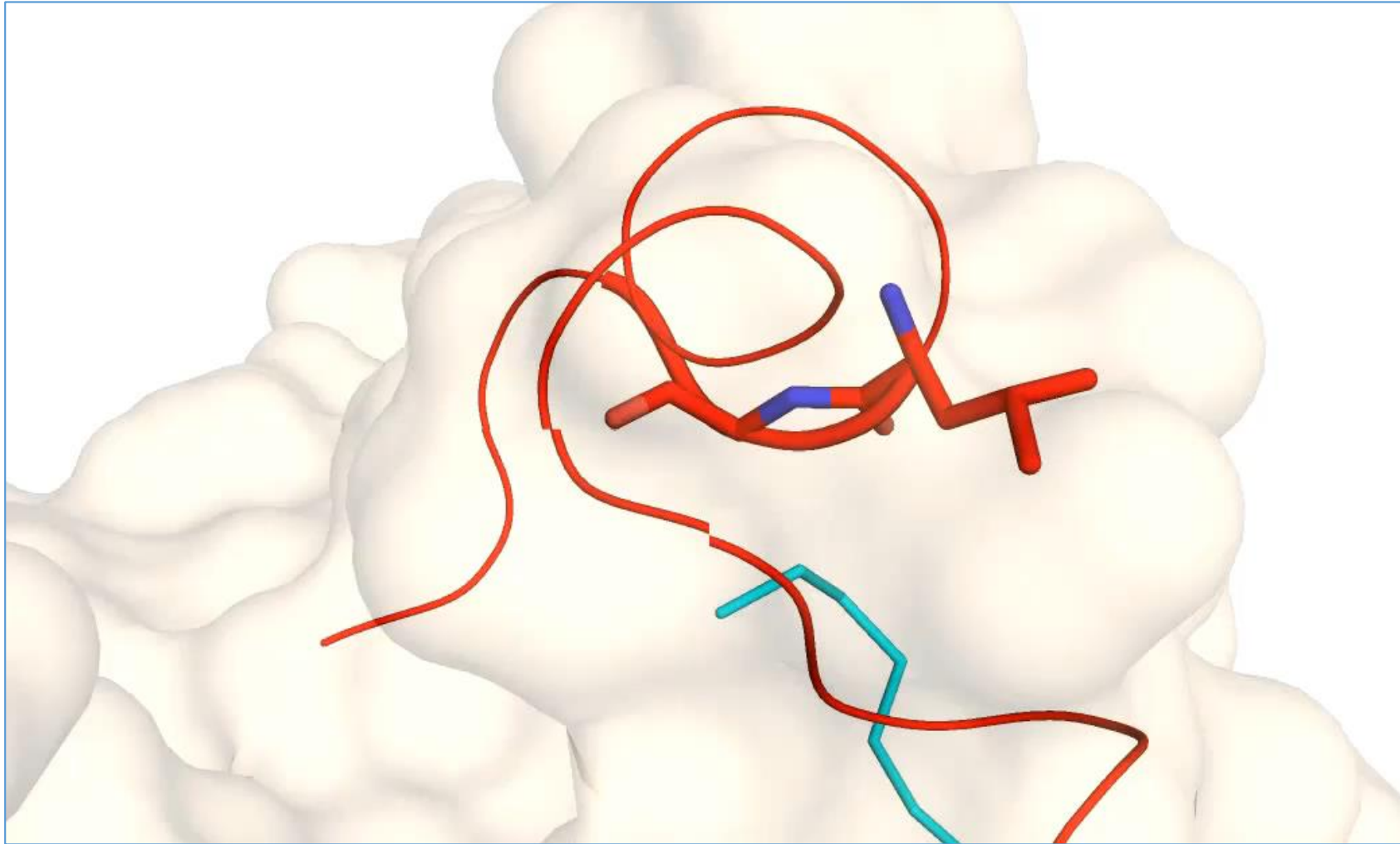
# Lactonase engineering towards short AHL



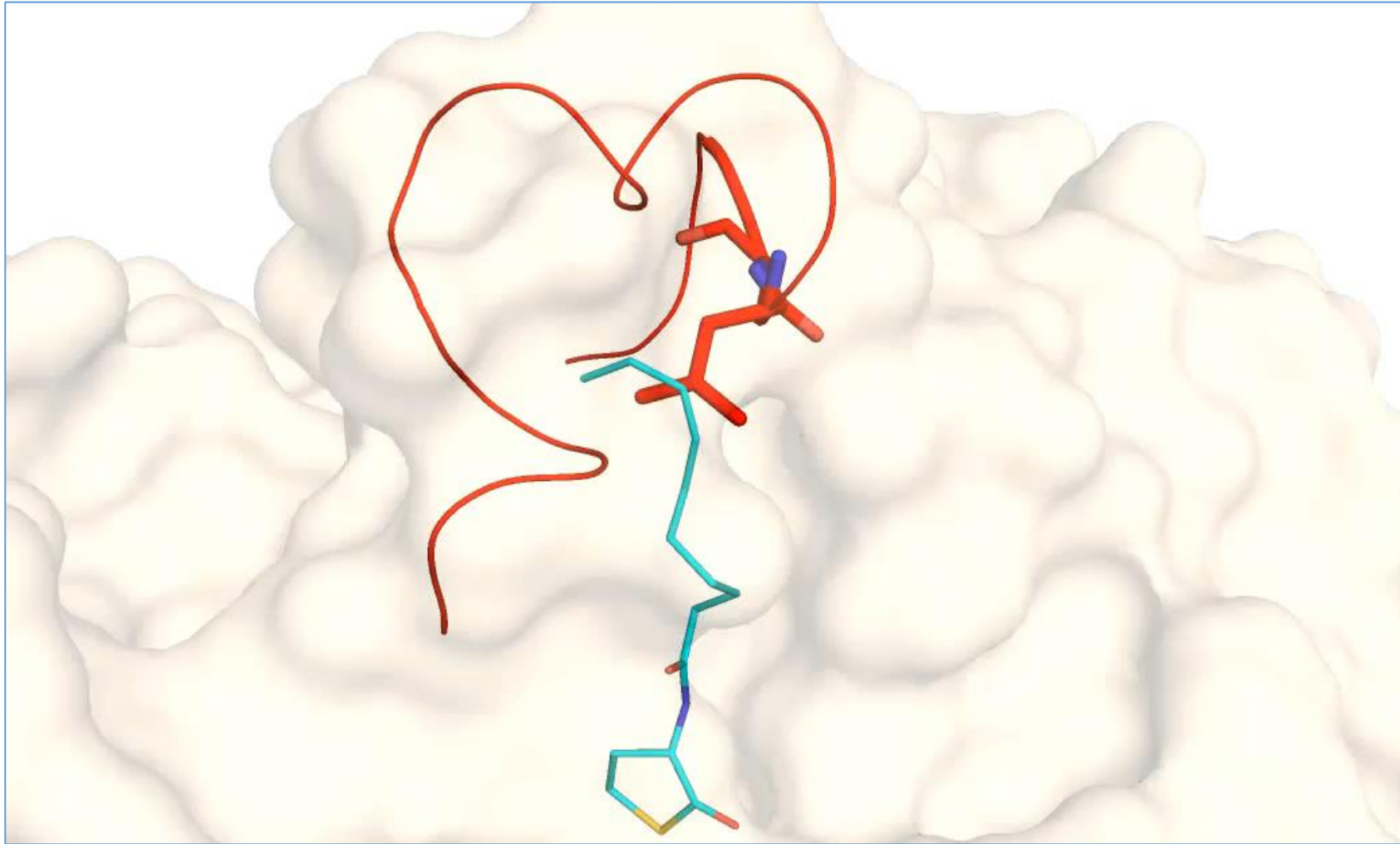
# Lactonase engineering towards short AHL



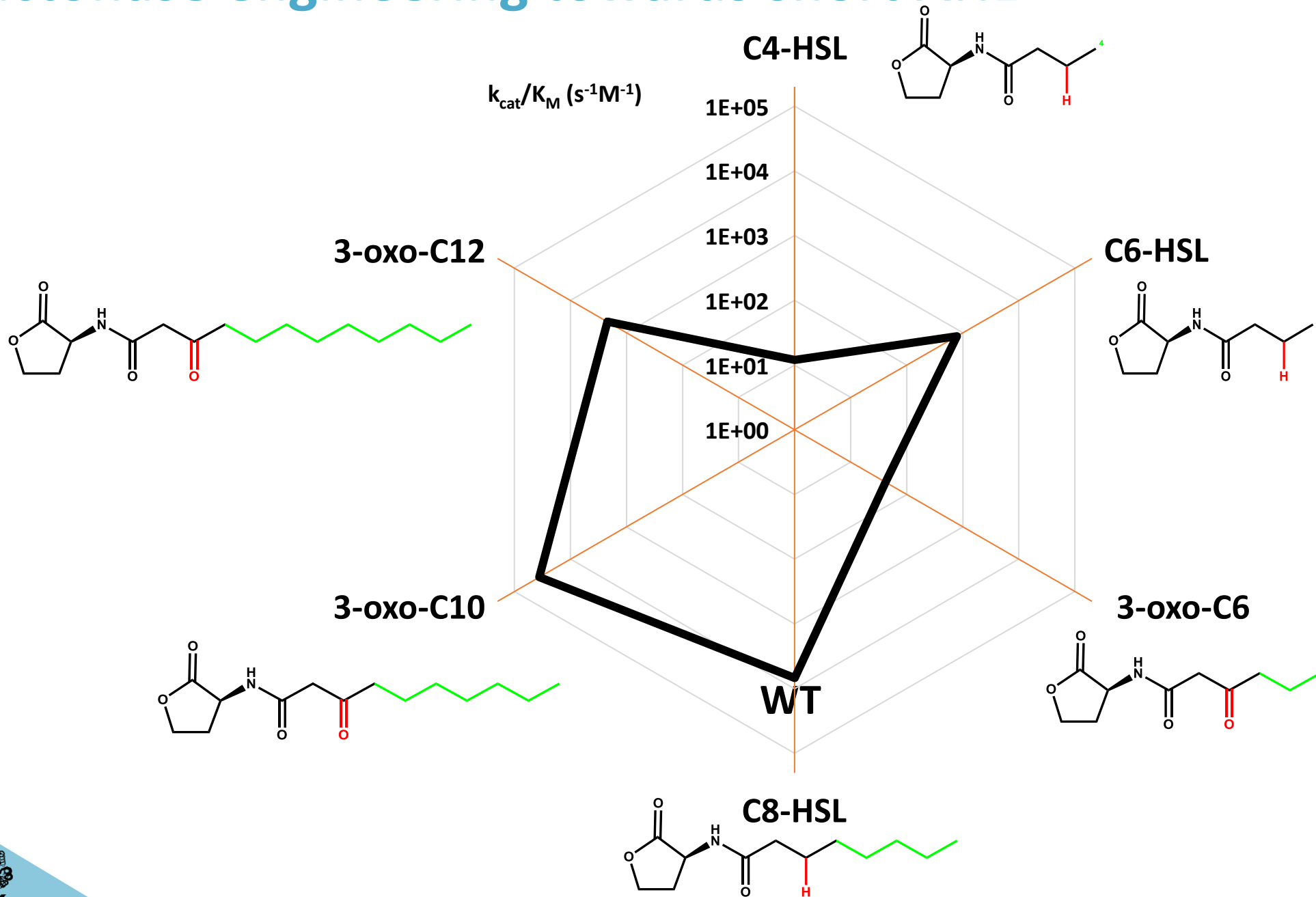
# Lactonase engineering towards short AHL



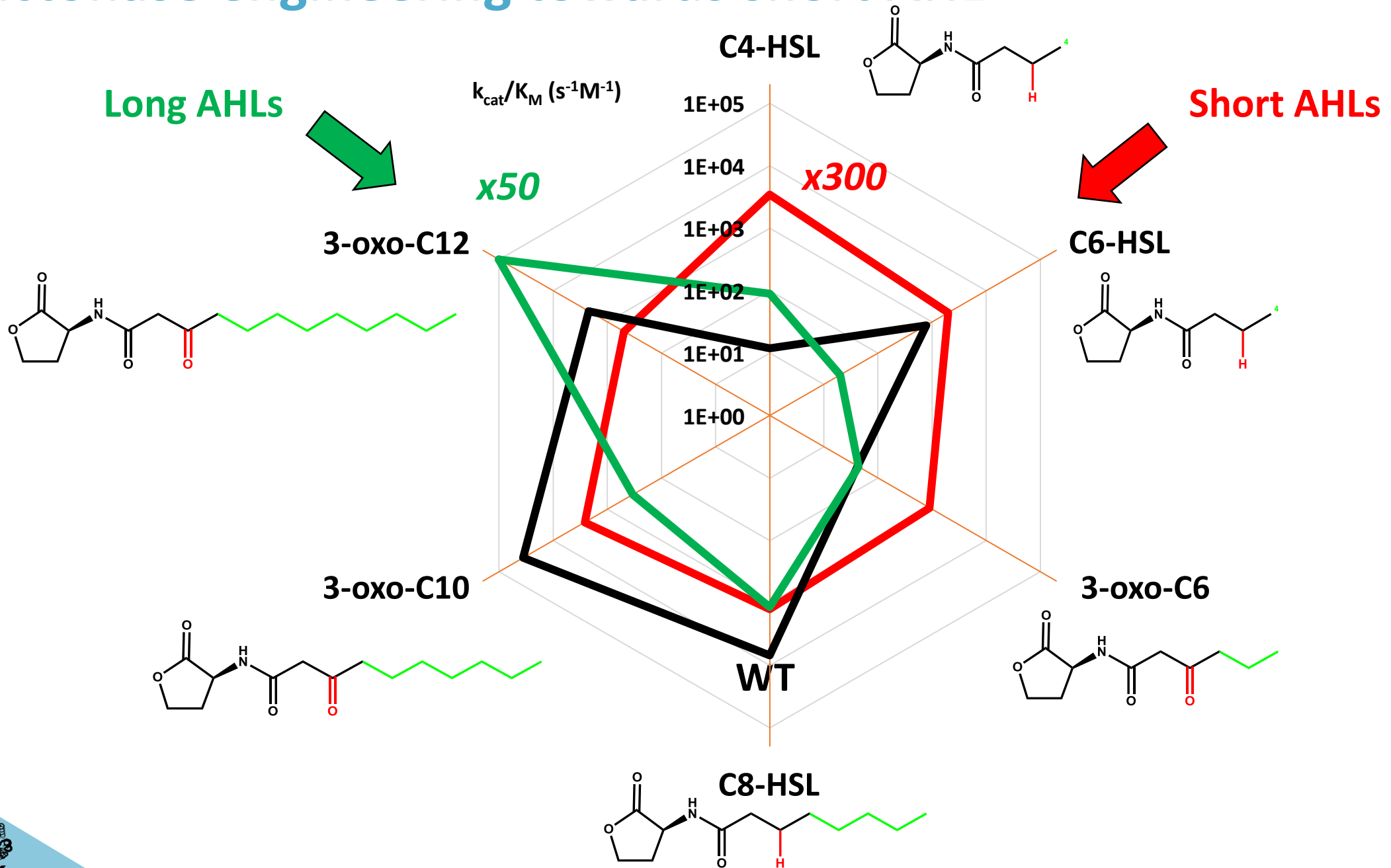
# Lactonase engineering towards short AHL



# Lactonase engineering towards short AHL



# Lactonase engineering towards short AHL



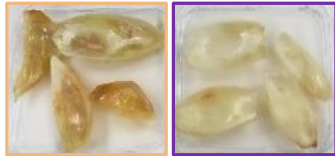
# An enzyme for every issue

*Erwinia amylovora*  
Impairs fireblight



Untreated Enzyme

*Burkholderia glumae*  
Decreases bacterial blight symptoms

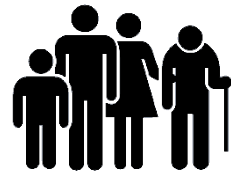


Untreated Enzyme

*Pectobacterium atrosepticum*  
Reduces black leg maceration



Untreated Enzyme



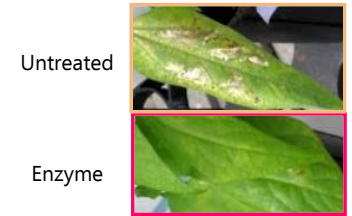
*Pseudomonas aeruginosa*

Decreases mortality in a rat pulmonary infection model  
Intratracheal treatment



x3 survival  
(Lactonase)

*Pseudomonas syringae*  
Inhibits bacterial canker



Untreated

Enzyme

*Serratia sp.*

Diminishes yellow vine symptoms

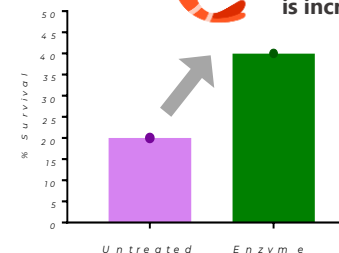


Untreated

Enzyme

*Vibrio parahaemolyticus*

Infected shrimp survival is increased



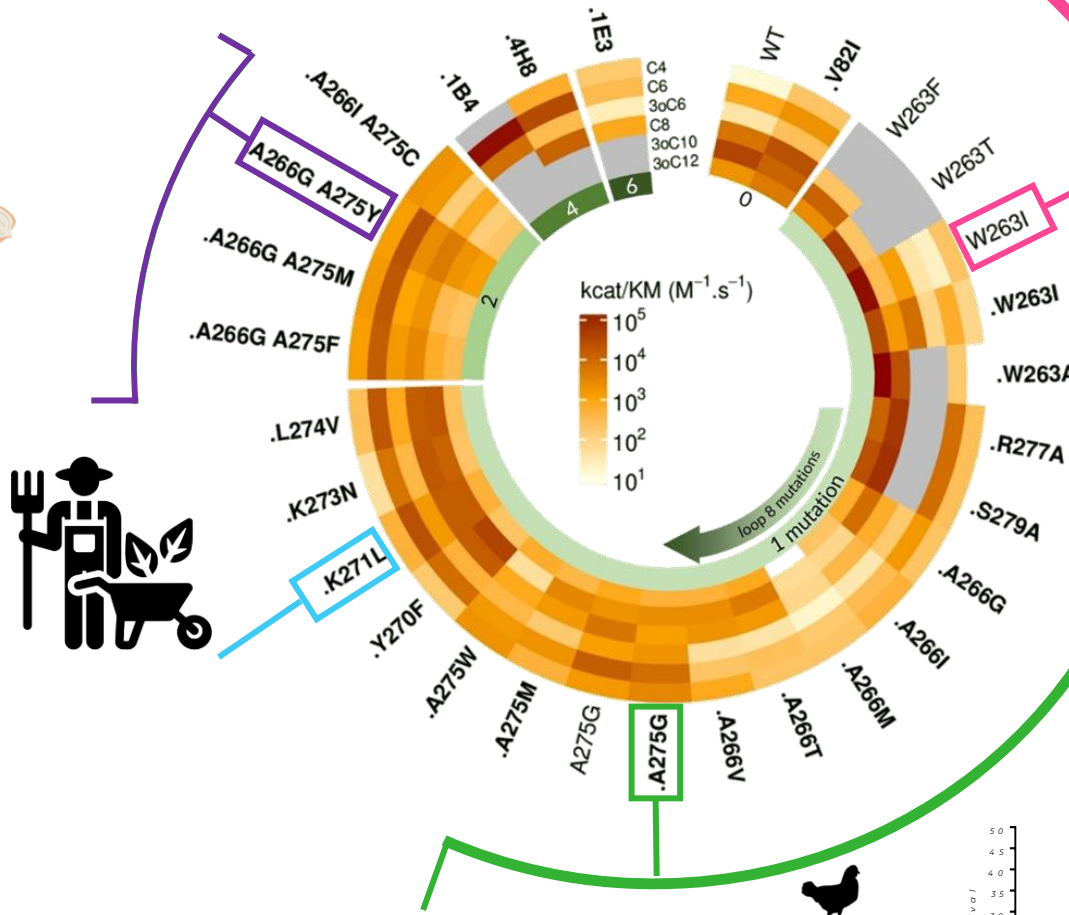
*Dickeya dadantii*

Prevents soft rotting



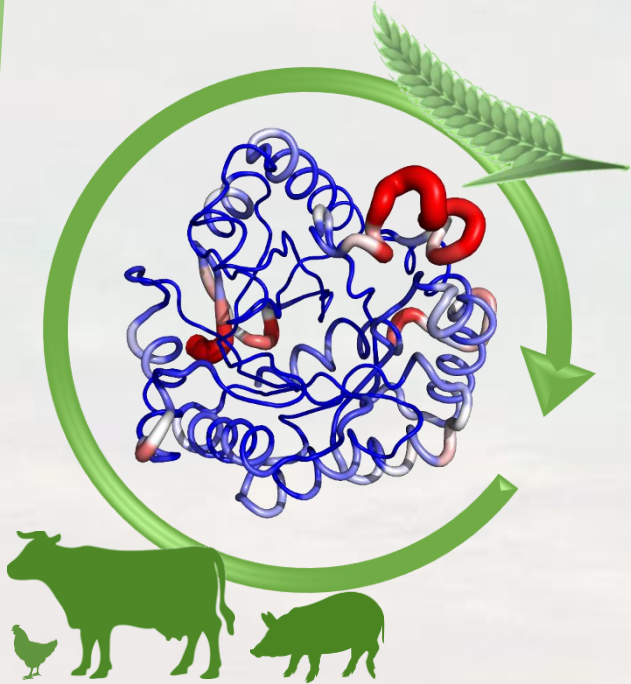
Untreated

Enzyme





# Our ambition



*Sustainable enzymes against bacterial diseases*



*Agriculture: Fighting phytopathogens and decreasing the use of phytopharmaceuticals*



## *Erwinia amylovora*



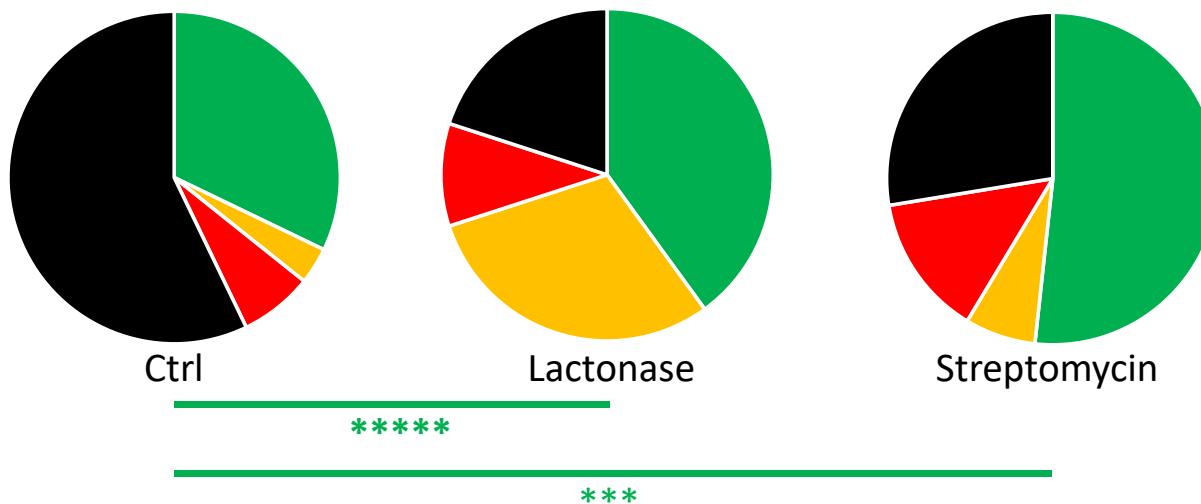
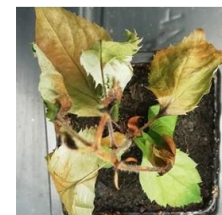
**Apples**  
*controlled conditions*

Treatment: 24h prior infection  
Infection of **Golden plantlets**  
( $10^8$  CFU/mL via infected scissors)



**Disease score** (21 dpi,  $n=30$ )

■ 0-20 ■ 25-50 ■ 50-70 ■ 75-100



***As efficient as streptomycin***



**La Pugère**

STATION D'EXPÉRIMENTATION  
EN ARBORICULTURE  
RÉGION SUD

## *Erwinia amylovora*



**Apples**  
*controlled conditions*

**Control (Not infected)**



**Control (Not treated)**



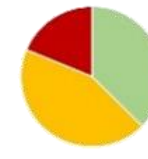
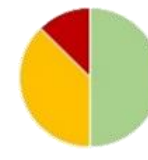
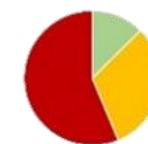
**Lactonase (0,1X)**



**Lactonase (0,01X)**



**Lactonase (0,001X)**

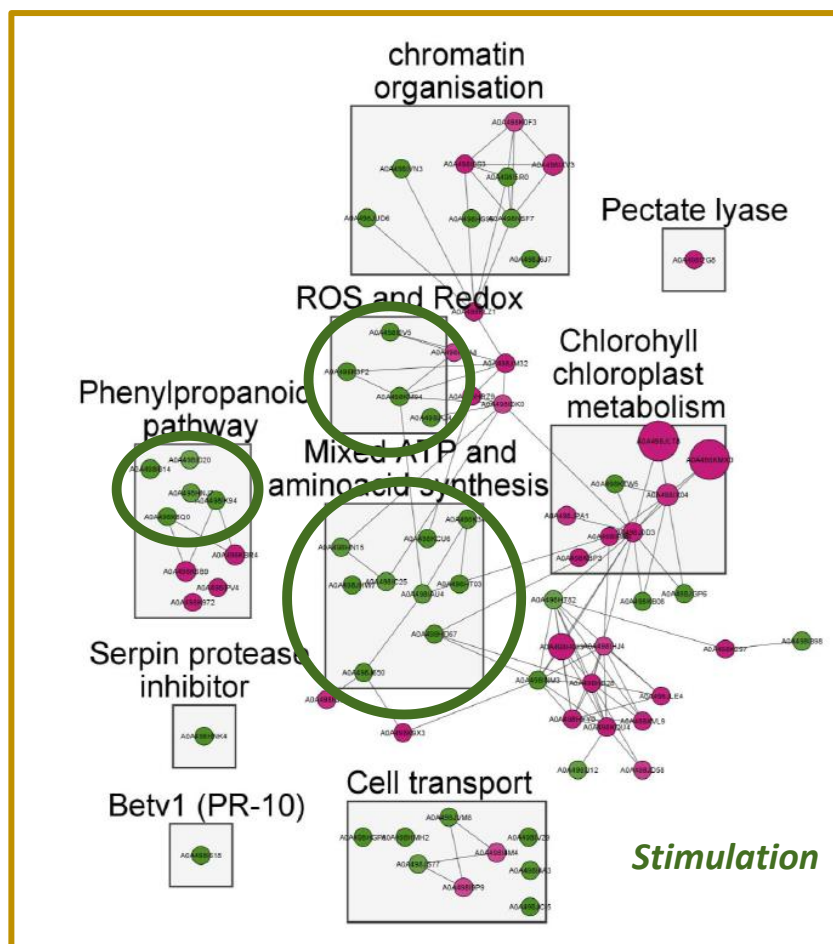


***Protection of apple flowers***

## *Erwinia amylovora*



**Apples**  
*controlled conditions*



***Proteomics analysis shows stimulation of plant health***

# Bacterial canker



## *Pseudomonas syringae*



**Beans**  
controlled conditions

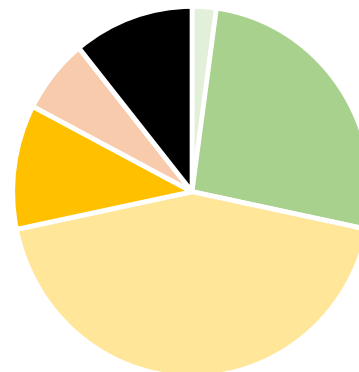


Disease score (5dpi –  $n=12$ )

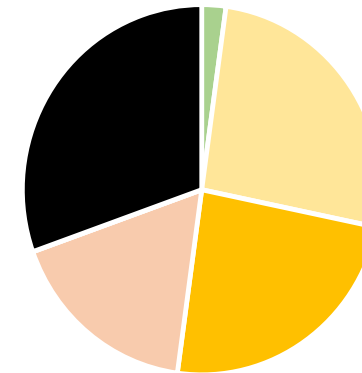
0 1 2 3 4 5 6



Ctrl



Lactonase



Copper

***More efficient than copper***

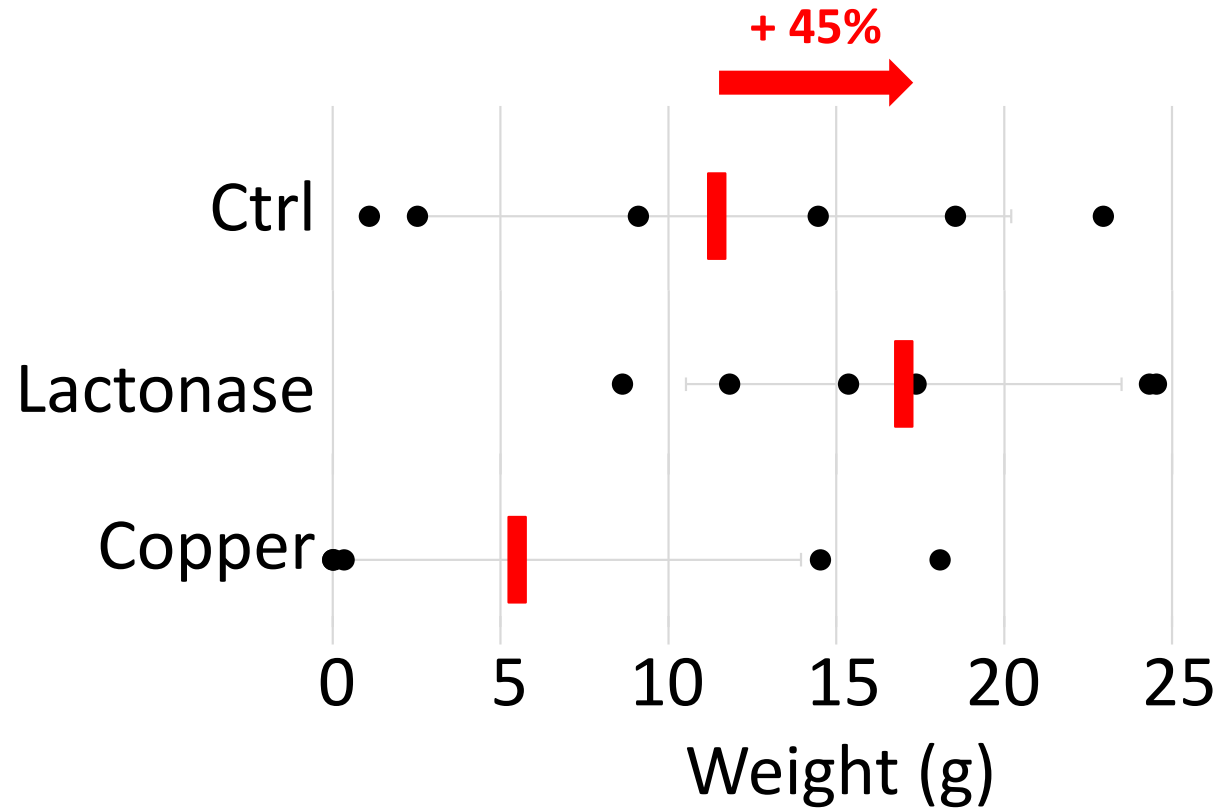
# Bacterial canker



*Pseudomonas syringae*



**Beans**  
controlled conditions



***Increase in biomass productivity***

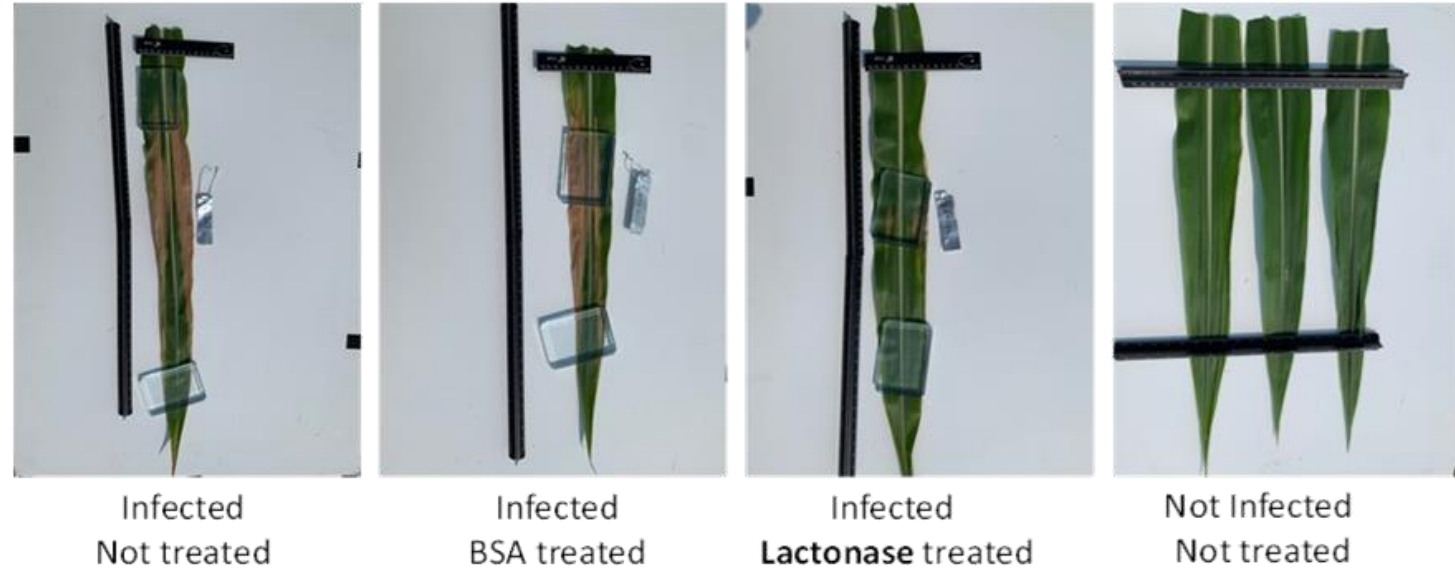
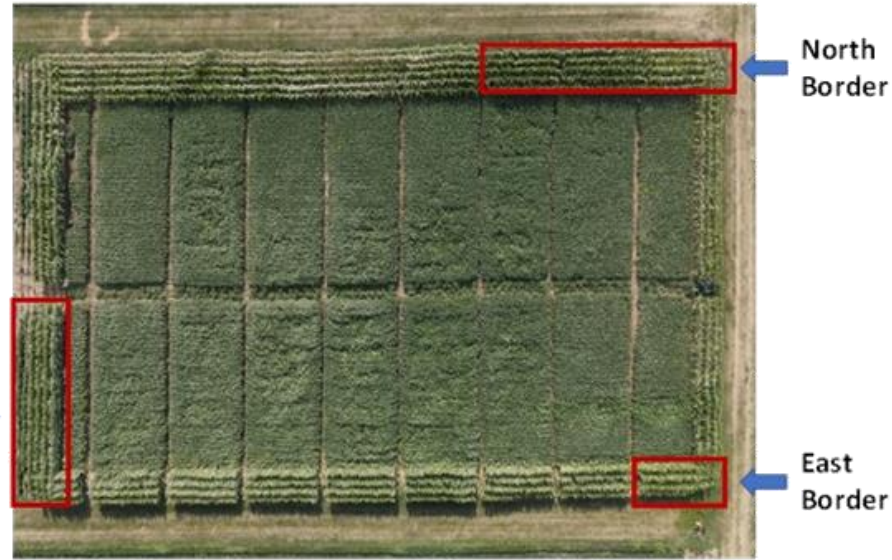
# Bacterial wilt and leaf blight



*Clavibacter michiganensis*



**Corn**  
*controlled conditions*



***Efficient protection in field experiment***

# A safe solution

No toxicity in rats (oral and dermal administration)

OECD423/OECD402

LD50 > 2000mg/kg



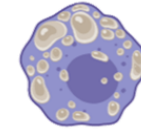
No irritation on reconstructed human skin epidermidis

OECD439 – Episkin™ Model

96% (>50%) mean viability (MTT)



No cytotoxicity on macrophages

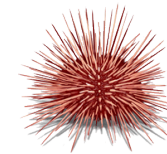


Urchin larva (*Paracentrotus lividus*)

EC50 > 10 mg/L

EC10 > 10 mg/L

NOEC 10 mg/L



Oyster larva (*Crassostrea gigas*)

EC50 > 10 mg/L

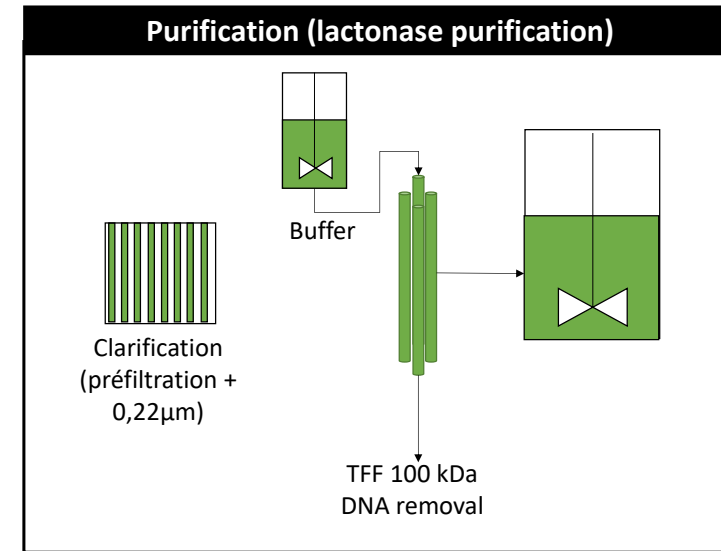
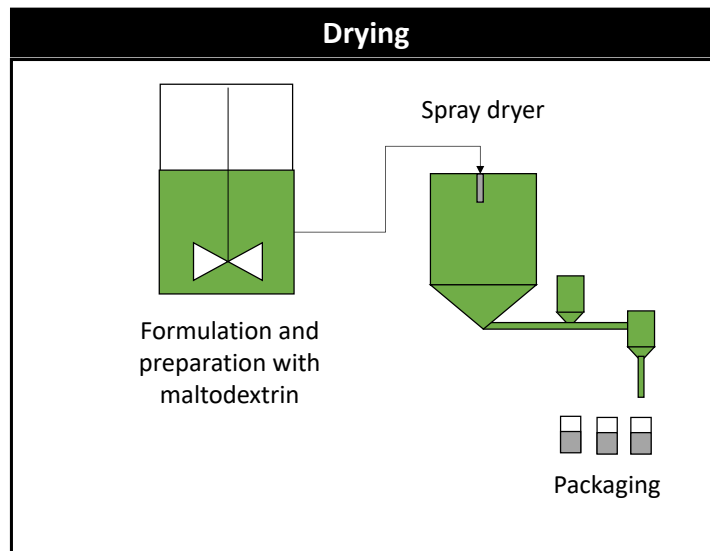
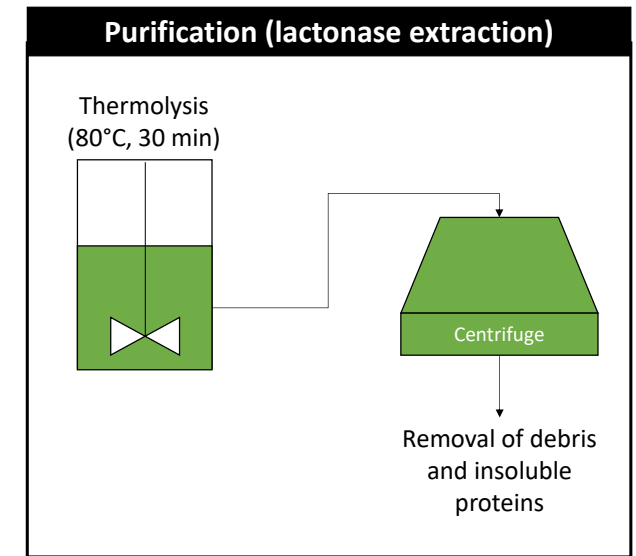
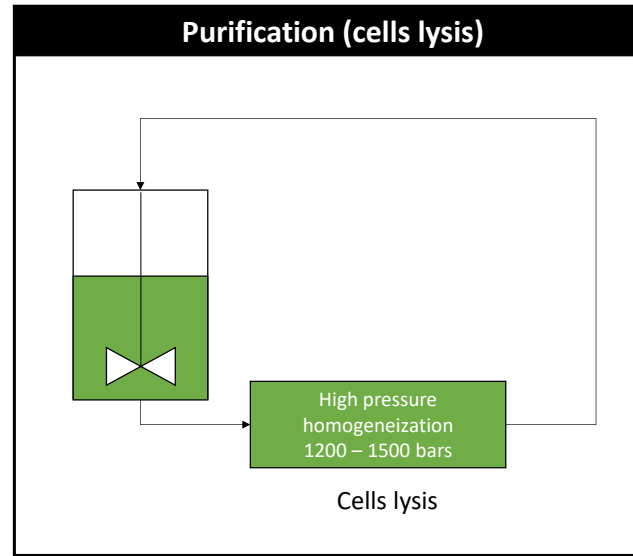
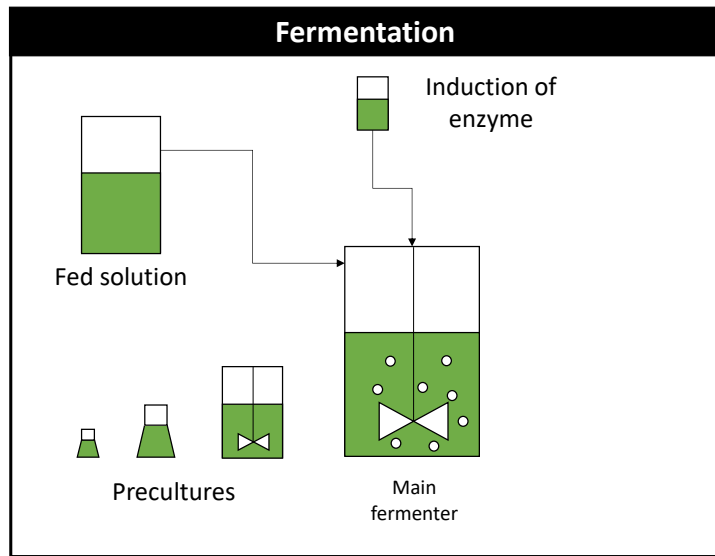
EC10 2,9-3,5 mg/L

NOEC 0,1 mg/L





# Production process

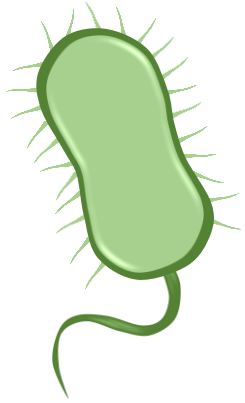


**Stable product**

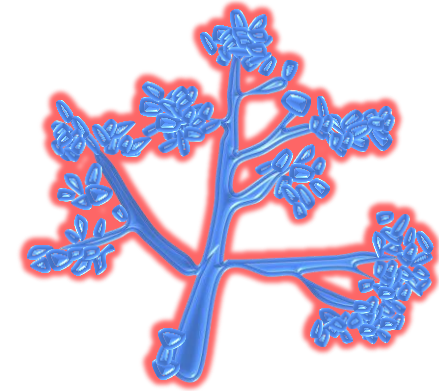
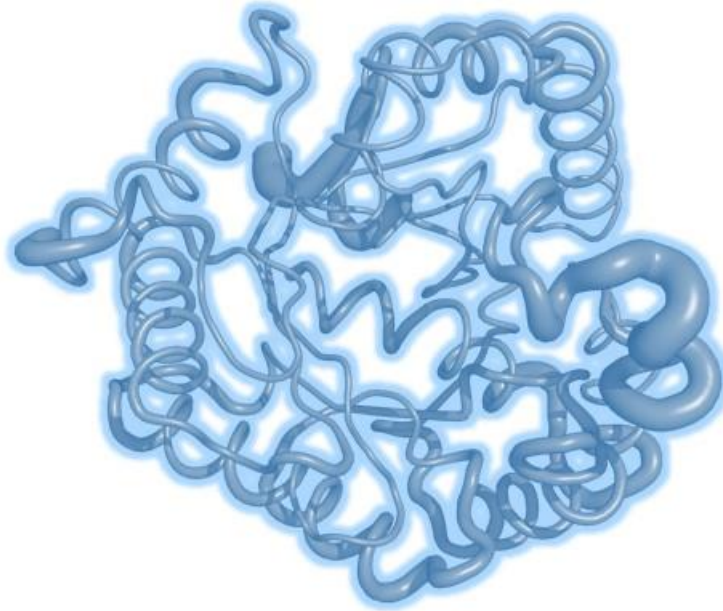
**GMO Free**

**Optimizing process before scaling-up**

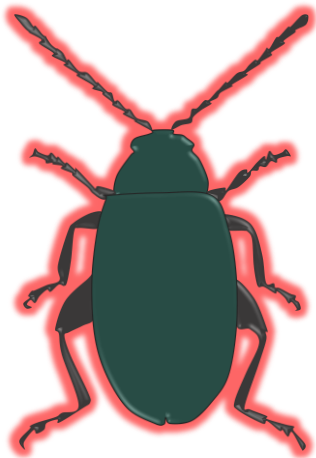
# Enzyme against phytopathogens



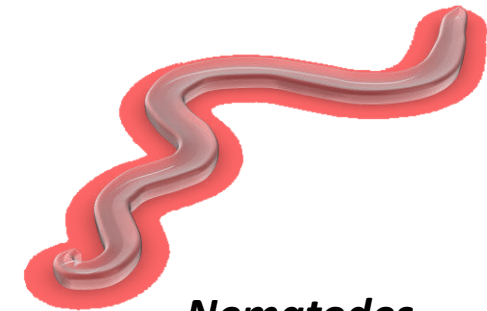
**Bacteria**



**Fungi**



**Insects**



**Nematodes**

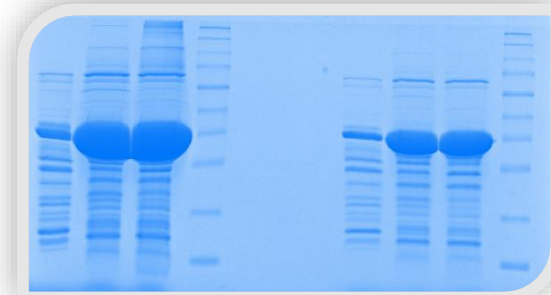
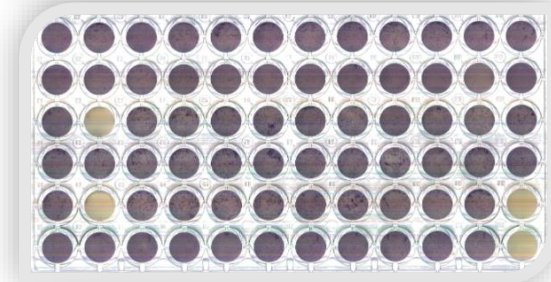
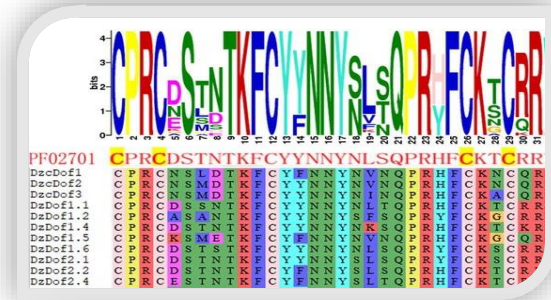
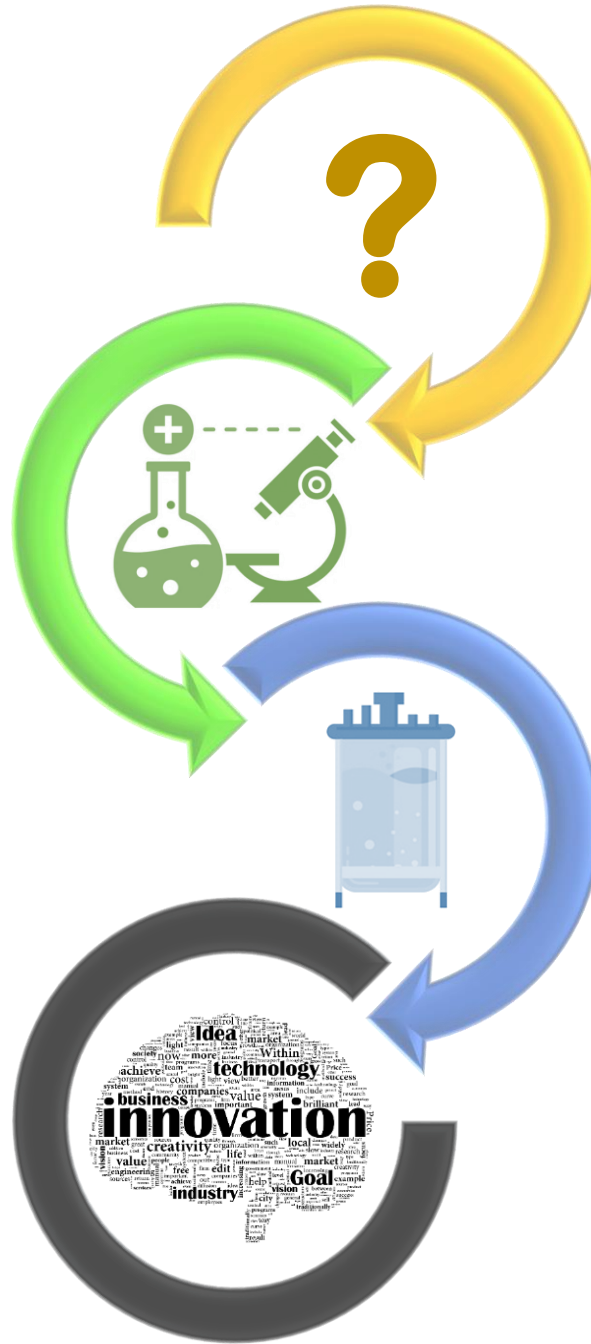
# Innovation pipeline

Searching enzymatic solutions

Enzyme engineering and validation

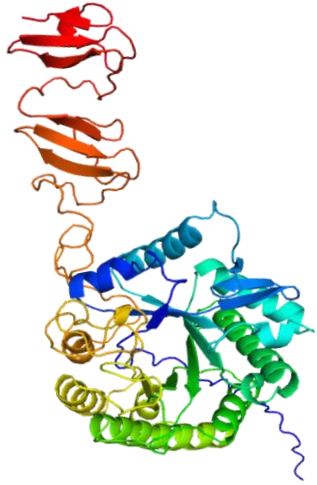
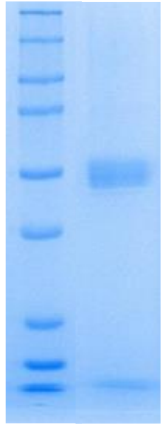
Scale-up

*Towards enzymes with fungicide and insecticide properties*

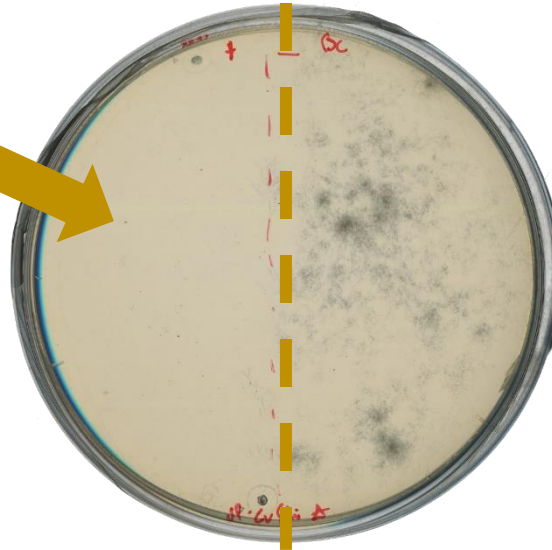


# A declinable know-how

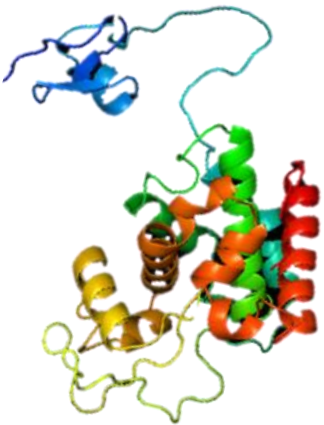
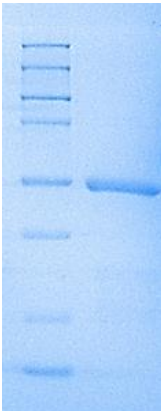
New antifungal enzymes    Control NT



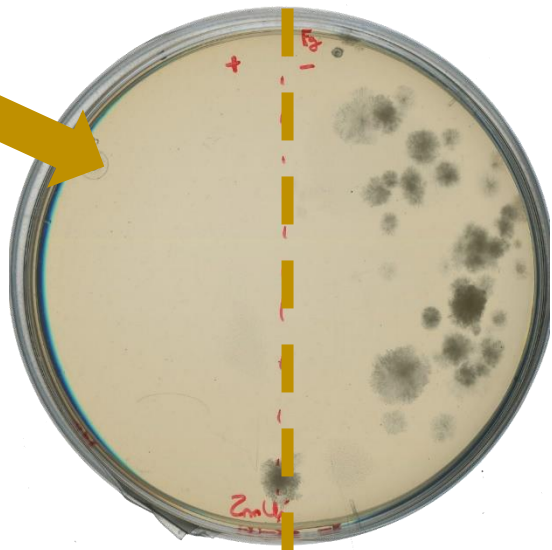
Inhibition



*Botrytis cinerea*



Inhibition



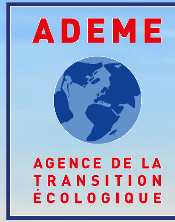
*Fusarium graminearum*

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*Thanks for your attention*

Email: [david.daude@gene-greentk.com](mailto:david.daude@gene-greentk.com)

Phone: +33 (6) 26 784 214

Address: 19-21 Boulevard Jean Moulin, 13005 Marseille

Website: [www.gene-greentk.com](http://www.gene-greentk.com)

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