

**An enzymatic extract of the proliferative red seaweed *Solieria chordalis*  
reduces the virulence of *Pseudomonas aeruginosa*  
and  
stimulates the immune system of *Caenorhabditis elegans*.**

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Bedoux Gilles<sup>1</sup>, Bourgougnon Nathalie<sup>1</sup>.

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# Context

- Since 2002, **more than 2 000 tons** of *Solieria chordalis* had to be removed from beaches in South Brittany.

(Neveu 2016)

- Between 2002 and 2015: annual cost for seaweed collection : **~ 105 000 US\$**

(Neveu 2016)

## Economical and ecological problems



# Objective

Active Water Soluble Extracts  
for the prevention of bacterial infections in  
animals, plants and humans

Using the pathogenicity model  
*Caenorhabditis elegans* –  
*Pseudomonas aeruginosa*



An environmentally friendly  
extraction method:  
**Enzyme-Assisted Extraction (EAE)**



Proliferative macroalga:  
***Solieria chordalis***



# Objective



Proliferative macroalga:

*Solieria chordalis*



# Part I : *Solieria chordalis*

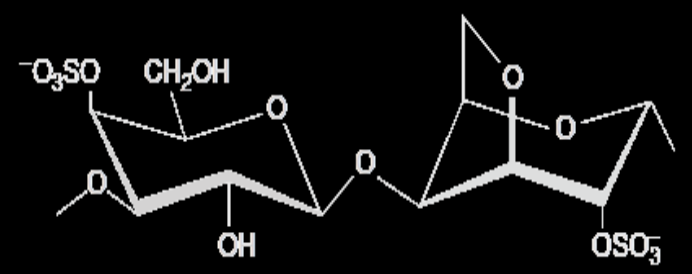


## Classification :

Empire : Eukaryota  
 Kingdom : Plantae  
 Phylum : Rhodophyta  
 Class : Florideophyceae  
 Order : Gigartinales  
 Family : Solieriaceae  
 Genus : *Solieria*  
 Species : *Solieria chordalis*.



Photos during scuba diving in South Brittany on october/november 2014 (Littoralg project)



l-carrageenan

○ **Sulfated polysaccharides** ~50 % of the dry matter with numerous applications. (Bondu *et al.* 2010, Hardouin *et al.* 2013, Prajapati *et al.* 2014)



5-8 m depth

# Objective



An environmentally friendly  
extraction method:  
**Enzyme-Assisted Extraction (EAE)**



Proliferative macroalga:  
***Solieria chordalis***





# Part II : Enzyme-Assisted-Extraction



## Production of 2 Water Soluble Extracts (WSE)



without enzyme  
**(TN)**

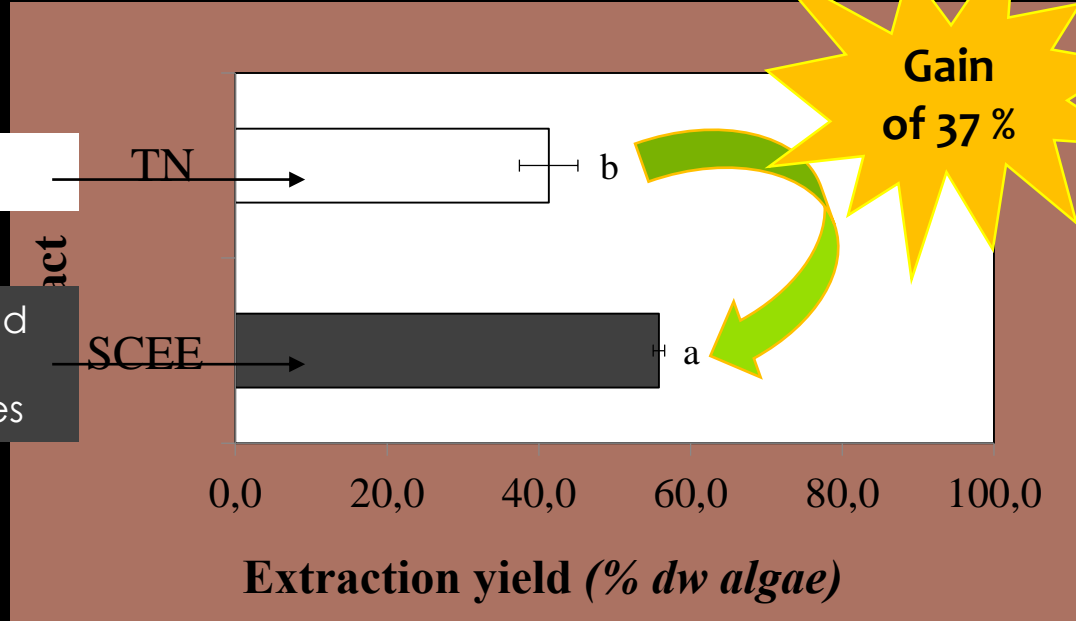
or  
with 2,5 % of enzymes  
**(SCEE)**

3h, at 50°C, in distilled water

Advantages of EAE
No organic solvent
Shorter time production
Water Soluble Bioproducts
Enzyme can be reused

+++ ash

+++ proteins and sulfated polysaccharides



# Objective

Active Water Soluble Extracts  
for the prevention of bacterial infections in  
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Using the pathogenicity model  
*Caenorhabditis elegans* –  
*Pseudomonas aeruginosa*



An environmentally friendly  
extraction method:  
**Enzyme-Assisted Extraction (EAE)**



Proliferative macroalga:

***Solieria chordalis***



# Part III: Application of WSE in the prevention of bacterial infections

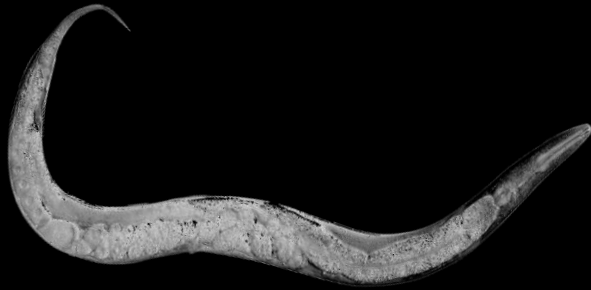
## Pathogenicity model

*C. elegans* -  
*P. aeruginosa* :

Antibacterial activity

Immuno-stimulant activity

*Caenorhabditis elegans*  
(nematode size: 1 mm)



### Innate Immune System

Antibacterial effectors  (antibacterial proteins, lysozyme)	Subsistence  (lipase, lectin)
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Water Soluble Extracts (WSE) of  
*S. chordalis*  
(**SCEE** and **TN**)

*P. aeruginosa*,  
strain PA14  
(bacteria size 1-5  $\mu\text{m}$ )



### Bacterial virulence

Virulence Factors

(hydrogen cyanide, superoxyde dismutase)

Regulation system :

**quorum sensing**

# Part III: Application of WSE in the prevention of bacterial infections

## ➤ Impact of WSE on the survival rate of infected *C. elegans*

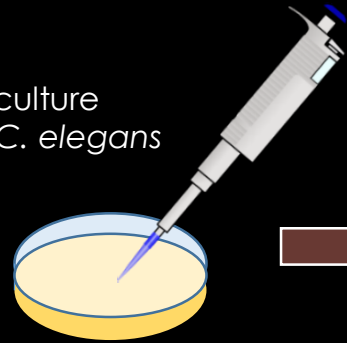
### Pathogenicity model

*C. elegans* - *P. aeruginosa* :

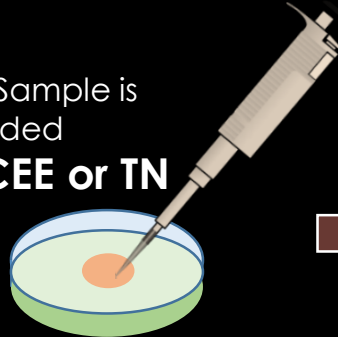
Antibacterial activity

Immuno-stimulant activity

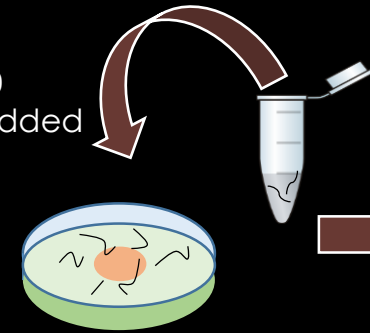
1) PA14 on culture medium of *C. elegans*



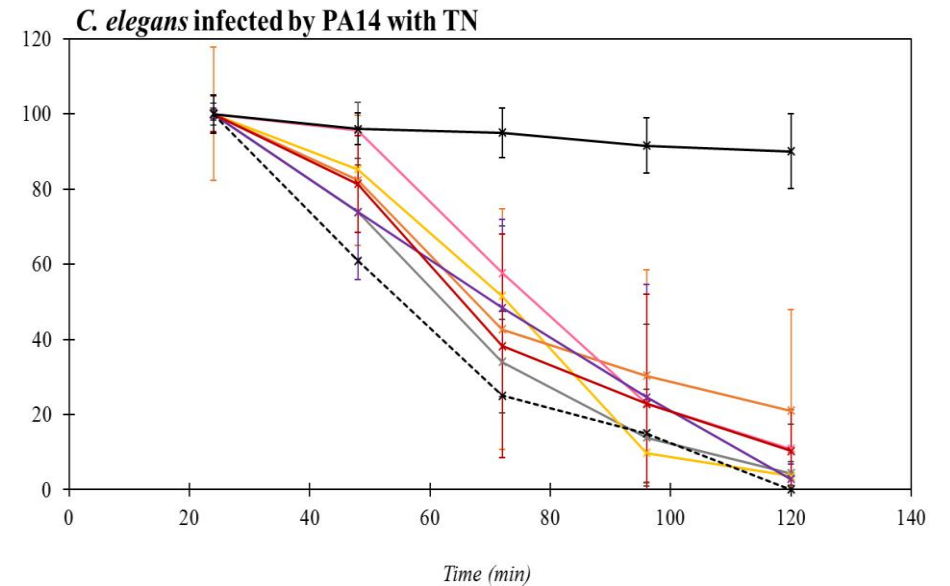
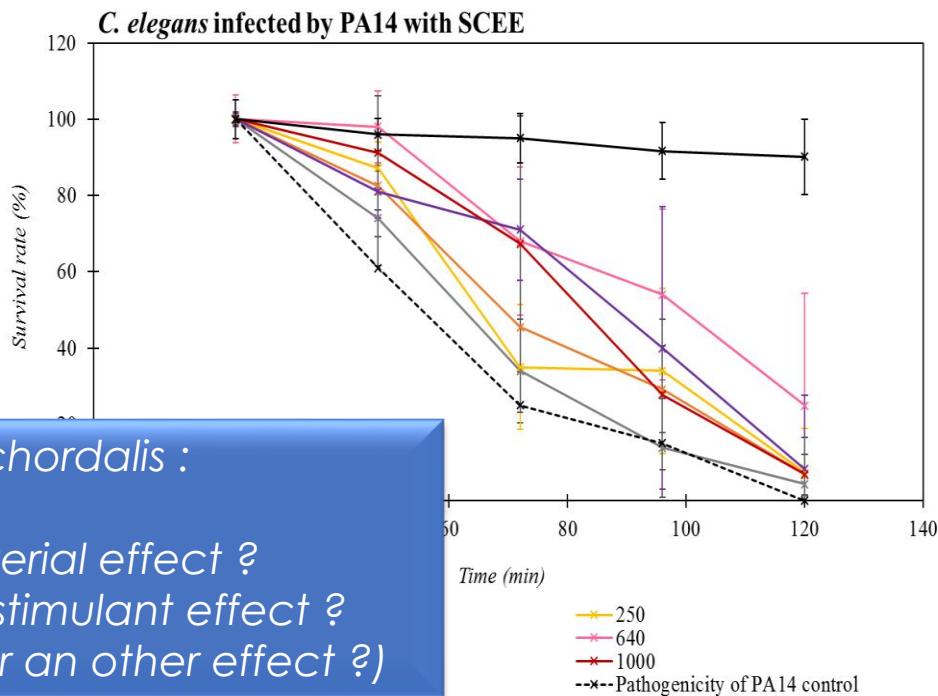
2) Sample is added  
**SCEE or TN**



3) Around 30 worms are added



4) Counting every 24h



### ➤ Do WSEs of *S. chordalis* :

have an antibacterial effect ?  
have an immunostimulant effect ?  
(both of them ? or an other effect ?)

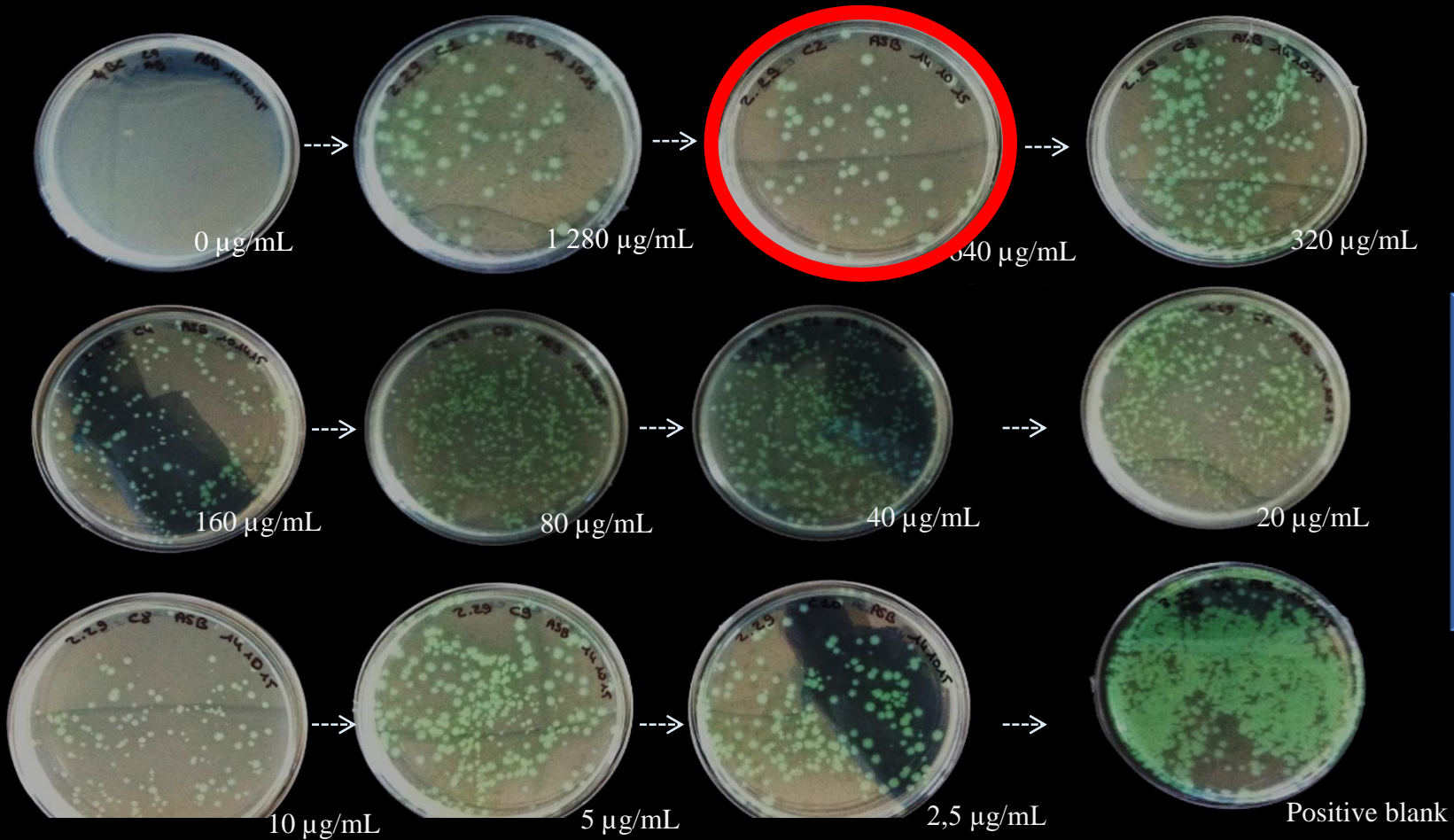
# Part III: Application of WSE in the prevention of bacterial infections

Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

Antibacterial activity

Immuno-stimulant activity

➤ Impact of SCEE on bacterial growth  
Microdilutions method (Wiegand *et al.* 2008)



*P. aeruginosa*,  
strain PA14



- SCEE (640 µg/mL) inhibits 69 % of PA14 growth.
- TN (640 µg/mL) inhibits 46.5 % of PA14 growth.



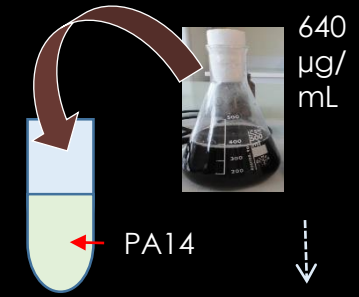
# Part III: Application of WSE in the prevention of bacterial infections

Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

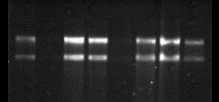
**Antibacterial activity**

Immuno-stimulant activity

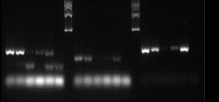
## ➤ Impact of SCEE on gene expression of PA14 virulence (ΔΔCt Analysis)



RNA extraction of PA14



Reverse transcription into cDNA



qPCR



**Bacterial virulence analysis**

Genes coding for **quorum sensing**

Genes coding for virulence factors



# Part III: Application of WSE in the prevention of bacterial infections

Pathogenicity model  
*C. elegans* - *P. aeruginosa*

**Antibacterial activity**

Immuno-stimulant activity

## ➤ Impact of SCEE on gene expression of PA14 virulence

### ( $\Delta\Delta C_t$ Analysis)

Exponential bacterial growth  
(6h of contact)

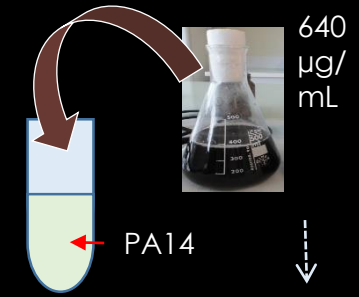
Stationary bacterial growth  
(24h of contact)

*rhII*  
*rhIR*  
*lasI*  
*lasR*

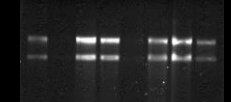
*rhII*  
*rhIR*  
*lasI*  
*lasR*

*hcnC*  
*aroE*  
*rpoN*  
*sodB*  
*sbe*

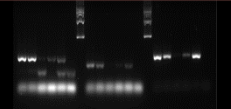
*hcnC*  
*aroE*  
*rpoN*  
*sodB*  
*sbe*



RNA extraction of PA14



Reverse transcription into cDNA



qPCR



**Bacterial virulence analysis**

Genes coding for **quorum sensing**

Genes coding for virulence factors

# Part III: Application of WSE in the prevention of bacterial infections

Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

Antibacterial activity

Immuno-stimulant activity

## ➤ Impact of SCEE on gene expression of PA14 virulence

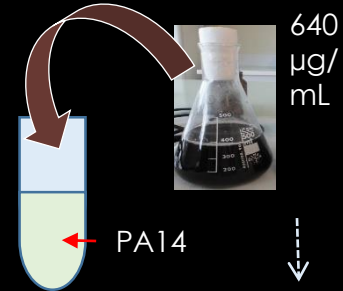
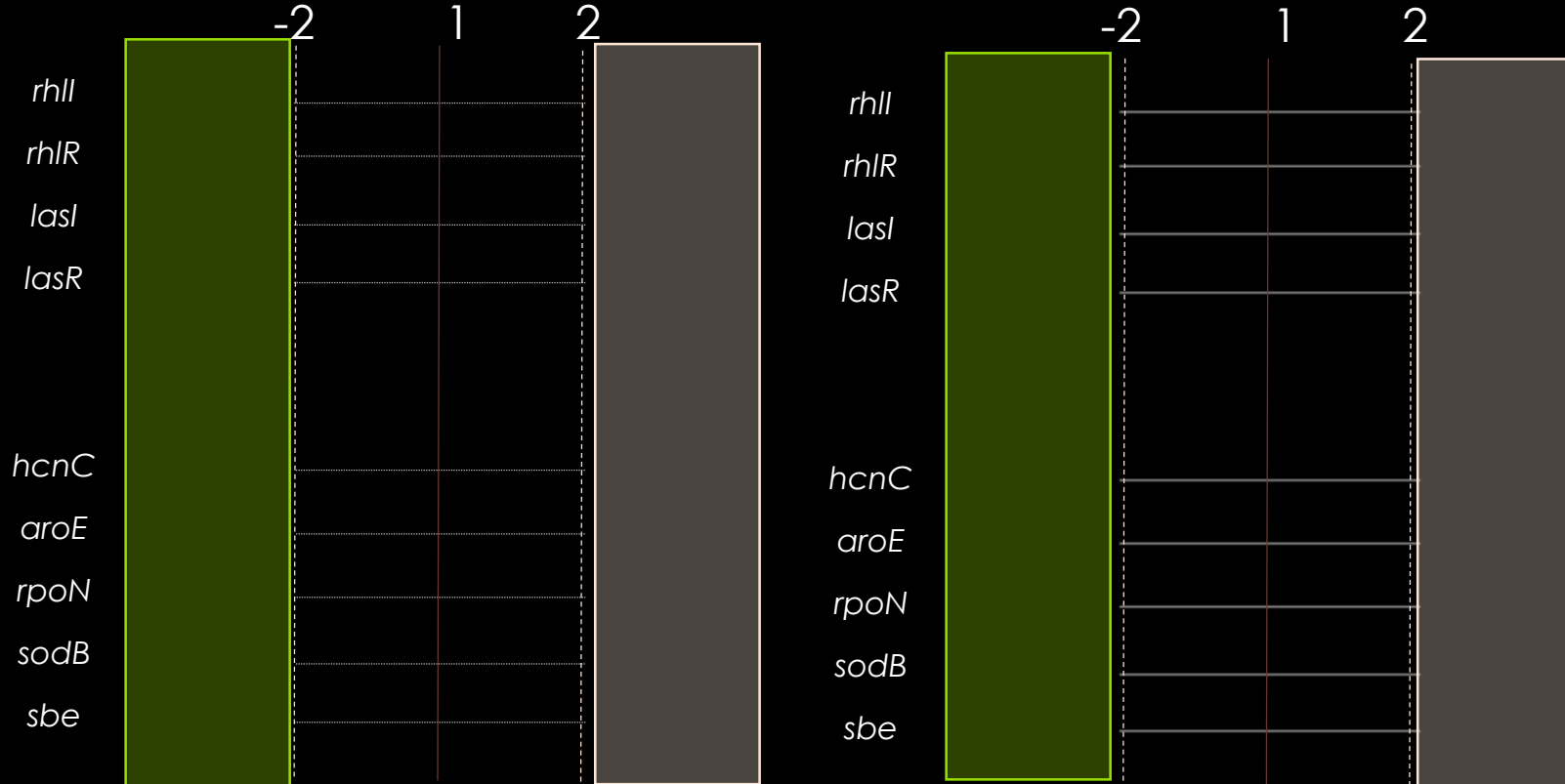
### ( $\Delta\Delta C_t$ Analysis)

Exponential bacterial growth  
(6h of contact)

Stationary bacterial growth  
(24h of contact)

Repression

Overexpression



RNA extraction of PA14

Reverse transcription into cDNA

qPCR

Bacterial virulence analysis

Genes coding for **quorum sensing**

Genes coding for virulence factors





# Part III: Application of WSE in the prevention of bacterial infections

Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

Antibacterial activity

Immuno-stimulant activity

### Legend

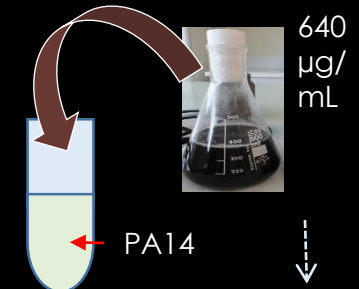
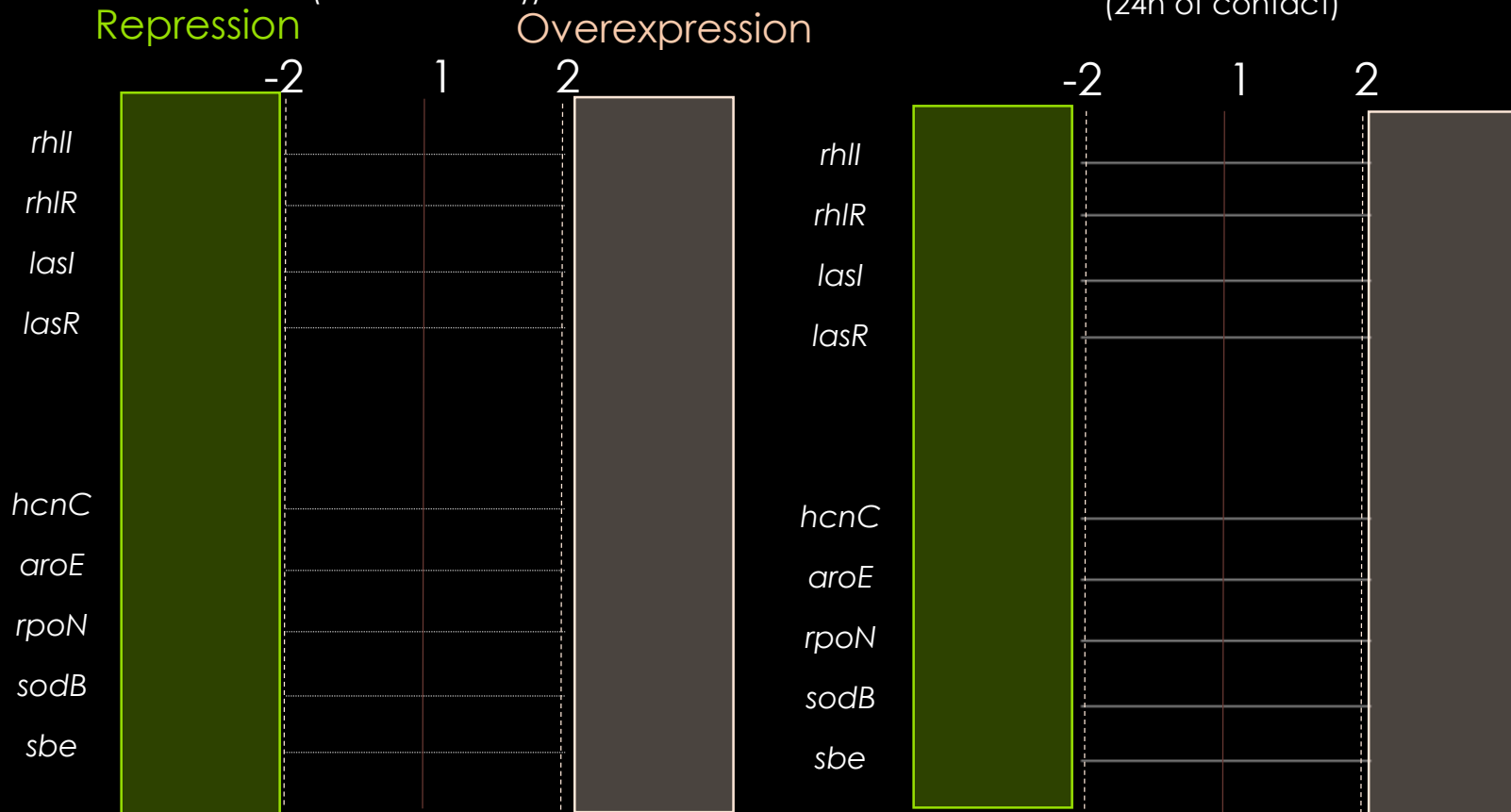
	PA14 in KB
	PA14 in KB and water
	PA14 in KB and SCEE 640 µg/mL
	PA14 in KB and TN 640 µg/mL

## ➤ Impact of SCEE on gene expression of PA14 virulence

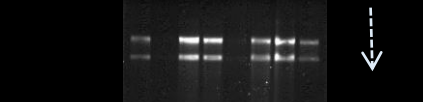
### ( $\Delta\Delta C_t$ Analysis)

Exponential bacterial growth  
(6h of contact)

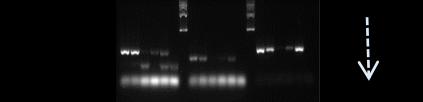
Stationary bacterial growth  
(24h of contact)



RNA extraction of PA14



Reverse transcription into cDNA



qPCR



Bacterial virulence analysis

Genes coding for **quorum sensing**      Genes coding for **virulence factors**

# Part III: Application of WSE in the prevention of bacterial infections

Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

Antibacterial activity

Immuno-stimulant activity

### Legend

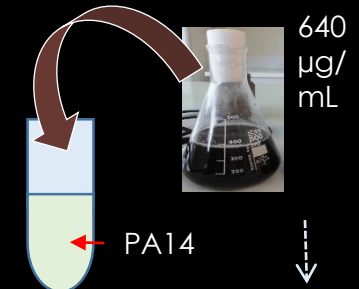
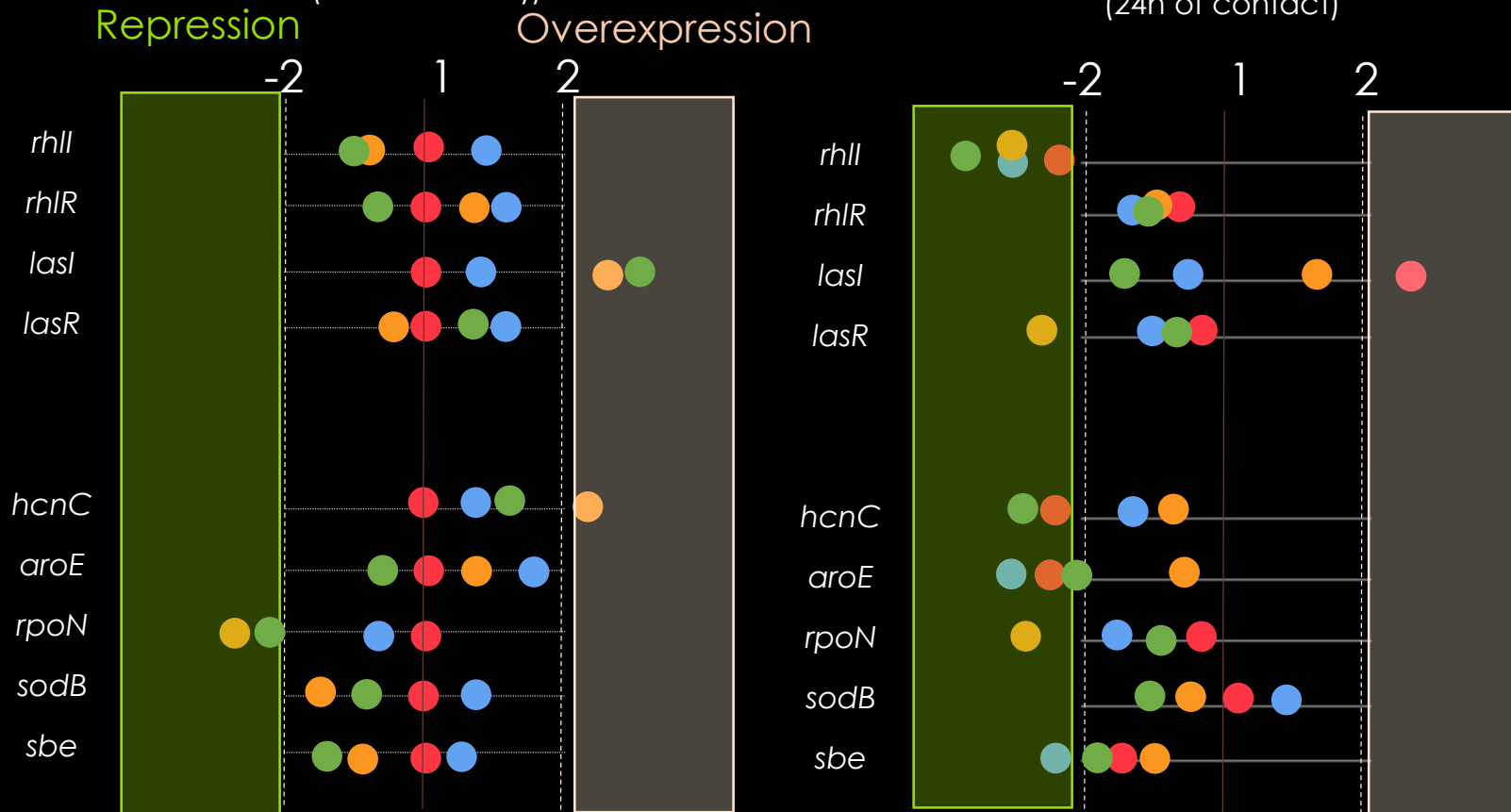
<span style="color: red;">●</span>	PA14 in KB
<span style="color: blue;">●</span>	PA14 in KB and water
<span style="color: orange;">●</span>	PA14 in KB and SCEE 640 µg/mL
<span style="color: green;">●</span>	PA14 in KB and TN 640 µg/mL

## ➤ Impact of SCEE on gene expression of PA14 virulence

### ( $\Delta\Delta C_t$ Analysis)

Exponential bacterial growth  
(6h of contact)

Stationary bacterial growth  
(24h of contact)



RNA extraction of PA14

Reverse transcription into cDNA

qPCR

Bacterial virulence analysis

Genes coding for <b>quorum sensing</b>	Genes coding for virulence factors
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An enzymatic extract of the proliferative red seaweed *S. chordalis* reduces the virulence of *P. aeruginosa* and stimulates the immune system of *C. elegans*.





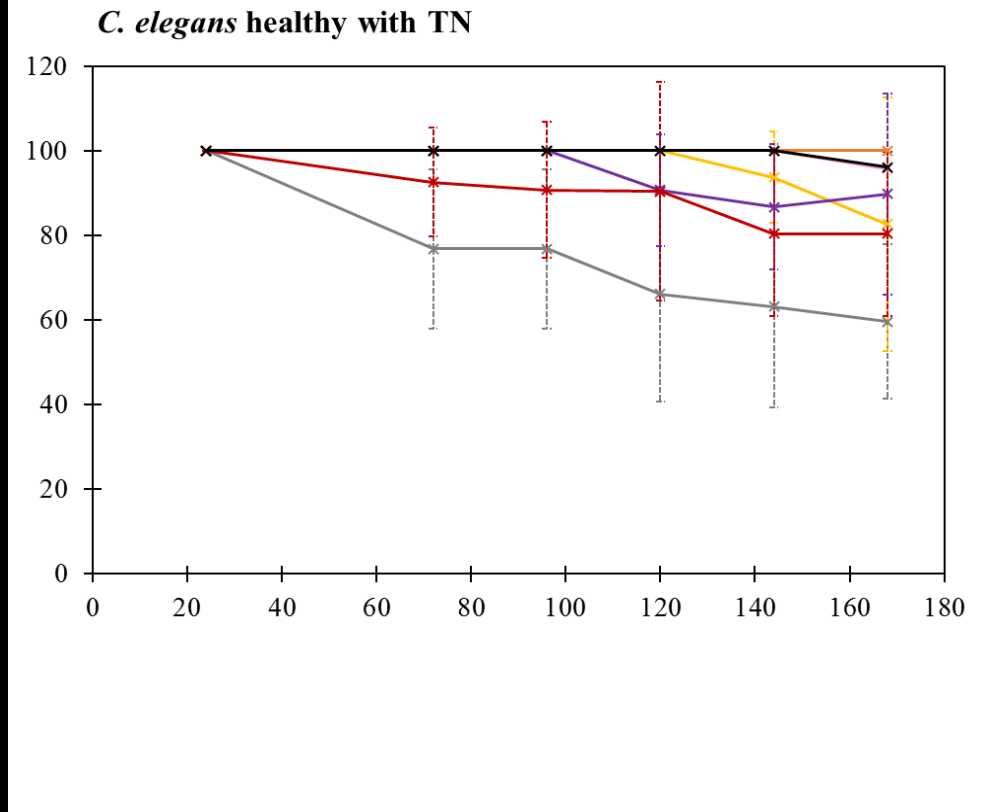
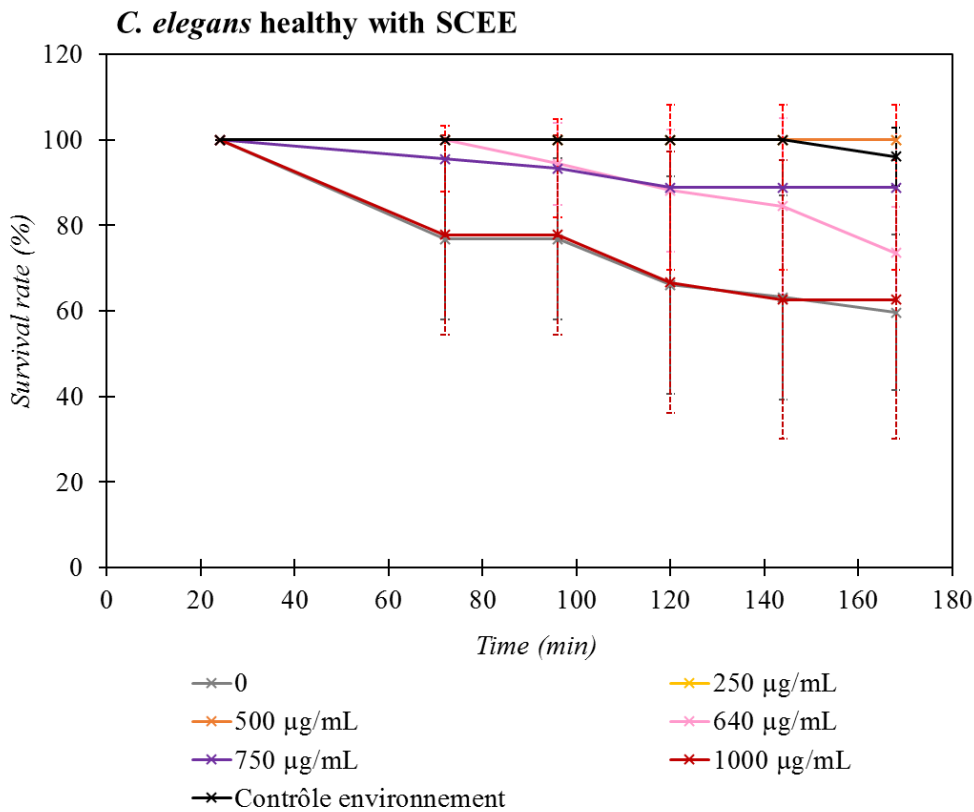
# Part III: Application of WSE in the prevention of bacterial infections

## ➤ Impact of WSE on the survival rate of healthy *C. elegans*

Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

Antibacterial activity

Immuno-stimulant activity



# Part III: Application of WSE in the prevention of bacterial infections

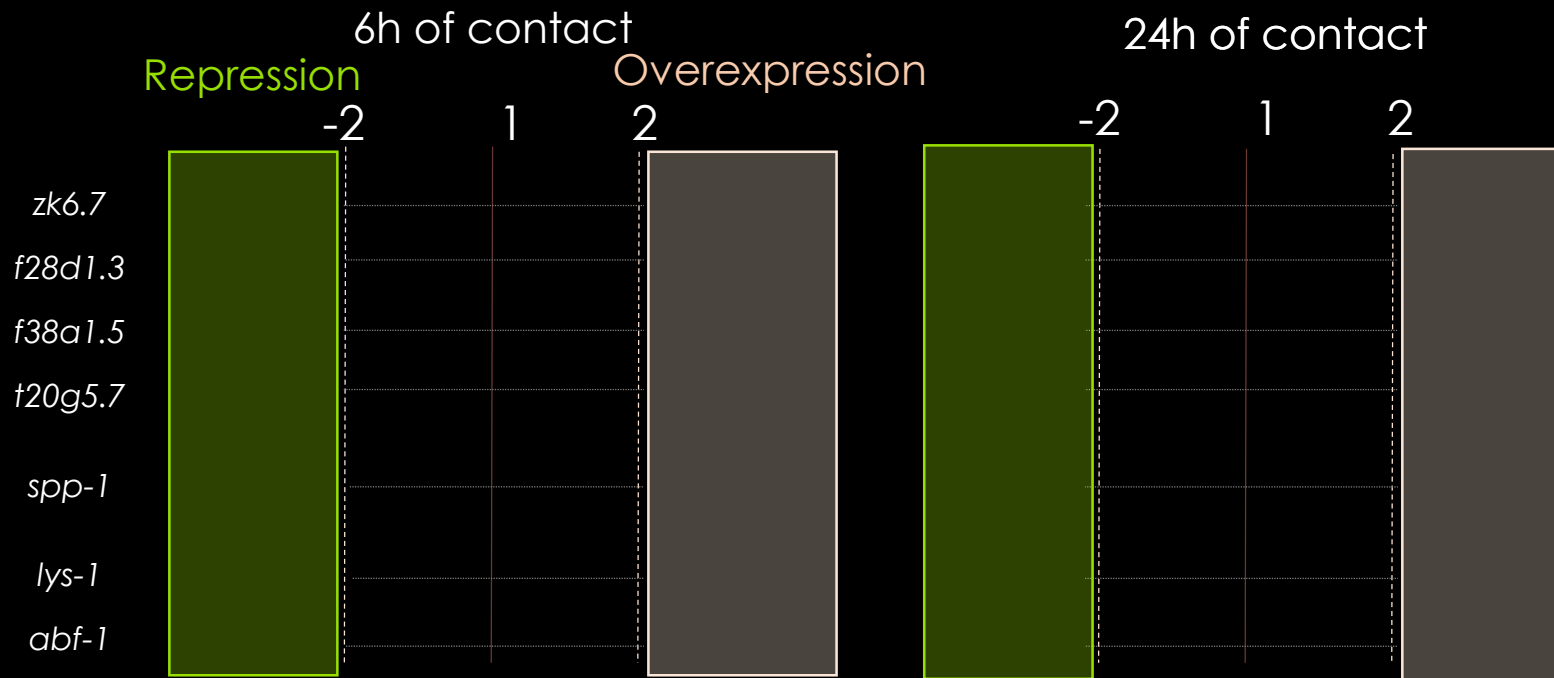
Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

Antibacterial activity

Immuno-stimulant activity

➤ Impact of WSEs on gene expression of innate immune system of **healthy and infected *C. elegans***

( $\Delta\Delta Ct$  Analysis)



RNA extraction of  
*C. elegans*

Reverse transcription  
into cDNA

qPCR



Innate Immune System analysis

Stress resistance genes

Antibacterial effector genes

# Part III: Application of WSE in the prevention of bacterial infections

Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

Antibacterial activity

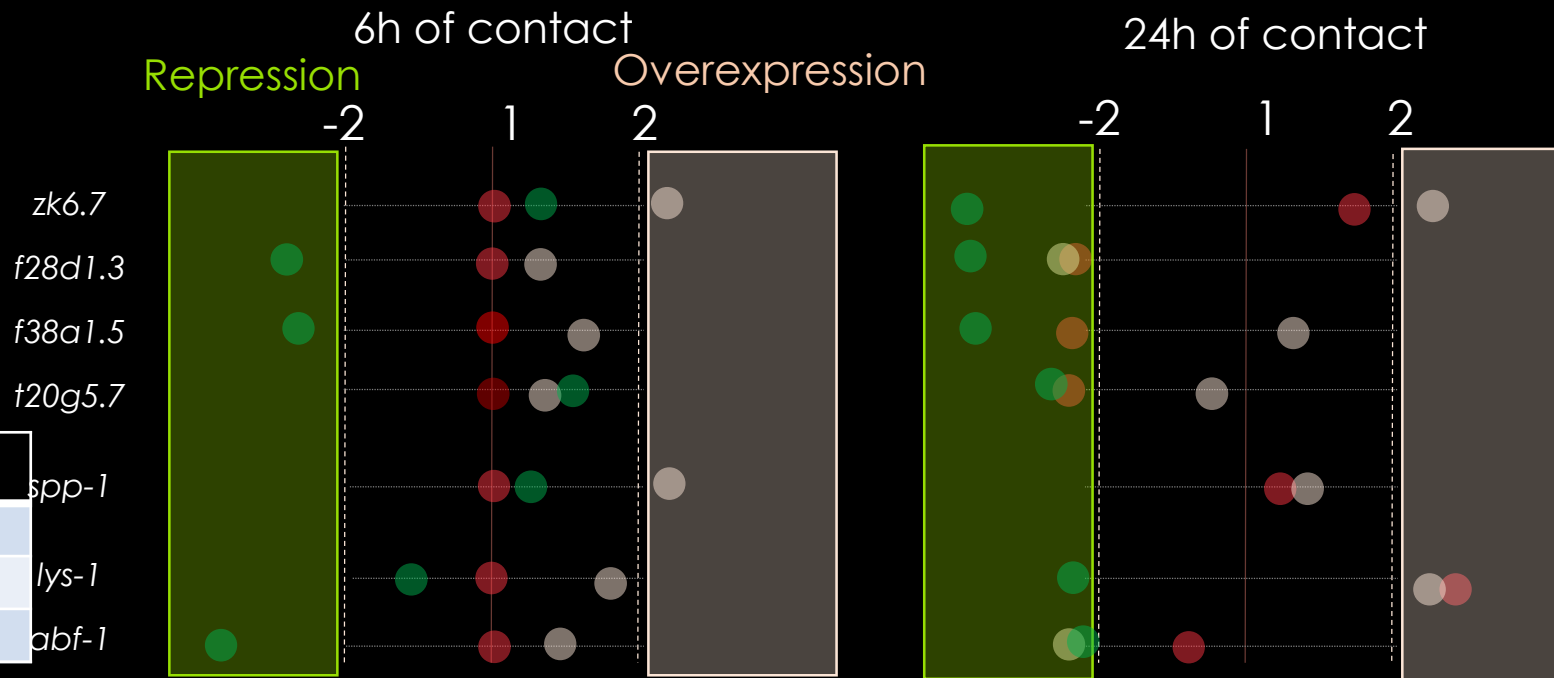
Immuno-  
-stimulant activity

### Legend

- Water + *C. elegans*
- SCEE + *C. elegans*
- TN + *C. elegans*

➤ Impact of WSEs on gene expression of innate immune system of **healthy and infected *C. elegans***

( $\Delta\Delta C_t$  Analysis)



RNA extraction of *C. elegans*

Reverse transcription into cDNA

qPCR



Innate Immune System analysis

Stress resistance genes

Antibacterial effector genes

# Part III: Application of WSE in the prevention of bacterial infections

Pathogenicity model  
*C. elegans* -  
*P. aeruginosa*

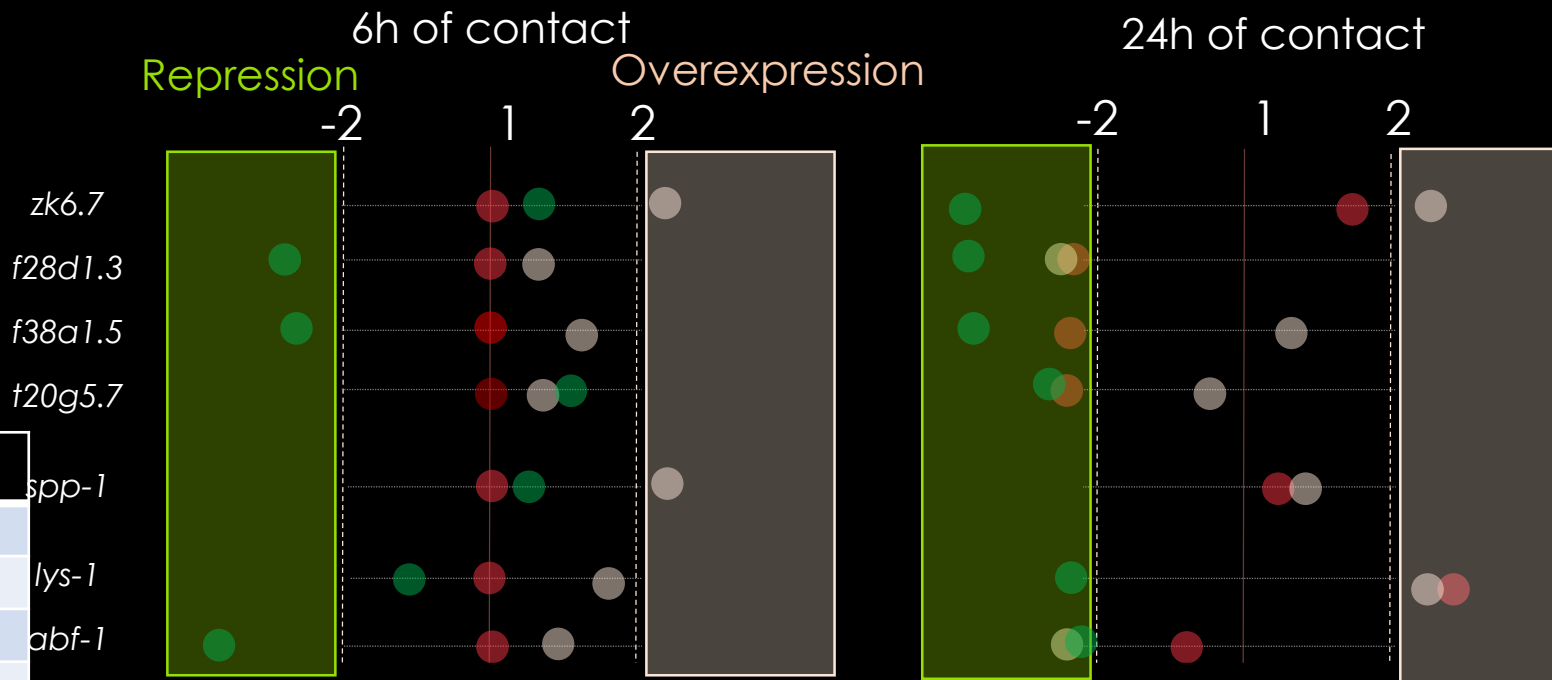
Antibacterial activity

Immuno-  
-stimulant activity

Legend	
●	Water + <i>C. elegans</i>
●	SCEE + <i>C. elegans</i>
●	TN + <i>C. elegans</i>
✗	Water + PA14 + <i>C. elegans</i>
✗	SCEE + PA14 + <i>C. elegans</i>
✗	TN + PA14 + <i>C. elegans</i>

➤ Impact of WSEs on gene expression of innate immune system of **healtly and infected *C. elegans***

( $\Delta\Delta$ Ct Analysis)



RNA extraction of  
*C. elegans*

Reverse transcription  
into cDNA

qPCR



Innate Immune System  
analysis

Stress  
resistance  
genes

Antibacterial  
effector  
genes

# Part III: Application of WSE in the prevention of bacterial infections

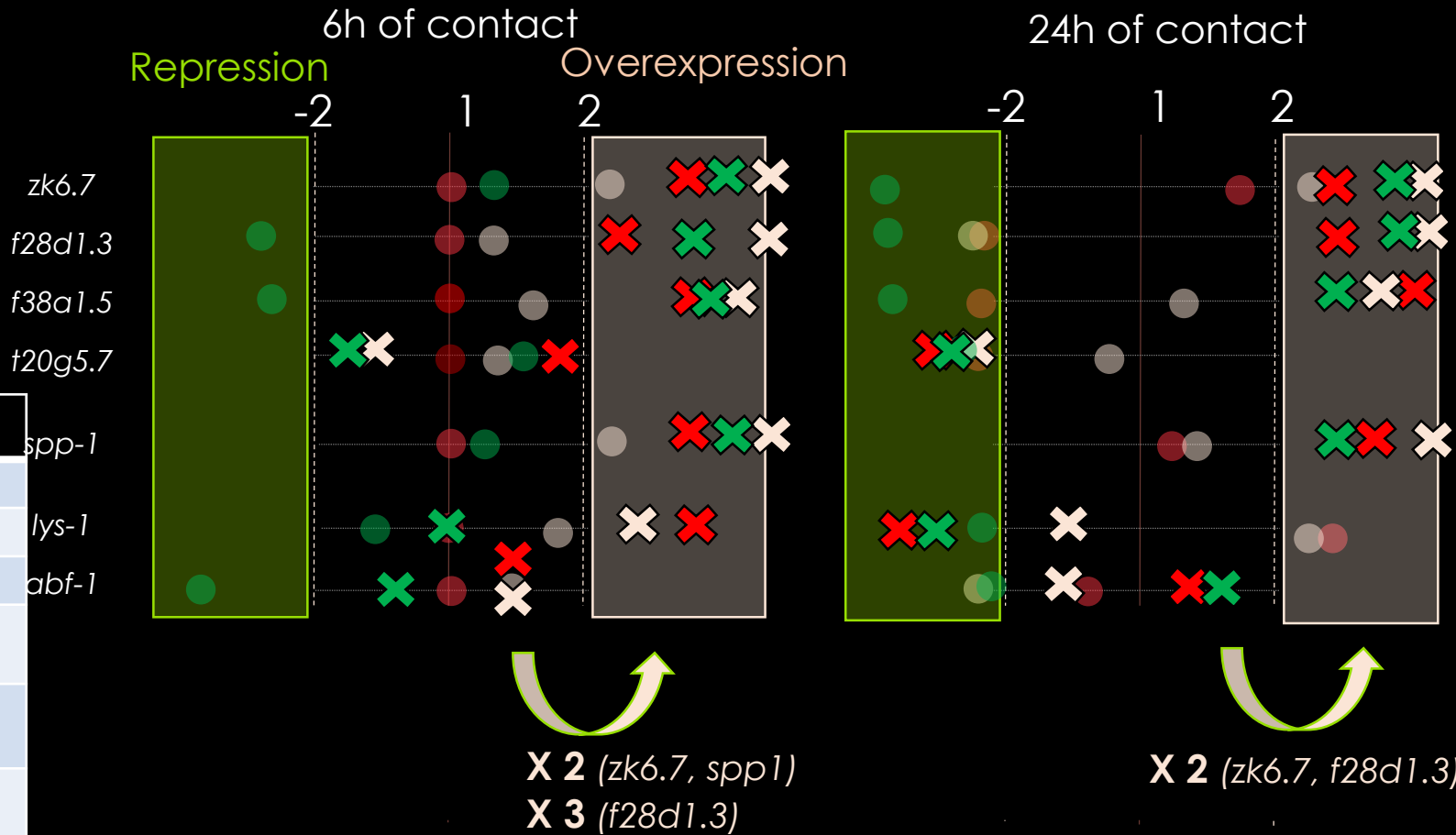
Pathogenicity model  
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➤ Impact of WSEs on gene expression of innate immune system of **healthy and infected *C. elegans***

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RNA extraction of  
*C. elegans*

Reverse transcription  
into cDNA

qPCR

Innate Immune System  
analysis

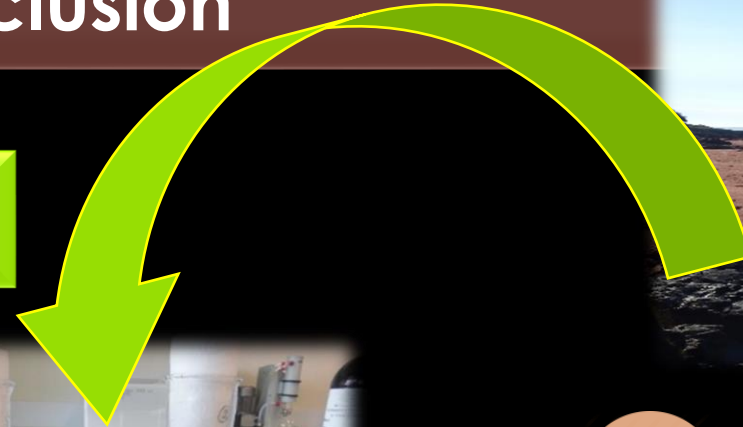
Stress  
resistance  
genes

Antibacterial  
effector  
genes



# Conclusion

Water Soluble Extracts from *S. chordalis*



*C. elegans*

*P. aeruginosa* PA14



QS

System *las*

Virulence factors

*rpoN*

Antibacterial effectors

*f38a1.5*

Stress resistance

*zk6.7*

*spp-1*  
*f28d1.3*



In accord with literature (Kandasamy et al. 2012; Liu et al. 2013; Kandasamy et al. 2015 Kulshreshtha et al. 2016) and confirms the study of Bondu et al. 2010.

French Team



Pr. Bourgougnon Nathalie  
and Dr. Bedoux Gilles

Canadian Team



Dr. Prithiviraj Balakrishnan

Thanks for your  
attention





# Part II : Enzyme-Assisted-Extraction

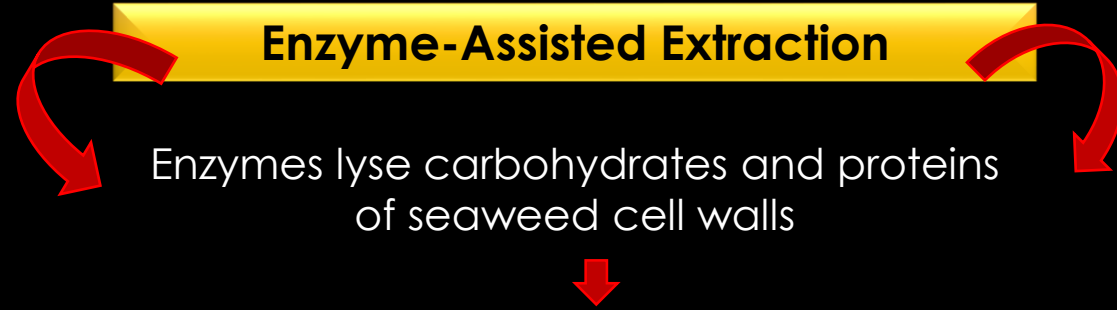


Industrial application

**Environmentally friendly extraction:**  
Enzyme Assisted Extraction (EAE)

Biological activity  
and/or physico-  
chemical properties

Available quantity  
and biochemical  
composition of  
seaweed biomass



<u>Advantages of EAE</u>
No organic solvent
Shorter time production
Water Soluble Bioproducts
Enzyme can be reused

(Jeon *et al.*, 2012; Hardouin *et al.*, 2014)



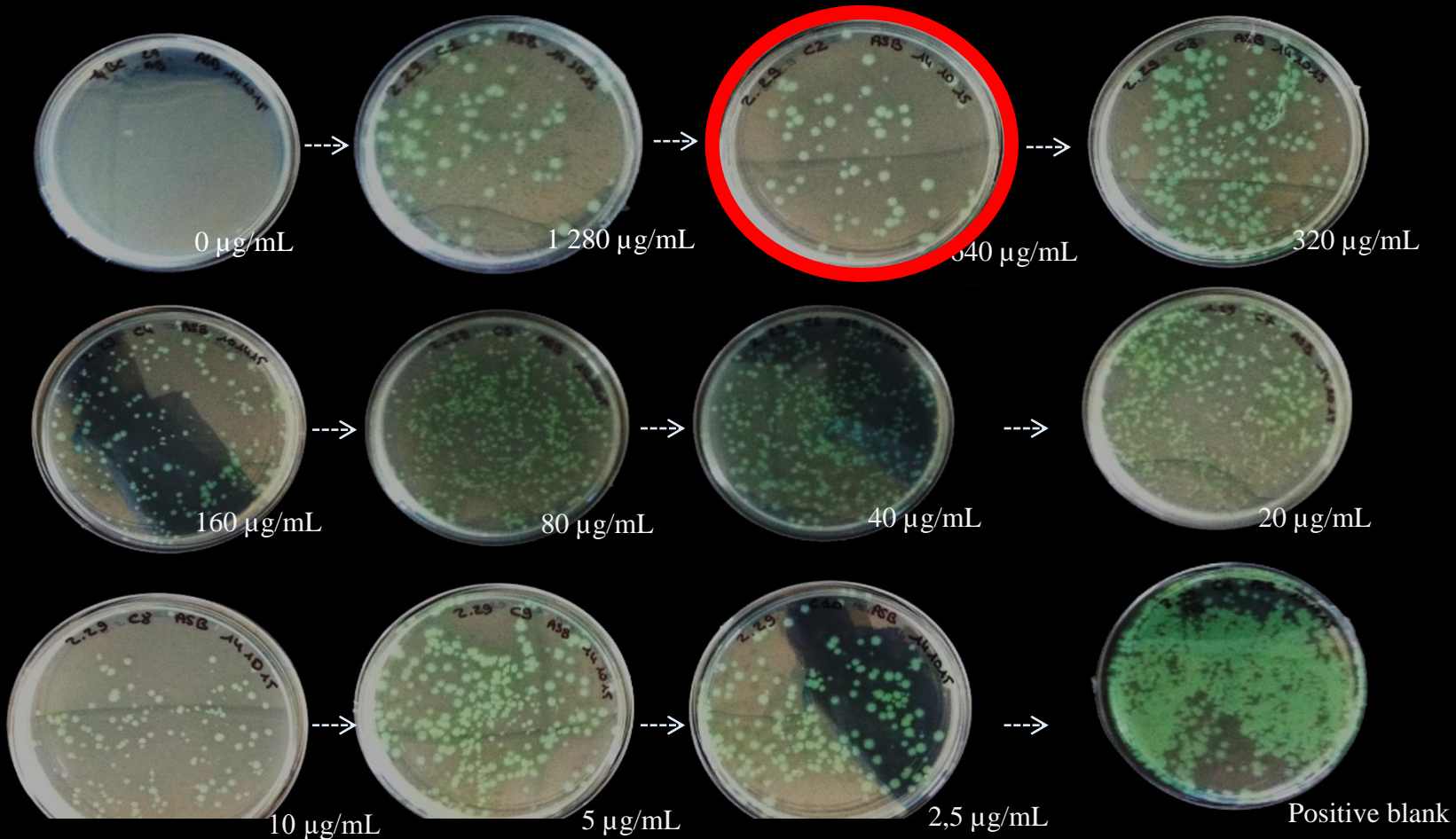
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Pathogenicity model  
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Antibacterial activity

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➤ Impact of SCEE on bacterial growth  
Microdilutions method (Wiegand *et al.* 2008)



*P. aeruginosa*,  
strain PA14



- SCEE (640 µg/mL) inhibits 69 % of PA14 growth.
- TN (640 µg/mL) inhibits 46.5 % of PA14 growth.
- Carbenicillin from 0,175 µg/mL inhibits more than 64 % of PA14 growth

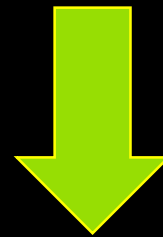


## Part II : Enzyme-Assisted-Extraction



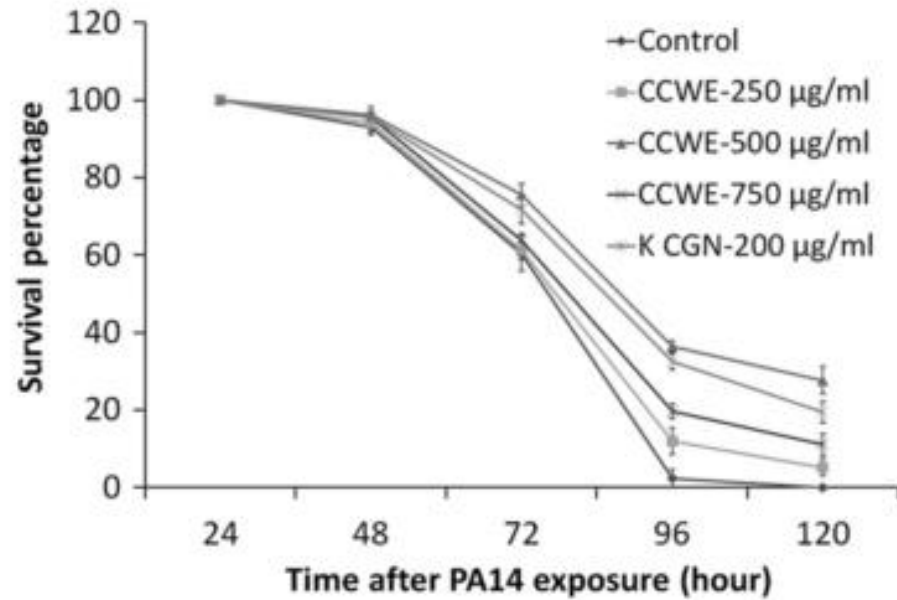
### Biochemical composition of *S. chordalis* and of extracts SCEE and TN

	Dry matter (% FW)	Lipids (% DW)	Ash content (% DW)	Proteins (% DW)	Uronic acids (% DW)	Sulfate groups (% DW)	Neutral sugars (% DW)
<i>S. chordalis</i>	10.0 ± 0.6	3.0 ± 0.04	43.3 ± 3.1	22.0 ± 2.0	3.1 ± 0.1	14.7 ± 1.0	31.7 ± 0.7

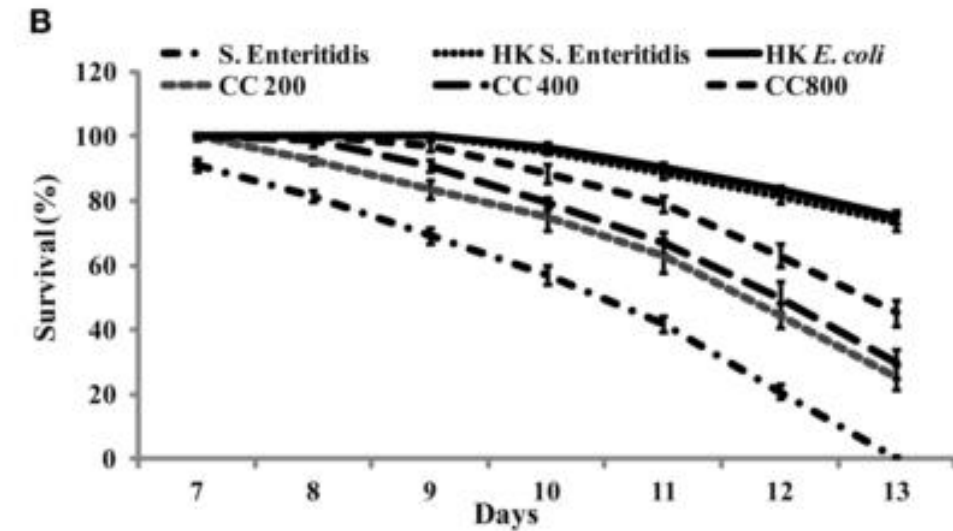
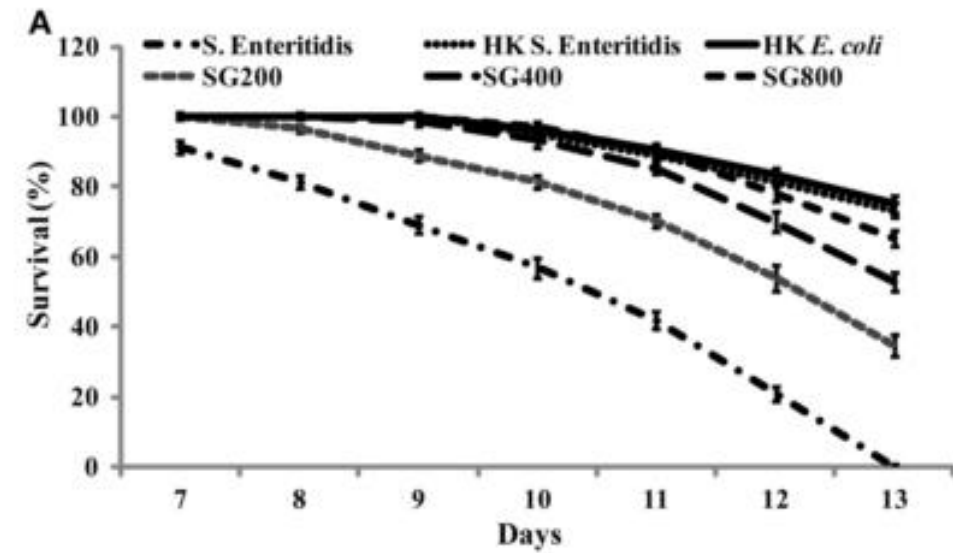
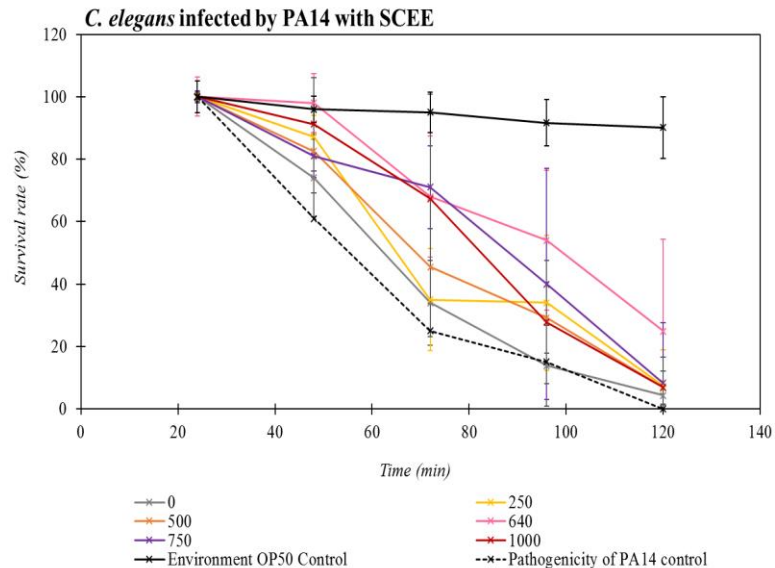


Hydrolysis without or with enzyme (EAE) at 50°C for 3h

	Others (% DW)	Ash content (% DW)	Proteins (% DW)	Uronic acids (% DW)	Sulfate groups (% DW)	Neutral sugars (% DW)
<b>TN</b>	9.2 ± 1.7	70.9 ± 1.0	4.5 ± 0.1	1.0 ± 0.1	1.6 ± 0.4	12.8 ± 0.1
<b>SCEE</b>	3.6 ± 4.2	56.5 ± 1.6	8.9 ± 2.0	0.3 ± 0.2	3.2 ± 0.1	27.5 ± 0.3



Liu J, Hafting J, Critchley AT, et al (2013) Components of the Cultivated Red Seaweed *Chondrus crispus* Enhance the Immune Response of *Caenorhabditis elegans* to *Pseudomonas aeruginosa* through the pmk-1, daf-2/daf-16, and skn-1 Pathways. *Appl Environ Microbiol* 79:7343–7350. doi: 10.1128/AEM.01927-13



Kulshreshtha G, Borza T, Rathgeber B, et al (2016) Red Seaweeds *Sarcodiotheca gaudichaudii* and *Chondrus crispus* down Regulate Virulence Factors of *Salmonella Enteritidis* and Induce Immune Responses in *Caenorhabditis elegans*. *Front Microbiol* 7:421. doi: 10.3389/fmicb.2016.00421