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COIMBRA

Ana Catarina Almeida Carregado e Martins Marques

THE ROLE OF SERRATOMOLIDES PRODUCED BY BACTERIA OF GENUS *SERRATIA* IN NEMATICIDAL ACTIVITY

Dissertação no âmbito do mestrado em Bioquímica orientada pela Professora Doutora Paula Maria de Melim Vasconcelos de Vitorino Morais e pelo Doutor Diogo Alexandre Neves Proença apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Junho de 2019

Faculdade de Ciências e Tecnologia
da Universidade de Coimbra

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AD MAJOREM DEI GLORIAM

Resumo

Bursaphelencus xylophilus, também conhecido como Nematode da Madeira do Pinheiro (PWN), é o agente patogénico da Doença do Pinheiro que afeta pinheiros em todo o mundo. A infeção espalha-se globalmente através do comércio internacional e localmente através de insetos vetores, ameaçando a economia mundial da madeira. Devido às alterações climáticas, um número crescente de países fica ameaçado pela Doença da Murchidão do Pinheiro (PWD), sendo necessário conhecer melhor este agente patogénico para prevenir a disseminação da infeção e limitar as perdas económicas e ecológicas. As bactérias do género *Serratia*, presentes na comunidade endofítica do pinheiro e transportadas pelo PWN, podem desempenhar um papel importante na PWD.

Este trabalho visa estudar como o nemátode *Bursaphelencus xylophilus* interage com estirpes bacterianas do género *Serratia* e avaliar o potencial nematicida de dois Serratomolides (Serrawettinas W1 and W2) produzidos por estirpes de *Serratia*.

A presença dos genes codificantes de Serrawettinas foi investigada em estirpes selecionadas de *Serratia*. Foram realizados testes de mortalidade com bactérias, sobrenadantes e amino lípidos extraídos, de modo a determinar o seu potencial nematicida contra *C. elegans* (nemátode modelo) e *B. xylophilus.* Foram realizados testes de atração com *C. elegans*. Foram realizadas análises bioinformáticas para compreender os processos de biossíntese das Serrawettinas e para esclarecer as interações proteína-ligando entre estes Serratomolides e a proteína recetora TOL1 de *C. elegans*. Os sobrenadantes concentrados das estirpes de *Serratia* contendo Serrawettinas foram capazes de matar mais de 77% de *B. xylophilus* após 72 h. Oito amino lípidos específicos demonstraram uma elevada atividade nematicida contra *B. xylophilus.* A análise dos clusters dos genes codificantes de Serrawettinas W1 e W2 revelou a presença de várias proteínas envolvidas no processo de biossíntese destes Serratomolides.

O trabalho permitiu concluir que várias estirpes de *Serratia*, os seus sobrenadantes e amino lípidos específicos foram capazes de demonstrar atividade nematicida contra *B. xylophilus.* O processo de biossíntese destes Serratomolides e as interações proteína-ligando entre estes e TOL1 merecem um estudo mais aprofundado.

PALAVRAS-CHAVE: Doença da Murchidão do Pinheiro, Nematode da Madeira do Pinheiro, *Bursaphelenchus xylophilus*, género *Serratia*, Serratomolides, Serrawettin W1, Serrawettin W2.

Abstract

Bursaphelencus xylophilus, also known as Pine Wood Nematode (PWN), is the pathogenic agent of Pine Wilt Disease that affects pine trees around the world. Infection is spreading globally through wood international commerce and locally by vector beetles, threatening wood world economy. As climate changes, more countries are becoming susceptible to Pine Wilt Disease (PWD) and, to prevent disease spread and limit economic and ecological losses, better knowledge about this pathogenic agent is needed. *Serratia* strains, present in endophytic community of pine trees and carried by PWN, may play an important role in PWD.

This work aims to better understand interaction between *Serratia* strains and *Bursaphelencus xylophilus* and to assess nematicidal potential of Serratomolides (Serrawettin W1 and W2) produced by *Serratia* strains.

Serrawettin genes presence was evaluated in selected *Serratia* strains. Mortality tests were performed with bacteria, supernatants and extracted amino lipids against *C. elegans* (model organism) and *B. xylophilus* to determine their nematicidal potential. Attraction tests were performed with *C. elegans*. Bioinformatic analysis were performed in order to better understand biosynthesis processes and to elucidate protein-ligand interactions between Serratomolides and *C. elegans* TOL1 receptor protein.

Concentrated supernatants of *Serratia* strains with Serrawettins were able to kill more than 77% of *B. xylophilus* after 72 h. Eight specific amino lipids showed a high nematicidal activity against *B. xylophilus*. Cluster analysis of Serrawettin W1 and W2 biosynthesis gene revealed several new proteins involved in Serratomolides biosynthesis process.

We conclude that some *Serratia* strains, their supernatants and specific amino lipids show nematicidal activity against *B. xylophilus.* Serratomolides biosynthesis process and protein-ligand interactions between TOL1 and Serratomolides deserve to be entirely studied.

***KEYWORDS**:* Pine Wilt Disease, Pine Wood Nematode, *Bursaphelenchus xylophilus*, *Serratia* genus, Serratomolides, Serrawettin W1, Serrawettin W2.

Abbreviations

A - Adenylation domain
Å - Armstrong
ACP - acyl carrier protein
AIY - Amphid interneurons
ANOVA - Analysis of Variance
APS - Ammonium Persulfate
ARB - Arbor project
AT - acyltransferase
AWB - Amphid wing "B" cells
BLAST - Basic Local Alignment Search Tool
BLASTP - Basic Local Alignment Search Tool Protein
bp - base pairs
C - Condensation domain
CAA - Casamino Acid medium
cm - centimeter
Da - Dalton
ddH2O - distilled water
DNA - Deoxyribonucleic acid
EPPO - European and Mediterranean Plant Protection Organization
g - grams
G/C - Guanine Cytosine content
GHF - glycosyl hydrolase family
GROMACS - Groningen Machine for Chemical Simulation
h - hours
H_2O - water
ha - hectare
HPLC - High Performance Liquid Chromatography
ID - Identification
IPTG - Isopropyl β-D-1-thiogalactopyranoside
K - Kelvin
kDa - kilodalton
kJ - kiloJoule
km - kilometer
KR - keto reductase
KS - ketosynthase
kV - kiloVolt
L3 - third larvae stage
LB - Lysogeny broth medium
LINCS - Linear Constraint Solver
LPSN - List of Prokaryotic Names
LPT - Living Tree Project
m - meter
M - Molar
m^3 - cubic meter
M9 - M9 minimal medium
Mb - mega base pairs
MD - Molecular Dynamics
mg - milligram
min - minutes
mL - milliliters
mm - millimeter
mM - milliMolar
mN - milliNewton
mol - mole
mol% - mole percentage
ms - millisecond
NCBI - National Center for Biotechnology Information
ng - nanogram
NGM - Nematode Growth Medium
nm - nanometer
nM - newton meter
NPT - constant number of particles, pressure, and temperature
NRPS - nonribosomal peptide synthetase
ns - nanosecond
NVT - constant number of particles, volume, and temperature
NZY - NZYTech
OD - Optical Density
ORF - open reading frame
PA - Phenylacetic acid
PCR - polymerase chain reaction
PKS - polyketide synthase
PME - Particle Mesh Ewald
PPTase - 4'-phosphopantetheinyl transferase
ps - picoseconds
pswP - PPTase gene
PWD - Pine Wilt Disease
PWN - Pine Wood Nematode
R2A - Reasoner's 2A agar medium
RMSD - Root-mean-square deviation
RMSF - Root mean square fluctuation
rpm - rotations per minute
rRNA - Ribosomal ribonucleic acid
s - seconds
SDS - Sodium Dodecyl Sulfate
SDS-PAGE - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

srw1 - Serrawettin W1 gene

srw2 - Serrawettin W2 gene

T - Thiolation domain

TE - Thioesterase

TEMED - Tetramethylethylenediamine

TLC - Thin Layer Chromatography

TOL-1 - Toll-like transmembrane receptor 1

tol-1 - Toll-like transmembrane receptor 1 gene

tRNA - Transfer ribonucleic acid

U - enzyme activity unit

UCCCB - University of Coimbra Bacteria Culture Collection

USA - United States of America

UV - Ultraviolet

V - Volt

v/v - volume per volume

VMD - Visual molecular dynamics

xg - G-force

X-Gal - 5-Bromo-4-chloro-3-indolyl-ß-D-galactopyranoside

µL - Microliter

µg - microgram

µM - microMolar

º C - Grau Celsius

1D - one-dimensional

2D - two-dimensional

3D - three-dimensi

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Introduction

A. Pine Wilt Disease Importance and Impact

Pine forests face a global threat, Pine Wilt Disease (PWD), which is spreading widely through vector beetles, capable of carrying pathogenic nematodes from tree to tree (**ZHAO, B. G. et al., 2008**).

Bursaphelenchus xylophilus is the pinewood nematode (PWN) responsible for this disease, in particular among the genus *Pinus* (**JONES, J. et al., 2008**).

Trees provide important products like timber, fibre and food and represent an important part of food and wood world economy. However, trees are susceptible to pests and diseases caused by virus, bacteria, fungi or insects (**BOYD, I. et al., 2013**). As globalization and climate change influence disease spread, researches are focusing on pathogens and diseases that damage trees and have a direct impact on global economy. These pests and diseases have detrimental consequences on ecosystems worldwide, diminishing services provided by trees, damaging timber and reducing fruit production (**BOYD, I. et al., 2013**). On top of that, they affect the ability of forests to sequester and store carbon and also reduce tree supported biodiversity. It is important to understand why some pathogens and insects become pests, to know how they cause tree diseases and to describe the molecular basis of their pathogenicity (**BOYD, I. et al., 2013**). In order to maintain healthy sustainable forests that provide needed products it is essential to protect ecosystems and susceptible species by recognizing pest risks and preventing disease consequences.

Portuguese forest sector is economically important for exportation and job creation, contributing to gross domestic product growth. In 2016, Portuguese forest sector represented 7.44% of all exportations. Wood subsector continues to be one of the most relevant exportation contributors (**SILVA, J. C., 2007**).

In Portugal, this disease was first reported in 1999, initially affecting Setúbal area (**MOTA, M. et al., 1999**) and gradually spreading to central and northern country regions (**RODRIGUES, J., 2008**), causing a massive destruction of pinewoods, with significant economic and environmental losses (**TÓTH, Á., 2011; PROENÇA, D. N. et al., 2017b**).

After 2017 extensive wildfires, new territories were found to be positive for PWN presence and new strategies of intervention were defined. A large number of trees was affected by wildfires, making it difficult to clean and remove remaining affected trees from national forest. Around 9.3 million m^3 of conifers were burned during this period. One important constraint is the extremely high number of symptomatic trees remaining after wildfires and the possibility for PWD to spread. Priority has been given to buffer zones and other risky adjacent areas (Decreto-Lei n.º 95/2011, de

8 de agosto, alterado e republicado pelo Decreto-lei n.º 123/2015, de 3 de julho e Declaração de Retificação n.º 38/2015, de 1 de setembro) (**MANUEL RODRIGUES, J., 2017**).
Wood international trade is one of the most common ways of spreading diseases to new areas around the world, especially to those, like Mediterranean area, where susceptibility is particularly high. Untreated wood may act as a source of pests such PWN and its vectors. Before being exported, wood should be treated to diminish the risk of pest spreading (Decreto-Lei n.º 123/2015) (**D'ERRICO, G. et al., 2014**).

B. Pine Wilt Disease International Distribution

Pine Wood Nematode originated in North America but was first reported in Japan where it rapidly caused a major pinewood catastrophe (**YANO S., 1913**). Before PWD devastation, Japan´s pine forests were full of *Pinus densiflora* (red pine) but since this nematode´s arrival a healthy pine tree can hardly be found. Disease subsequently spread to other Asian countries, like China, Korea and Taiwan, causing severe economic damages (**FUTAI, K., 2008**).
The initial incidence report was from Nagasaki City, on Kiushu Island, in 1905. Disease spread to mainland in 1921, where affected tree number kept on increasing and, by 1940, forests were largely devastated. During 1950 and 1960 decades, Japanese authorities implemented some control measures in order to slow disease spread. By 1970 decade, a governmental project found that PWN was responsible for this disease and needed a vector, *Monochamus* spp. beetles. New control tactics were established to interfere with disease cycle, using insecticides sprays to prevent vector spread. These methods were not effective enough and disease continued spreading to northern regions (**FUTAI, K., 2008**).
In China, PWD was first detected in 1982, near Nanjing City, and rapidly spread to ten provinces, becoming a serious forest and economical problem. In 2006, China's total damaged area reached 23.000 ha (**ZHAO, B. G. et al., 2008**).
In North Korea pines used to represent 23.5% of forest area and pine trees were cultural and spiritually important for Korean people. Despite efforts to achieve disease control, PWD spread to North Korea and then to South Korea, where progression was slower due to a correct disease management. By 2006, white pine *Pinus koraiensis* was also described as a PWD host (**SHIN et al., 2008**).
PWN was later reported in Nigeria and Mexico from 1990 to 1995 (**KHAN F. A., & GBADEGESIN R. A., 1991; DWINNEL L. D., 1993**).
A 1972 study, in Japan, showed that *B. xylophilus* was the causal agent of PWD (**MAMIYA, Y., & KIYOHARA, T., 1972**). In 1984, a shipment of wood from North America to Finland was found to carry PWN and this finding caused alarm. After this event, European authorities developed rigorous inspections, particularly on wood products from North America. Yet, no control was made on wood products from Asia (**MOTA, M. M., & VIEIRA, P., 2008**).

Portugal was the first European country to detect PWD, *B. xylophilus* and its vector *M. galloprovincialis* (**MOTA, M. et al., 1999**). Most probably, PWN entered Portugal through international trade of wood products, such as timber, from Japan or China. International trade of wood products continues to be the most common way to spread PWN and PWD.

International agreements to prevent disease expansion and more detailed scientific knowledge (mainly at a molecular level) are paramount to efficiently control nematode and vector spread, in order to protect forests, ecosystems and, ultimately, pine-based economy (**RODRIGUES, J., 2008**).

It was predicted that some areas and forests in South Europe, southern areas of North America and East Asia are vulnerable to PWD due to clime conditions, forest tree content and proximity to infected areas (**HIRATA, A. et al., 2017**). Factors like storm damages, global warming, imported wood and insect's migration increase disease spreading risk in Hungary and Russia (**TÓTH, Á., 2011**). But, in Europe, until this date, PWD was only found in Portugal, affecting districts such as Setúbal (**MOTA, M. et al., 1999**), Coimbra (**RODRIGUES, J., 2008**) and Madeira island (**FONSECA, L. et al., 2012**), and in Spain (**ROBERTSON, L. et al., 2011**).

C. Pine Wood Nematode

The genus *Bursaphelenchus* includes more than 100 species worldwide and is a member of phylum Nematoda and family Aphelenchoididae (**HUNT, D. J., 2008**). Most species of this genus are mycophagous and are transmitted by vectors through maturation, feeding or oviposition, with beetle insect being the most common vector. Only two species in this genus are plant parasitic nematodes: *Bursaphelenchus cocophilus* and *Bursaphelenchus xylophilus* (**D'ERRICO, G. et al., 2014**). Steiner and Buhrer first classified PWN as *Aphelenchoides xylophilus*, in Louisiana, USA, in 1934, but Nickle's group (**NICKLE, W. R. et al., 1981**) reclassified this species as *Bursaphelenchus xylophilus* due to a typical bursa present in the male tail.

B. xylophilus have four incisures in the lateral field, females have a long vulval flap and males have seven caudal papillae. A distinguishing feature of this species is a round female shape tail (**D'ERRICO, G. et al., 2014**).

PWN is feared worldwide for causing PWD and, in Europe, is listed as a quarantine pest in EPPO A2 list (**CENTRE FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL., 2004**).

In North America, PWN is not a pathogen of native pine species and does not cause PWD in native pine species, but causes PWD in non-native species like *P. nigra* or *P. sylvestris* (**WINGFIELD M. J. et al., 1984**).

Portugal has a Mediterranean climate characterised by dry summers and cold winters, offering ideal conditions for *B. xylophilus* and its vector *M. galloprovincialis*. It is imperative to understand nematode and vector life cycles and dynamics to calculate spreading risk in different areas of Portugal (**D'ERRICO, G. et al., 2014**).

In phylum Nematoda, ability to parasitize evolved independently resulting in a wide range of strategies. Most species of genus *Bursaphelenchus* are fungal feeders, including *B. xylophilus* which is a migratory endoparasite. This species is uniquely capable to feed on live trees as well as on fungi, suggesting a recent type of parasitism. Genes involved in parasitism include some to accomplish plant cell wall degradation, probably through fungi horizontal gene transfer (**KIKUCHI, T. et al., 2011**).

Five major taxonomic groups of phylum Nematoda have been described; *C. elegans* belongs to clade 9, group V and *B. xylophilus* belongs to clade 10, group IV (**KIKUCHI, T. et al., 2011**). PWN has six pairs of nuclear chromosomes, with a 74.5 Mb genome total size, smaller than *C. elegans*, but with a higher content of G+C mol%. *B. xylophilus* mitochondrial genome is similar in size and content to *C. elegans*. Chromosomal rearrangements analyses were made between the two species and identified a similar pattern, where large *B. xylophilus* scaffolds contain orthologous genes from a single *C. elegans* chromosome (**KIKUCHI, T. et al., 2011**).

C. elegans was the first multicellular organism with a complete genome sequence available and provided important information on biological processes. *C. elegans* was selected for genetic research because it is easy to keep, observe, analyse and manipulate, grows fast, has a simple life cycle and continues to be considered an excellent animal model (**BLAXTER, M., 2011; BRENNER, S., 1974**).

D. Pine Wilt Disease

B. xylophilus normal life cycle includes a propagative mode and a dispersal mode, using a *Monochamus* species as vector.

In propagative mode, fourth-stage larvae carried by a beetle vector invade already dead or dying trees using holes cut by the vector to lay its eggs (**WINGFIELD, M.J., 1983; CENTRE FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL., 2004**).

Production of enzymes, such as cellulases, able to degrade cell wall, is critical for plant pathogens. PWN has a distinct capacity to produce, in its esophageal gland cells. This kind of enzymes that are secreted through a stylet, facilitates nematode penetration and migration over plant tissues. Genes underlying this process in PWN belong to glycosyl hydrolase family and are phylogenetically more similar to fungal cellulases, suggesting horizontal gene transference from fungi (**KIKUCHI, T. et al., 2004**).

Feeding on fungi, nematode larvae moult into adult stage and start rapid multiplication. After initial invasion, as population ceases to grow and begins declining, probably due to lack of food, a different type of larva (third-stage larva) develops: this is a survival stage larva, capable of resisting adverse conditions and apt for dispersal (**CENTRE FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL., 2004**).

Dispersal mode is, most likely, consequence of a reduction of food availability. Third-stage larvae, and fungal hyphae, gather and surround *Monochamus* vector's pupal chambers and, close to the time of beetle emergence, moult to fourth-stage larvae (**CENTRE FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL., 2004**).

Three terpenes, produced by insect larvae and able to attract nematodes, have been described (**ZHAO, L. L. et al., 2007**). Young beetles emerge and brush against the chamber, pick up nematode's larvae and fly out, carrying fourth-stage larvae inside their tracheal system and on their body surfaces.

This life cycle is similar to other *Bursaphelenchus* spp. cycles, being the most common in native pine species of North America (**WINGFIELD, M.J., 1983**). However, transmission is different on non-native and susceptible pine species, where nematodes are transmitted by young adult vectors, shortly after emergence from pupal chambers, while they feed on young pine shoots.

Nematodes carried by vectors enter young shoots through feeding wounds and multiply in resin canals, attacking epithelial plant cells. Native North America pines probably developed physical and/or biochemical barriers to prevent this type of invasion (**CENTRE FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL., 2004**).

But in non-native and susceptible pine species, after three weeks of infection, trees start to show disease signs, caused by reduced oleoresin exudation that results in weaker defence mechanisms. This weakened tree state attracts adult insects that gather on the trunk to mate. At this phase, pine wilting intensifies and tree needles start to lose their green colour and become yellow (**CENTRE FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL., 2004**).

Monochamus spp. lay their eggs in oviposition scars in the bark of host trees and these eggs hatch, depending on temperature, within 4-12 days. Mature adults are attracted to weakened trees and to recently fell logs, by monoterpenes and ethanol, where they mate and resume oviposition. Females excavate new conical scars in the bark using their mandibles and deposit one egg per scar. Females can live 83 days and continue to lay eggs until they die, laying up to a maximum of 215 eggs in a lifetime (**KOBAYASHI, F., et al., 1984**). Unfortunately, vector created chambers make the wood unsalable (**CENTRE FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL., 2004**).

Reduction of oleoresin production is the first sign of PWD and can be detected, before any visible signs appear, by making a 10-15 mm diameter hole through the bar and cambium (**ODA, K., 1967**). An early visible sign is needle's wilting, initially involving only one branch but, later, affecting the whole tree (**MAMIYA, Y., 1983**). Transpiration decreases and ultimately stops. Trees usually die 30-40 days after infection, invaded throughout the trunk, branches and roots by millions of PWN (**WINGFIELD, M.J., 1983**).

PWN can move actively inside wood tissues but, without vectors, they are incapable of moving from one host tree to a new host tree. Beetles can fly for up to 3.3 km but dispersal usually occurs in a few hundred meters radius only (**KOBAYASHI, F. et al., 1984**).

Introduction of PWN into a new country occurs when large pieces of untreated timber, with enough moisture to carry vectors and nematodes, are imported. As larger pieces of wood increase vector

insects' survival time, round wood poses a greater risk than wood chips. Apparently, the only known effective treatment for PWN infected wood and its vectors is heat treatment: all parts of wood have to reach 56 ºC for, at least, 30 min (**CENTRE FOR AGRICULTURE AND BIOSCIENCE INTERNATIONAL., 2004**).

For a long-term disease establishment, PWN needs to contact with native vectors. This is only possible if PWN infects wood containing larvae of a potential native vector (**MCNAMARA, D.G., & STOØEN, M., 1988**).

There is no current way to control PWN after tree infection and damaged trees have to be removed and destroyed. Insecticides against vectors are the only way to achieve some level of disease control in affected areas. Governments of infected countries spent a large amount of money with control programmes (**IKEDA, T., 1984**).

Researchers are trying to find effective alternatives to maximize disease control, using biological control agents for PWN and vectors (for example, with trap systems). New forms of PWD control may help forest recuperation and may contribute to reduce infection risk in Europe.

E. Bacteria Associated with PWN and/or Endophytes of Pine Trees

Plant tissues and intercellular spaces can be colonised by many different bacteria and provide a very complex plant-microbial ecosystem.

By infecting pine trees, PWN interacts with their endophytic microbial community, comprising all those bacteria which can colonize internal pine tissues without causing apparent harm (**PETRINI, O., 1991**). For example, *Pinus sylvestris* has a wide range of endophytic bacteria genera, such as *Methylobacterium*, *Pseudomonas*, *Bacillus* and *Paenibacillus* (**IZUMI, H. et al., 2008; PIRTTILÄ, A. M. et al., 2000; STRZELCZYK, E., & LI, C. Y., 2000**).

Endophytic microbial community density is lower than rhizosphere microbial community density but, usually, both communities are instrumental for plant welfare and their actual composition varies depending on plant species (**DING, T., & MELCHER U., 2016**).

Within the endophytic microbial community, we can distinguish bacteria that are obligate endophytes (usually less diverse and related to nitrogen fixation function) from bacteria that are opportunistic endophytes (**PROENÇA, D. N. et al., 2017b**).

In Portugal, maritime pines, *P. pinaster*, endophytic community is mainly composed by class Gammaproteobacteria (**PROENÇA, D. N. et al., 2017a**), expressing genes that suggest an association with nitrogen fixation and denitrification. In domain Archaea, the phyla *Euryarchaeota*, *Thaumarchaeota* and *Crenarchaeota* were also found in this endophytic community (**PROENÇA, D. N. et al., 2010**). Although several studies were performed to describe the endophytic microbial diversity, it is still unclear if observed variations are related to different sample locations and soil

characteristics or to other factors. New studies, using new techniques like next generation sequencing, may better elucidate PWD impact in pine endophytic community (**PROENÇA, D. N. et al., 2017b**).

PWN was shown to carry several bacteria, most present on its surface where it can carry an average of 290 bacteria per adult (**reviewed in PROENÇA, D. N. et al., 2017b**). PWN bacterial diversity varies depending on forest area, country and *Pinus* spp.. Species from genera *Pantoea*, *Peptostreptococcus*, *Enterobacter*, *Serratia*, *Staphylococcus*, *Buttiauxella*, *Stenotrophomonas* and *Pseudomonas* were isolated from PWN in China (**ZHAO, B. G., & LIN, F., 2005; TIAN, X. et al., 2010**). In Republic of Korea, species such as *Burkholderia arboris*, *Brevibacterium frigoritolerans*, *Enterobacter asburiae*, *Ewingella americana* and *Serratia marcenscens* were found to be associated with PWN (**KWON, H. R. et al., 2010**). In Portugal, bacteria associated with PWN belong mostly to the genera *Pseudomonas*, *Burkholderia*, *Enterobacteria*, *Serratia*, *Ewingella*, *Pantoea* and *Erwinia* (**PROENÇA, D. N. et al., 2010**). Next-generation sequencing showed that the most abundant genera in PWN were *Stenotrophomonas* genera and unclassified genera belonging to families *Pseudomonadaceae* and *Rhizobiaceae* (**XIANG, Y. et al., 2015**).

Recent studies showed PWN bacterial community structure to be quite different from that of infected trees and very similar to that of insect vector, suggesting that PWN microbiome may be, at least in part, inherited from its vector (**ALVES, M. et al., 2018**).

Interestingly, strains belonging to genus *Pseudomonas* were associated with PWN collected from all infected pine species samples in every country studied (**PROENÇA, D. N. et al., 2014**). Bacteria may be pathogenic and carried by PWN or endophytic and activated by *B. xylophilus* presence through a mutualistic effect. No single bacteria effect was proved to be significant or to act as single agent responsible for Pine Wilt Disease (**PROENÇA, D. N. et al., 2017b**).

However, rapid wilt was related not only to nematode´s presence but also to bacteria carried by PWN, especially belonging to genus *Pseudomonas*, involved in toxin production (**OKU, H. et al., 1980**).

Bacteria capable of phenylacetic acid (PA) production were also suggested as pathogenic. Several studies regarding pathogenic bacteria and nematodes interaction were performed in order to better understand the infective process. Han and colleagues concluded that a combination of nematodes with pathogenic bacteria caused pine wilt development (**HAN, Z. et al., 2003**). Zhao and colleagues discovered that even non-pathogenic nematode species, when in contact with pathogenic bacteria, could cause pine wilt. Further studies revealed that PWD might be caused by a *Bacillus* and *Stenotrophomonas* arrangement (**ZHAO, B. G. et al., 2009; ZHAO, B. G. et al., 2011**). Seventeen isolated bacteria carried by PWN from disease trees where phytotoxin producers, the majority belonging to genera *Pseudomonas* (**ZHAO, B. G. et al., 2003**). However, these studies suffer from serious limitations since most of them were made in vitro and only a few strains of a wide microbial diversity were studied. It is not clear how these bacteria interact with pine trees in real conditions.

Most species identified as related to PWD are common members of the endophytic community and of soil and water environment. Some endophytic bacteria are able to produce secondary metabolites

capable of controlling plant pathogens and of promoting plant growth. For instance, metabolites like siderophores (iron chelators and plant growth promoters) and lipases, that benefit plants by providing PWD protection. Some of these bacteria are currently being studied for their nematicidal properties (**PROENÇA, D. N. et al., 2017b**).

Previous studies concluded that some bacteria, associated with PWN, have nematicidal activity and may contribute to defend host trees. Strains of families *Enterobacteriaceae* and *Pseudomonadaceae* express genes associated to nematicidal activity and growth plant promotion (**PROENÇA, D. N. et al., 2017b**).

In Portugal, 78.5% of bacterial community associated with the vector is composed by *Proteobacteria* and *Serratia* is the most abundant genus. *Serratia* was also the most abundant genus found in the thorax of *M. galloprovinciallis* (44%) (**VICENTE, C. S. et al., 2013**).

Several proteins have been discovered, such as proteases, from different bacteria, like *Brevibacillus laterosporus*, *Bacillus nematocida* and *Stenotrophomonas maltophlia*, that act against PWN. Many *Serratia* strains are able to produce extracellular proteins, for example, serralysin-like proteases (**PROENÇA, D. N. et al., 2019a**). A particular serine protease produced by *Serratia* sp. A88Copa13 is responsible for its nematoxicity and is biochemically different from others previously characterized (**PAIVA, G. et al., 2013**).

Serratia strains were isolated from PWN (**PROENÇA, D. N. et al., 2012**). Strains from this genus were also reported as pathogenic to PWD vector, *Monochamus* (**SHIMAZU, M., 2009**), and *Serratia* sp. A88Copa13 revealed a very active nematicidal effect towards PWN (**PAIVA, G. et al., 2013**). These findings suggest that *Serratia* strains may play an important part in future PWD control because of their potential to produce active chemical compounds and bio-products against both PWN and its vector.

F. The Genus *Serratia*

Serratia species occupy different habitats such as water, soil, plants, insects and vertebrates (**MATSUYAMA, T. et al., 1992**). Twenty species are known to belong to this genus, for instance: *S. marcescens*, *S. liquefaciens*, *S. proteamaculans*, *S. grimesii*, *S. plymuthica, S. rubidaea, S. odorifera, S. ficaria, S. entomophila, S. fontícola* - LPSN database (**PARTE, A. C., 2014**).

Serratia species are a Gram-negative, facultatively anaerobic, rod-shaped bacteria and *S. marcescens* is the type species for *Serratia* genus, of family *Enterobacteriaceae*. Some *Serratia* species produce a red pigment, prodigiosin (**WILLIAMS, R.P., & QUADRI, S.M.H., 1980**), a secondary metabolite formed by enzymatic condensation. Non-pigmented strains lack this condensing enzyme (**DING, M. J., & WILLIAMS, R.P., 1983**). Other pigments produced by strains of the genus *Serratia* are pyrimine, or ferrorosamine A, and a yellow diffusible pigment (**GRIMONT, P. A. D., & GRIMONT F., 1978**).

Both pigmented and non-pigmented strains of *Serratia marcescens* produce biosurfactants. These surfactants were identified as Serratomolides: Serrawettin W1, a cyclodepsipeptide (**WASSERMAN, H. H. et al., 1961**), Serrawettin W2 a surface-active exolipid (**MATSUYAMA, T. et al., 1992**) and Serrawetin W3 (**MATSUYAMA, T. et al., 1986**).
As some *Serratia* strains are capable of producing a red pigment, several medical studies were conducted using these strains as tracers (**MAHLEN, S. D., 2011**).
Some *Serratia* strains are potentially pathogenic and several species of *S. marcescens* can infect humans, causing acute urinary retention and pulmonary vascular congestion (**reviewed in MAHLEN, S. D., 2011**).
Several *Serratia* strains are able to produce Siderophores, high affinity iron-chelating compounds (**NASCIMENTO, F. et al., 2018; PROENÇA, D. N. et al., 2019b**). Some *Serratia* strains associated with PWN were already described as Siderophores producers (**PAIVA, G. et al., 2013; PROENÇA, D. N. et al., 2017b**). Siderophores are able to promote plant growth and can play an important role in plant defence (**PROENÇA, D. N. et al., 2017b**). Some *Serratia* strains with nematicidal activity also produced Siderophores (**PAIVA, G. et al., 2013**).

G. Serratomolides

Serratomolides, such as Serrawettin W1, W2 and W3, are able to reduce surface tension of extracellular mediums. They are extracellular lipopeptides and can reach up to 17% of bacterial mass dry weight (**MATSUYAMA, T. ET AL, 1985**). Surfactants with a microbial origin are superior to synthetic surfactants due to a less toxic potential, useful for industrial applications (**MATSUYAMA, T. et al., 1992**).
Serrawettins are extracellular products capable of enhancing flagellum dependent and independent spread growth in a surface environment. Mutants for Serratomolides genes failed to form colonies and these lipopeptides were suggested as promoters of a new type of spreading growth (**MATSUYAMA, T. et al., 1992**).
Serratia strains are able to live in plants and invertebrates and the surfaces in these habitats have a variety of characteristics: hydrophobic, hydrophilic, fractal, smooth, axenic, among others. In order to colonise these surfaces, bacteria have special strategies, such as Serratomolides production. Bacteria are so small that intermolecular forces are strong enough to drive their movements and Serrawettins excretion lightens surface tension (**MATSUYAMA, T. et al., 1992**).
Serrawettins surface activity is established: Serrawettin W1 has a surface activity of 32,2 mN/m, Serrawettin W2 of 33,9 mN/m and Serrawettin W3 of 28,8 mN/m (**MATSUYAMA, T., & NAKAGAWA, Y., 1996**). Other lipopeptides analogous to Serrawettin W2, different in residue 4 and 5 of the amino acid chain, were suggested and named Serrawettin W4, W5 and W6 (**MOTLEY, J. L. et al., 2017**).

As prodigiosin, Serrawettins are thermoregulated. Production occurs at 30 ºC and ceases at 37 ºC (**LI, H. et al., 2005**). Serratomolides have complex structures and are synthesize by non-ribosomal enzymatic processes.

Wasserman first described Serrawettin W1 in many pigmented *S. marcescens* strains as an antibiotic (**WASSERMAN, H. H. et al., 1961**). Serrawettin W1 has a symmetric structure composed of two serine molecules and two 3-hydroxydecanoic acids (**MATSUYAMA, T. et al., 1992**). The biosynthesis of this Serratomolide is thought to occur by condensation of two molecules of serratamic acid (D-3hydroxydecanoyl-L-serine). PPTase (4'-phosphopantetheinyl transferase) encoded by *pswP* gene plays a role on Serrawettin W1 biosynthesis. Serrawettin W1 gene belongs to non-ribosomal peptide synthetase (NRPS) family. The *srw1* gene has 4,476 bp, presents a large open reading frame (ORF) and a condensation, adenylation, thiolation and thioesterase domains in functional order.

Biosynthesis of serrawettin W1 was determined: the first step is the activation of L-serine by adenylation through adenylation domain. Then, activated L-serine binds as a thioester to thiolation domain that was previously phosphorylated by PPTase. The 3-D-hydroxydecanoyl moiety binded to acyl carrier protein (ACP) will react to L-serine thiolation domain, creating an amide linkage by ACP detach. This concludes the formation of serratamic acid. The role of ACP is not well described. Since this molecule is a symmetric molecule with two serratamic acids, it is necessary to bind a second serratamic acid to the free phosphorylated thiolation domain. An inter-molecular ester linkage bond between two serratamic acids produces a symmetrical circular Serrawettin W1. Serrawettin W1 formation has no peptide bonds and the synthesis is made by a uni-modular NRPS (**LI, H. et al., 2005**).

Serrawettin W2 is a cyclic peptide containing a single fatty acid (3-hydroxydecanoic acid) and five amino acids: D-leucine (N-bonded to the carboxylate of the fatty acid)-L-serine-L-threonine-D-phenylalanine-L-isoleucine (bonded to fatty acid group). This lipopeptide promotes flagellum spreading growth, contributing specifically to surface bacterial translocation. Hydroxyl groups of serine and threonine may be hydrophilic parts of this pentapeptide (**MATSUYAMA, T. et al., 1992**). The production of Serrawettin W2 is known to be dependent on the action of *pswP* gene expression PPTase (**PRADEL, E. et al., 2007**). In genome of *S. surfactantfaciens* YD25T, a hybrid polyketide synthase (PKS-NRPS) gene cluster was found to be involved in biosynthesis of Serrawettin W2.

This peptide has antimicrobial activity against many bacteria and fungi and antitumor activity against Hela cells. Serrawettin W2 has a molecular mass of 731.2 Da (**SU, C. et al., 2016**).

Biosynthesis gene cluster is characterized by a hybrid PKS-NRPS system. PKS includes domains that express an acyltransferase (AT), ketosynthase (KS) and a keto reductase (KR). NRPS gene has five modules, each with a specific condensation (C), adenylation (A) and thiolation (T) domains. Serrawettin W2 pathway was deduced: first, PKS and other undetermined proteins synthesize a C10 unit fatty acid, precursor material released in fatty acyl-CoA form. Then, *srw2* gene, which is more complex than *srw1* gene and has five modules, encodes the core W2-peptide chain with 5

amino acids. The N-terminal C-domain of initiation module catalyses the condensation of fatty acyl-CoA with leucine. After peptide initiation, chain elongation continues to serine, threonine and phenylalanine. In the last step, this oligopeptide is transferred to leucine active site in C-terminal of the last module. The whole organization is consistent with catalysing chain elongation to create a cyclopeptide and release it to extra-membrane space, through extracellular vesicles (**SU, C. et al., 2016**).

Serrawettin W3 structure is not well defined but degradation analysis indicates presence of a dodecanoic acid and five amino acids: threonine, serine, valine, leucine, and isoleucine (**MATSUYAMA, T. et al., 1992**).

Serrawettin W1 and Serrawettin W2 will be the two Serratomolides studied in this work.

To better understand nematod-bacteria interaction, three types of studies can be performed: avoidance test, attraction test and mortality test.

Studies with *C. elegans* and *S. marcescens* were made by Pradel and colleagues, in order to determine if the nematode avoids its bacterial pathogen (**PRADEL, E. et al., 2007**). Some results suggested an olfactory ability of nematodes to discriminate between odours and to modify their behaviour through olfactory learning. The model nematode has 30 chemosensory neurons that are able to detect bacterial odour through G-proteins coupled with chemoreceptors. *C. elegans* can be attracted by several metabolites, such as amino acids and autoinducers in order to seek food sources. However, *C. elegans* is also able to discriminate different species of bacteria, including those with pathogenic potential, in order to avoid them. Some pathogenic bacteria, for instance *S. marcescens*, elicit a biphasic nematode behaviour in which it initially enters into the bacterial lawn but later remains near the edge of the lawn, a behaviour called lawn avoidance (**PRADEL, E. et al., 2007**). Toll-like transmembrane receptor, TOL1, AIY neurons and the two AWB sensory neurons may be involved on avoidance processing (**PRADEL, E. et al., 2007**).

One study revealed that Serrawettin W2 repeals *C. elegans*, suggesting that Serrawettin W2 chemically informs the nematode that *Serratia* species are near.

S. marcescens is very virulent to *C. elegans* and other nematodes, because it grows in the intestine, killing the animal after infection, and segregates enzymes capable of dissolving nematode´s eggshells. Although this study provides results suggesting a correlation between Serrawettin W2 and *C. elegans*, this is not unequivocally proved. *C. elegans* is attracted by *S. marcescens* from distance but when it comes in contact with certain strains, the nematode develops avoidance behaviour (**PUJOL, N. et al., 2001**).

Some studies described that Serrawettin W2 is directly sensed as an aversive nematode stimuli. Avoidance response depends on AWB chemosensory neurons attached to a G-protein receptor. Serrawettin W1 and W3 molecules differ on nematode´s avoidance response. Other receptors might be involved on avoidance response, such as *tol-1* gene, important to discriminate *Serratia* strains. The three serratomolides induced *C. elegans* avoidance, but Serrawettin W3 had a higher avoidance effect and Serrawettin W1 a lower effect (**PRADEL, E. et al., 2007**).

H. Objectives

PWD threatens Portuguese forests and in consequence, wood exportations and forest based economy. We aim to better understand the role played, in PWD, by some *Serratia* strains, and by Serratomolides they produce, in order to assess their nematicidal potential against PWN. To achieve this, seven specific goals were established:

1- Determine the presence of Serrawettin genes in nineteen selected *Serratia* strains from UCCCB collection.
2- Determine bacteria-nematode interactions through mortality tests (*C. elegans* and *B. xylophilus*), and attraction and avoidance tests (*C. elegans*), using bacterial strains and respective supernatants.
3- Create disruptive *srw1* and *srw2* mutants in selected *Serratia* strains to assess the nematicidal impact of Serrawettins.
4- Clone *srw1* and *srw2* genes into *E. coli* DH5α to obtain purified Serrawettin W1 and W2 and test their nematicidal potencial.
5- As Serrawettins are amino lipids, extract amino lipids produced by *Serratia* strains with Serrawettin genes to understand amino lipid-nematode interactions through mortality tests in *C. elegans* and *B. xylophilus* and attraction tests in *C. elegans.*
6- Analyse Serrawettin W1 and W2 gene clusters, through AntiSMASH data base, to better understand and determine Serrawettins biosynthesis process.
7- Perform molecular dynamic simulations between TOL1 protein from *C. elegans* and Serrawettins in order to identify residues with higher probability of protein-ligand interactions.

Material and methods

A. Amplification and purification of *srw1* and *srw2* genes

A1. Bacterial strains, media and growth conditions

Twenty bacterial strains, 19 *Serratia* strains (7 strains isolated from Portuguese *P. pinaster* trees endophytic community (**PROENÇA, D. N. et al., 2017a**), 4 PWN carried strains from Portugal (**PROENÇA et al., 2010**), 5 PWN carried strains from USA (**PROENÇA, D. N. et al., 2014**) and 3 similar to *S. plymuthica*) and one PWN carried *Pseudomonas* strain from Portugal (**PROENÇA, D. N et al., 2010**), used as control, were studied for lipopeptides Serrawettin W1 and Serrawettin W2 presence.
Strains were grown on R2A agar medium for 24 h at 30 ºC for bacterial DNA extraction.
For mortality, attraction and avoidance tests, bacterial strains were grown in Erlenmeyer's flasks containing 50 mL CAA liquid medium at 30 ºC for 24 h, at 180 rpm (**PAIVA, G. et al., 2013**). Four mL of each bacterial growth were stored to be used on attraction and avoidance tests. Bacterial growths were centrifuged at 12.100 rpm, at 4 ºC, for 10 min, and 4 mL of each supernatant were stored to be used in attraction, avoidance and mortality tests. Remainder supernatant was collected and concentrated with vacuum at -40 ºC to be used on mortality tests.

A2. DNA extraction and quantification

Genomic DNA extraction from all strains was performed using NZY Microbial gDNA Isolation kit, according to manufacturer´s instructions. DNA samples were quantified using NanoDrop technology (Thermo Scientific™, USA).

A3. Primers design

To partially amplify *srw1* and *srw2* genes using PCR, new specific primers were designed. 69 *Serratia* genomes available on NCBI (**Table 1**) were individually introduced in AntiSMASH database (**BLIN, K. AT AL., 2019**) to identify all secondary metabolites gene clusters. Some of those secondary metabolites gene clusters presented DNA sequences similar to Serrawettins' genes: six genomes with *srw1* gene cluster and six with *srw2* gene cluster (**Table 1**). These genes, when aligned with known *srw1* and *srw2* genes, provided an idea of genetically conserved regions.

Table 1 Analysis of *Serratia* strains with *srw1* and *srw2* similar genes identified through AntiSMASH software. AntiSMASH software was used in 69 *Serratia* genomes. We confirmed the presence of genes similar to *srw1* in six genomes and the presence of genes similar to s*rw2* in other six genomes, marked with (X).

Bacterial strain	Accession number	*srw1*	*srw2*
Serratia sp. AS12	CP002774.1		
Serratia sp. AS13	CP002775.1	X	
Serratia sp. FGI94	CP003942.1		
Serratia sp. ATCC 39006	CP025084.1		
Serratia sp. FS14	CP005927.1		
Serratia sp. SCBI	CP003424.1		X
Serratia sp. YD25	CP016948.1		X
Serratia sp. ATCC 39006	CP025085.1		
Serratia sp. SSNIH1	CP026383.1		X
Serratia sp. MYb239	CP023268.1		
Serratia sp. 1D1416	CP032738.1		
Serratia sp. 3ACOL1	CP033055.1		
Serratia sp. P2ACOL2	CP033162.1		
Serratia sp. LS-1	CP033504.1		
Serratia sp. FDAARGOS_506	CP033831.1		
Serratia sp. JKS000199	LT907843.1		
Serratia sp. S4	APLA00000000.1		
Serratia sp. HMSC15F11	LWNG00000000.1		
Serratia sp. M24T3	AJHJ00000000.1		
Serratia sp. DD3	AYKS00000000.2		
Serratia sp. H1n	AYMN00000000.1		
Serratia sp. H1w	AYMQ00000000.1		
Serratia sp. Ag1	JQEI00000000.1		
Serratia sp. Ag2	JQEJ00000000.1		
Serratia sp. TEL	LDEG00000000.1		
Serratia sp. ISTD04	MBDW00000000.1		
Serratia sp. S119	MSFH00000000.1		
Serratia sp. OLMTLW26	MNBD00000000.1		
Serratia sp. OLLOLW30	MKYT00000000.1		
Serratia sp. OLBL1	MORD00000000.1		
Serratia sp. OLEL1	MORG00000000.1		
Serratia sp. OLFL2	MORH00000000.1		
Serratia sp. OLHL2	MORI00000000.1		
Serratia sp. OLJL1	MOWO00000000.1		
Serratia sp. OLDL1	MORE00000000.1		
Serratia sp. OLCL1	MORE00000000.1		
Serratia sp. OLIL2	MOWN00000000.1		
Serratia sp. OSPLW9	MSTM00000000.1		
Serratia sp. OMLW3	MSTK00000000.1		
Serratia sp. OLAL2	MSTL00000000.1		
Serratia sp. OPWLW3	MTCE00000000.1		
Serratia sp. OPWLW2	MTCF00000000.1		
Serratia sp. TKO39	PETD00000000.1		
Serratia sp. SSNIH4	PQJU00000000.1		
Serratia sp. SSNIH5	PQJS00000000.1		
Serratia sp. SSNIH3	PQJT00000000.1		
Serratia sp. SSNIH2	PQJR00000000.1		
Serratia sp. Nf2	PYTW00000000.1		
Serratia sp. S1B	PYUJ00000000.1		
Serratia sp. JKS296	OCMX00000000.1		
Serratia sp. C-1	CAQO00000000.1		
Serratia sp. 506_PEND	JVEL00000000.1		
Serratia sp. Leaf50	LMLI00000000.1		
Serratia sp. PWN146	LT575490.1		X
Serratia sp. 2880STDY5682895	FCJL00000000.1		
Serratia sp. 2880STDY5682894	FCJA00000000.1		
Serratia sp. 14-2641	LXKR00000000.1		
Serratia sp. BW106	MCGS00000000.1		
Serratia sp. S40	QYYG00000000.1		
Serratia sp. GLFA	ARZD00000000.1		
Serratia sp. UBA10706	DPNH00000000.1		
Serratia sp. LCN16	FBSD00000000.1		
Serratia grimesii	LT883155.1	X	
Serratia marcescens UMH8	CP018927.1	X	

Table 1 (continuation)

Bacterial strain	Accession number	*srw1*	*srw2*
Serratia marcescens IOMTU 115	AB894481.1	X	
Serratia sp. FS14	CP005927.1	X	
Serratia marcescens WW4	CP003959.1	X	
Serratia marcescens RSC-14	CP012639.1		X
Serratia liquefaciens	AF039572.1		X

Specific primers for *srw1* and *srw2* genes were designed, complementary to genetically conserved regions. Four primers for *srw1* gene were designed: srw1_1F, srw1_2F, srw1_2R and srw1_7R. Two groups for *srw2* gene were formed according to their similarities. Five primers were designed for group A of *srw2*: srw2A_1F, srw2A_9F, srw2A_9R, srw2A_10R and srw2A_11R. Seven primers were designed for group B of *srw2*: srw2B_1F, srw2B_7F, srw2B_10F, srw2B_7R, srw2B_10R, srw2B_11R, and srw2B_13R. Four PCR were performed to study the presence of *srw1* and *srw2* genes (**Table 2**).

Table 2 Primers information to test the presence of *srw1* gene (PCR A) and *srw2* gene (PCR B, C and D).

Primer	Temperature cycling (30x)	Primer sequence	Length (bp)
PCR A	95 ºC for 60 s 56 ºC for 60 s 72 ºC for 210 s	srw1_1F: 5´-GTGTCCGCTTATTCYCTSAC-3´ srw1_7R: 5´-TGAATGGCRTGCAGCGAATG-3´	3,037
PCR B	95 ºC for 60 s 56 ºC for 60 s 72 ºC for 210 s	srw2B_1F: 5´-TCGCCCGGATARGCCGGATC-3´ srw2B_13R: 5´-GGSGATCCTSAAAGCCGGCGG-3´	3,352
PCR C	95 ºC for 60 s 56 ºC for 60 s 72 ºC for 180 s	srw2A_1F: 5´-ATGAACAAACAMACTGATGTG-3´ srw2A_10R: 5´-CCGCGCCTGCGCTTCRAACAG-3´	2,652
PCR D	95 ºC for 60 s 56 ºC for 60 s 72 ºC for 180 s	srw2A_1F: 5´-ATGAACAAACAMACTGATGTG-3´ srw2A_11R: 5´-TTTGACCTGCGCTTCRAACAG-3´	2,652

A4. Polymerase Chain Reaction and DNA sequence analysis

To screen for the presence of *srw1* and *srw2* genes in *Serratia* strains, PCRs were performed with selected primers. For each PCR, a mix was prepared containing, for each sample, 6.25 µL of MasterMix (NZYTaq II 2× Green Master Mix 0.2 U/µL), 0.2 µM of forward and reverse primers and 16.25 µL of ddH_2O. 1 µL of DNA samples (**Table 5**) were added to a PCR tube containing the correspondent mix. For every mix a negative control was made with no DNA. PCR primers and gene length are summarized in **Table 2**. According to gene size and G+C mol% content of primers, temperature of annealing was adjusted. Electrophoreses were performed on 1 % agarose gels at 90 V to separate and extract *srw1* and *srw2* partial genes. For PCR products that showed a band in the correspondent molecular size, DNA bands were removed and purified, using Omega´s Gel DNA Extraction Kit, according to manufacturer´s instructions. DNA samples after gel extraction were sequenced by STABVIDA and the resulting sequences were compared with sequences available in EMBL/GenBank database using BLAST network services (**ALTSCHUL, S.F., et al., 1990**).

A5. Phylogenetic analysis

One evolutionary tree was designed based on 16S rRNA gene sequence comparison of 64 bacterial strains:

- *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^{T}, AS13 and Leaf50 and *Pseudomonas* strain M47Tronco1;
- strains with *srw1* gene cluster retrieved from ANTISMASH: *Serratia* strains ATCC 13880, CDC_813-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EGD-HP20, WW4, FS14, TEL NODE_13, BXF1, A2, AS9 and AS12;
- strains with *srw2* gene cluster retrieved from ANTISMASH: *Serratia* strains PWN146, SSNIH1, SM39, SmUNAM836, Lr5/4 LG59, RSC-14, AH0650_Sm1 AG2, Db11, SCBI and YD25;
- LPSN species (**PARTE, A. C., 2014)** *S. aquatilis, S. oryzae, S. fonticola, S. fonticola DSM 22080, S. liquefaciens, S. grimesii, S. proteamaculans, S. myotis, S. proteamaculans, S. ficaria, S. vespertilionis, S. entomophila, S. odorifera, S. symbiotica, S. nematodiphila, S. marcescens, S. ureilytica*, *P. moorei, P. mohnii and P. aeruginosa*.

All sequences were included in 16S rRNA-based Living Tree Project (LTP) through Silva database (**PRUESSE, E. et al. 2012**). A phylogenetic tree was established using the Neighbor-Joining method in ARB software package version 5.5 (**LUDWIG, W., 2004**).

B. Bacteria-nematode interactions: Mortality, Attraction and Avoidance tests

Mortality, attraction and avoidance tests were performed to assess *C. elegans* reaction when in the presence of selected strains. Mortality tests with bacterial concentrated supernatants were performed in 48 multi-well plates. The percentage of dead nematodes after a period of 24 h, 48 h and 72 h were determined to see which bacteria have a nematicidal effect. Attraction and avoidance tests were performed on agar plates with *Serratia* and *Pseudomonas* strains versus *Escherichia coli* OP50, with supernatants of selected strains versus *E. coli* OP50 and with supernatants of selected strains versus *E. coli* OP50 supernatant. With attraction tests we assessed the percentage of nematodes drawn to bacteria or supernatants. With avoidance tests we determined the fraction of nematodes that escaped from bacteria or supernatants. These results provided us with an idea of *C. elegans´* behaviour when in the presence of select bacteria and supernatants.

B1. Maintenance of *C. elegans*

Nematodes were synchronized on Nematode Growth Medium (NGM) agar plates with *E. coli* OP50 (source of food) twice a week in order to renew the medium and maintain young and adult nematodes. To synchronize *C. elegans*, NGM agar plates with nematodes in different stages were washed up and down with 2 mL of ddH_2O and the water suspension with nematodes and eggs was transferred into a 15 mL sterile Falcon. A fresh NaOH Bleach solution with 0.5 mL of NaOH (2M) and 1 mL of 3 % bleach was added to the 2 mL of *C. elegans* suspension. This solution kills all nematodes, preserving eggs that have a protective shield. After 10 min with vigorous vortex every 2 min, nematode suspension with dead nematodes and viable eggs was centrifuged at 13,000 rpm for 1 min, supernatant was discarded and pellet was washed with 1 mL of pure water two times. Then, 150 µL of the final resuspended solution was distributed into new NGM plates with *E. coli* OP50. For attraction and avoidance tests, *C. elegans* synchronization took place 24 h before the experiment in order to use stage L3 larvae in all tests. Attraction and avoidance tests were performed with starved synchronized *C. elegans*: eggs hatched on NGM plates without *E. coli* OP50. For mortality tests, fed *C. elegans* were synchronized 48 h before tests (**STIERNAGLE, T., 2006**). For all experiments, Petri dishes with synchronized *C. elegans* were washed with 2 mL of ddH_2O and the volume was adjusted in order to have a nematode suspension with 20-30 *C. elegans* on 10 µL for attraction and avoidance tests and with 10 *C. elegans* on 10 µL for mortality tests.

B2. Nematicidal tests in *C. elegans* and *B. xylophilus*

Mortality tests were performed on 24 multi-well plates for both *C. elegans* and *B. xylophilus*. For *C. elegans´* mortality tests, 10 µL of a nematode suspension with approximately 10 *C. elegans* was inserted in the middle of the well. Initial number of *C. elegans* in each well was assessed. For *B. xylophilus´* mortality tests, 50 µL of ddH_2O was put on the middle of the well and for each well, exactly 10 young *B. xylophilus* were added using a disinfected eyelash. Every test was performed in triplicate with concentrated bacterial supernatants and once with supernatants. For each test, 500 µL of bacterial supernatant or bacterial concentrated supernatant were added. After 24 h, 48 h and 72 h, dead nematodes were assessed (**PAIVA, G. et al., 2013**). CAA medium (bacterial growth medium) and M9 medium (liquid medium used on *C. elegans* maintenance) were used as negative controls.

B3. Attraction tests in *C. elegans*

Attraction tests were performed on NGM plates divided in three parts and equidistant spots. For each bacteria strain, three different attraction tests were made: i) bacterial suspension on one side

and *E. coli* OP50 on the other side as a control; ii) supernatant of bacteria on one side and *E. coli* OP50 on the other side; iii) and supernatant of bacteria on one side and supernatant of *E.coli* OP50 on the other side. For bacterial suspension, 10 µL with OD_{600}=1.0 were placed on the correspondent side of NGM agar plates. Ten µL of supernatants obtained by bacterial growth centrifugation was used. After 30 min for drops to dry, 10 µL of a nematode suspension, with approximate 20-30 *C. elegans*, were added to the third side of NGM plate. Initial number of *C. elegans* in every plate was counted. Each attraction test was performed in triplicate. All NGM plates were sealed with Parafilm and were put at 19 ºC. After 2 h and 24 h, nematodes on each drop were assessed to see if bacteria or supernatants were able to attract more nematodes than control (**PRADEL, E. et al., 2007; PUJOL; N. et al., 2001; HSUEH, Y. ET AL, 2017**).

B4. Avoidance tests in *C. elegans*

NGM agar plates were divided in two parts and, as with attraction tests, three different avoidance tests were conducted: i) bacteria suspension on one side and *E. coli* OP50 on the other side as a control; ii) supernatant of bacteria on one side and *E. coli* OP50 on the other side; iii) supernatant of bacteria on one side and supernatant of *E.coli* OP50 on the other side. For each side, 10 µL of bacterial suspension or supernatant were dropped. After 30 min, to allow drops to dry, 10 µL of a nematode suspension with approximately 20-30 nematodes was added on top of each test drop. Initial number of *C. elegans* in every drop was counted. Avoidance tests were performed in triplicate. All NGM agar plates were sealed with Parafilm and were put at 19 ºC. Nematodes were counted after 2 h and 24 h and an avoidance fraction of *C. elegans* on test drop and on control drop was calculated for each case, in order to test if nematodes avoided bacteria or supernatant (**PRADEL, E. et al., 2007; PUJOL, N. et al., 2001**).

B5. Bacterial extracellular proteome by SDS-PAGE

Total protein concentration of supernatant and pellet, resultant from bacterial suspension centrifugation, was measured by using Bradford protein assay (**BRADFORD, M. M., 1976**). For supernatant protein quantification, 100 µL of supernatant samples were added to 1,000 µL of Bradford reagent and OD was measured at 595 nm. Protein concentration of concentrated supernatants, mentioned above, was quantified using the same method. Protein concentration from both supernatants and concentrated supernatants were also quantified using Nanodrop (mg/mL).
In order to observe the protein profile, present in concentrated supernatants, a SDS-PAGE was performed. A 12 % resolution gel was prepared with 11.7 mL of ddH_2O, 9 mL of 40% acrylamide solution, 7.5 mL of 1.5 M Buffer, 300 µL of a 10% SDS solution, 175 µL of APS 10% and 30 µL of TEMED. Isopropanol was added on top of resolution gel to align the surface. After

polymerization, isopropanol was withdrawn, the system was washed with dH_2O, and a 4% stacking gel was prepared with 2.95 mL of ddH_2O, 750 µL of a 40% acrylamide solution, 1.25 mL of 0.5 M Buffer, 50 µL of a 10% SDS solution, 175 µL of APS 10% and 30 µL of TEMED. 30 µL of each concentrated supernatant were mixed with 15 µL of Loading Buffer (0.25 mL/mL of 1M Tris-HCl with pH 6.8, 0.1 g/mL of SDS, 0.08 mL/mL of 0.1% bromophenol blue, 0.4 mL/mL of 100% glycerol, 0.2 mL/mL β-mercaptoethanol in water) and heated at 100 ºC for 10 min. After loading the gel with a molecular marker and samples, a 170 V current was applied for 5 h for big systems or 2 h for small systems. After electrophoresis, proteins were stained with a Coomasie Blue G-250 solution and discoloured with a discolouring solution (**PAIVA, G. et al., 2013**).

B6. Statistical analysis

In order to find statistical differences among mortality test results, a Two-way ANOVA was performed, separately, with data from *C. elegans´* mortality tests and with data from *B. xylophilus´* mortality tests. Subsequently, a Tukey Test was performed for each concentrated supernatant and CAA medium control to calculate statistical differences (**Annex 1**).
Attraction and avoidance test results were treated and a Chi-Square statistical test was performed to detect significant differences between case and control (**Annex 2**).
All statistical analyses were performed using R-program (**R DEVELOPMENT CORE TEAM, 2008**) and results were considered statistically different when $p < 0.05$.

C. Disruptive mutants

A Random Amplification of Polymorphic DNA (RAPD) was performed on *Serratia* strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, A88copa7, A88copa13, NBRC 102599^T and AS13. Each RAPD had 17.25 µL of ddH_2O, 6.25 µL of Master Mix (NZYTaq II 2x Green Master Mix 0.2 U/µL), 0.5 µL of OPA-03 primer 0.2 µM (Operon Technologies, USA) and 1 µL of genomic DNA.
One colony from each *Serratia* strain was grown overnight at 30 ºC, at 120 rpm on LB liquid medium with 50 mg/mL of kanamycin and, as control, on LB liquid medium without kanamycin. The mutagenic plasmid pK18mob with a kanamycin resistance cassette (**SCHÄFER, A. et al. 1994**) was chosen to induce a disruptive mutation on Serrawettins genes. *E. coli* S7 with pK18mob plasmid was grown overnight on 5 mL of LB liquid medium with 50 mg/mL of kanamycin and pK18mob vector was extracted according to Omega EZNA Plasmid DNA mini kit.
New primers complementary to StabVida results for Serrawettins partial genes were designed. Two restriction enzymes unable to interrupt Serrawettins´ partial genes, both present on multiple cloning site of pK18mob vector, were chosen (EcoRI and SphI from Takara) and the recognized enzyme

sequences were added to forward and reverse primers. A poly-A sequence was also added to the 3´ side of designed primers for mutagenesis (**Table 3**).

Table 3 Mutagenesis primers details. *Serratia* strains Arv-22-2.5c, Arv-22-2.6, NBRC 102599^T and AS13 have *srw1* gene and *Serratia* strains A88copa7 and A88copa13 have *srw2* gene. Primer sequences were based on StabVida sequencing from Serrawettin W1 and W2 partial genes. Forward and reverse primers were designed in order to have a restriction sequence recognized by EcoRI and SphI restriction enzymes. A 3´ poly-A tail was added to each primer. Serrawettins´ partial gene length is presented for each pair of primers in base pairs.

PCR	Temperature cycling (30x)	Primer sequence	Length (bp)
OPA-03	94 ºC for 60 s 40 ºC for 60 s 72 ºC for 90 s	OPA-03: 5´-AGTCAGCCAC-3´	
Arv-22-2.5c and Arv-22-2.6	95 ºC for 60 s 58 ºC for 60 s 72 ºC for 30 s	EcoRIsrw1A_F: 5´-AAA**GAATTC**TCTTTAACCCCGATATCCCCTTATA-3´ SphIsrw1A_R: 5´-AAA**GCATGC**GTTTTTTATCCCTGCTTTTAATTGT-3´	454
NBRC 102599^T and As13	95 ºC for 60 s 58 ºC for 60 s 72 ºC for 30 s	EcoRIsrw1B_F: 5´-AAA**GAATTC**TCGACCAATCTTTGATCCCGAGATC-3´ SphIsrw1B_R: 5´-AAA**GCATGC**TTAATTGCTTATAACGATCCATGAT-3´	427
A88copa7 and A88copa13	95 ºC for 60 s 58 ºC for 60 s 72 ºC for 30 s	EcoRIsrw2_F: 5´-AAA**GAATTC**ACGGGTGCTCAGGCCGCGGCGGGGA-3´ SphIsrw2_R: 5´-AAA**GCATGC**TAGCGCGGCGCGGCAGCGCGGCATC-3´	368
ligation confirmation	95 ºC for 60 s 55 ºC for 60 s 72 ºC for 60 s	M13F: 5´-GTAAAACGACGGCCAG-3´ M13R: 5´-CAGGAAACAGCTATGAC-3´	480

PCRs with primers designed for mutagenesis were performed with Supreme Taq (5 U/µL), each one with 6 µL of dNTP´s, 3 µL of Magnesium 50 mM, 1 µL of each specific primer (0.2 µM), 0,3 µL of Taq Supreme, 17,2 µL of ddH$_2$O water and 1,5 µL of DNA (**Table 5**). Specific size band from PCR amplification was cut off. DNA was extracted according to OMEGA Gel extraction DNA kit and quantified with NanoDrop technology.

Restriction with EcoRI and SphI enzymes was performed both in inserts and pK18mob plasmid. For each restriction reaction, 2 µL of 1x buffer, 0.6 µL of each restriction enzyme (10 U/µL) and 15 µL of insert or plasmid (**Table 8**) were incubated at 37 ºC for 3 h. Three µL of loading buffer were added to restriction reactions and samples were loaded on a 2 % electrophoresis gel at 90 V. Bands with a slightly smaller size than PCR amplification and pK18mob vector were cut, DNA was extracted according to OMEGA Gel extraction DNA kit and quantified with NanoDrop Technology.

Ligation reaction between pK18mob vector and inserts was performed with 1.5 µL of plasmid (**Table 8**), 10 µL of insert (**Table 8**), 1.5 µL of T4 ligase 5 U/µL (T4 DNA Ligase, Thermo Scientific™) and 7.5 µL of 1x ligase buffer.

After 2 h, at room temperature, 50 µL of *E. coli* DH5α competent cells were added to each ligation reaction. To transform *E. coli* DH5α competent cells, after 20 min at 4 ºC, cells were incubated for 47 s on a 42 ºC bath and, immediately after, returned to 4 ºC for 2 min. 250 µL of sterile LB liquid medium were added to transformed cells and, after 90 min of incubation at 37 ºC, cells were

centrifuged at 13,300 rpm for 1 min. Volume was adjusted to 100 µL and pellet was resuspended. 100 µL with transformed cells were spread on LB solid medium with 50 mg/mL of kanamycin plates and incubated, overnight, at 37 ºC.

Colonies from LB solid medium with 50 mg/mL of kanamycin plates were transferred to 5 mL of LB liquid medium with 50 mg/mL of kanamycin and were incubated, overnight, at 120 rpm, at 37 ºC. Plasmid extraction was performed according to Omega E.Z.N.A DNA Plasmid Mini Kit I, followed by quantification using NanoDrop Technology. Ligation confirmation was performed by PCR, using pK18mob vector primers M13F and M13R (Thermo Scientific^{TM}), with MasterMix, as previously described.

Electrocompetent cells of *Serratia* strains were prepared as follows. An isolated colony was pre inoculated on LB liquid medium overnight, at 120 rpm, at 30 ºC. Bacteria were transferred from pre inoculum to 50 mL of LB liquid medium in order to obtain an OD_{600} = 0.07. After bacterial growth reached an OD_{600} = 0.6, inocula were transferred to ice and centrifuged at 12,100 rpm for 10 min at 4 ºC. Supernatants were discarded. Pellets were resuspended on ice water and centrifuged twice at 12,100 rpm for 5 min at 4 ºC. Last pellets were washed with an ice 10% glycerol solution and centrifuged at 12,100 rpm for 5 min at 4 ºC. Glycerol supernatants were discarded and 200 µL of ice glycerol solution was added. Pellets were resuspended and 50 µL aliquots were stored at -80 ºC for future use.

Aliquots with *Serratia* electrocompetent cells were placed on electroporation cuvettes with 2 µL of appropriate plasmid-insert ligation. Electroporation was performed with 2.5 kV for 5.9 ms. Transformed cells were recuperated on 1 mL of SOC medium and incubated at 30 ºC for 60 min. After a 1 min centrifugation at 13,300 rpm, volumes were adjusted to 100 µL and pellets were resuspended. Transformed cells were spread on R2A plates with 50 mg/mL of kanamycin.

Putative mutant DNA from transformed *Serratia* strains was extracted. A 1 µL loop of each transformed *Serratia* was resuspended on 50 µL of lysis buffer. Cells were centrifuged at 13,300 rpm for 5 min after two exposure cycles of 10 min at -80 ºC and 10 min at 100 ºC. Supernatants were transferred to new Eppendorfs. 100 µL of sterile water and 300 µL of chloroform/isoamyl alcohol 24:1 solution were added and samples were vortexed. After centrifugation at 13,300 rpm for 15 min, upper phases were transferred to new Eppendorfs. 500 µL of 70% ethanol were added and samples were vortexed. After a 13,300 rpm centrifugation for 15 min, supernatants were discarded and remaining ethanol was evaporated at 70 ºC. DNA was eluted with 30 µL of sterile water. DNA from *Serratia* A88copa13 mutant 17.8 was extracted according to NZY Microbial gDNA Isolation kit NZYTech.

PCR for each DNA extraction from transformed *Serratia* strains was performed with 17.25 µL of ddH_2O, 6.25 µL of MasterMix (NZYTaq II 2x Green Master Mix 0.2 U/µL), 0.5 µL of each primer (0.2 µM) (**Table 3**) and 1.5 µL of DNA. Samples were loaded on a 2% electrophoresis gel at 90 V.

D. Heterologous expression in *E. coli*

Using AntiSMASH alignment of Serrawettin genes from different *Serratia* strains (*Serratia* strains AS13, NBRC 102599^T, grimesii, UMH8 and WW4 for *srw1* gene and *Serratia* strains PWN146, SSNIH1 and UMH9 for *srw2* gene), new primers were designed for Serrawettin W1 and W2 total genes. Expression vector pET30a with a kanamycin resistance cassette was chosen. Two restriction enzymes unable to interrupt Serrawettins´ genes and present on multiple cloning site of pET30a expression vector were chosen (SalI and NotI from Takara) and a recognized sequence was added to forward and reverse primers. A poly-A sequence was also added to the 3´ side of designed primers for expression. **Table 4** presents expression primers details.

Table 4 Expression primers details. *Serratia* strains Arv-22-2.5c, Arv-22-2.6, NBRC 102599^T and AS13 have *srw1* gene and *Serratia* strains A88copa7 and A88copa13 have *srw2* gene. Primers were based on AntiSMASH sequence alignment of *srw1* gene on *Serratia* strains AS13, NBRC 102599^T, grimesii, UMH8 and WW4 for and of *srw2* gene on *Serratia* strains PWN146, SSNIH1 and UMH9. Forward and reverse primers were designed in order to have a restriction sequence recognized by SalI and NotI restriction enzymes. A 3´ poly-A tail was added to each primer. Length of Serrawettins genes for each pair of primers are represented in base pairs.

PCR	Temperature cycling (30x)	Primer sequence	Length (bp)
Arv-22-2.5c and Arv-22-2.6	94 ºC for 60 s 57 ºC for 60 s 72 ºC for 300 s	srw1_FT_ALL_SalI: 5´-AAA**GTCGAC**GTGTCCGCTTATTCYCTGACRACCG-3´ srw1_RT_Arv_NotI: 5´-AAA**GCGGCCGC**TCAAGGAAGGTTGCCGAGCATCGCC-3´	3,948
NBRC 102599^T and As13	94 ºC for 60 s 57 ºC for 60 s 72 ºC for 300 s	srw1_FT_ALL_SalI: 5´-AAAGTCGACGTGTCCGCTTATTCYCTGACRACCG-3´ srw1_RT_AS13_NBRC_NotI: 5´-AAA**GCGGCCGC**TCAATGAATRTCGTCGAGYATCAYC-3´	3,948
A88copa7 and A88copa13	94 ºC for 60 s 57 ºC for 60 s 72 ºC for 1080s	srw2_FT_NotI: 5´-AAA**GCGGCCGC**ATGAACAAACAAACTGATGTGAAGA-3´ srw2-RT-NotI: 5´-AAA**GCGGCCGC**TTACGCCTGTYCGAGCTCG-3´	17,853
Ligation confirmation	95 ºC for 60 s 58 ºC for 60 s 72 ºC for 60 s	T7promoter: 5´-TAATACGACTCACTATAGGG-3´ srw2_2R: 5´-GCCAGGCTGCGCTGYGGRTC-3´	2,062

PCRs with primers designed for *srw1* gene were performed with Supreme Taq, as previously described. Insert concentrations are resumed in **Table 10**. PCRs with primers designed for *srw2* gene were performed with Supreme NZYLong 2x Green Master Mix (0.2 U/µL) each containing 34.5 µL of ddH_2O, 12.5 µL of Supreme Master Mix, 1 µL of each primer (0.2 µM) and 2 µL of DNA (**Table 10**).

Restriction with SalI and NotI enzymes was performed both in Serrawettin W1 inserts and pET30a plasmid. For each restriction reaction, 4 µL of 1x buffer, 2 µL of 1x BSA, 0.5 µL of each restriction enzyme (10 U/µL) and 10 µL of insert or plasmid (**Table 10**) were incubated at 37 ºC for 3 h. 3 µL of loading buffer were added to restriction reactions and samples were loaded on a 2 % electrophoresis gel, ran at 90 V. Bands with restricted inserts and vector were cut and DNA was extracted according to OMEGA Gel extraction DNA kit.

Ligation reactions between pET30a vector and *srw1* total genes were performed with 1.5 µL of pET30a plasmid (10 µg/µL), 10 µL of insert (1 µg/µL), 1.5 µL of T4 ligase (T4 DNA Ligase (5 U/µL), Thermo Scientific™) and 7.5 µL of ligase 1x buffer.
After overnight incubation at 16 ºC, 50 µL of *E. coli* DH5α competent cells were added to each ligation reaction. To transform *E. coli* DH5α competent cells, after 20 min at 4 ºC, cells were incubated for 47 sec on a 42 ºC bath and, immediately after, returned to 4 ºC for 2 min. 250 µL of sterile LB liquid medium were added to transformed cells and after 90 min of incubation at 37 ºC, cells were centrifuged at 13,300 rpm for 1 min. Volumes were adjusted to 100 µL and pellets were resuspended. 100 µL with transformed cells were spread on LB solid medium plates with kanamycin at 50 mg/mL and incubated at 37 ºC overnight.
Colonies from LB solid medium plates with kanamycin at 50 mg/mL were transferred to 5 mL of LB liquid medium with kanamycin at 50 mg/mL and were incubated, overnight, at 120 rpm, at 37 ºC. Plasmid extraction was performed using Omega E.Z.N.A DNA Plasmid Mini Kit I. Ligation confirmation was performed with PCR using pET30a primer T7promoter and Serrawettin W1 srw1_2R primer (**Table 4**).

E. Lipopeptides extraction and purification

Serratia strains Arv-22-2.5c, Arv-22-2.6, A88copa7, A88copa13, NBRC 102599^T and AS13, expressing Serrawettins, were grown on 100 mL of R2A liquid medium at 120 rpm and 30 ºC with an initial OD_{600} of 0.07. After 24 h, OD_{600} was read and cultures were centrifuged at 12.100 rpm for 30 min. Supernatants were transferred to clean frosted Erlenmeyers and were adjusted to pH 2 with a 12 M HCl solution. 100 mL of a chloroform-methanol 2:1 v/v solution were added to each supernatant and these were agitated for 2 h. Organic phases were transferred, after phase separation, to clean frosted flasks (**KORENBLUM, E. et al., 2012**).
Organic phases were concentrated using a rotary evaporator at 60 ºC and residual liquid was concentrated with nitrogen. Crude extracts with lipids were resuspended with 2 mL of chloroform-methanol 2:1 v/v solution.
Polar lipids were separated from other lipids trough a column with 1 g of activated silica-gel 60. Columns were washed with 4 mL of methanol and 4 mL of a chloroform-methanol 9:1 v/v solution before 2 mL of eluted crudes were passed through the silica column. Particles unable to bind to silica-gel were rescued on a flask. Apolar lipids were eluted with 7 mL of a chloroform-methanol 9:1 v/v solution and were saved on a flask. Polar lipids were eluted with 7 mL of methanol and saved on a flask (**KORENBLUM, E. et al., 2012**). Flasks with polar lipids were concentrated with nitrogen and resuspended in 250 µL of methanol.
Purified polar lipids were examined with 1D Thin Layer Chromatography (TLC). TLCs were prepared in triplicate for each sample and were performed on silica plates 10 x 10 cm with 15 µL of each sample along 3 cm. Chloroform - acetic acid - methanol - water (80:15:12:4) solvent was

used to run TLCs. When TLC plates dried, ninhydrin reagent was sprayed on one of three plates and plates stayed at 120 ºC for 5 min to reveal amino lipids (**KÜGLER, J. H. et al., 2015**).
Positive spots with amino lipids were scraped from the other two plates without dye and extracted with 3 mL of chloroform-methanol 2:1 v/v solution. After vortex and centrifugation at 13,300 rpm for 1 min to remove silica gel, chloroform-methanol 2:1 v/v solutions with individualized polar lipids were transferred to clean flasks and evaporated with nitrogen. Extractions from one TLC plate were used to attraction and mortality tests and were resuspended with 750 µL of 0.1 M NaCl solution. Extractions from a second TLC plate were used for HPLC analysis and were resuspended with 100 µL of methanol.
Mortality tests were performed on *C. elegans* and *B. xylophilus*. To assess which amino lipid had higher nematicidal effect, dead nematodes percentage was determined after a period of 24 h and 48 h (**PAIVA, G. et al., 2013**). Three attraction tests were performed with *C. elegans*: i) with amino lipids versus 0.1 M NaCl solution; ii) with amino lipids versus respective supernatant; iii) and with amino lipids versus *E. coli* OP50. With attraction tests we assessed the percentage of nematodes drawn to amino lipids. These results provided us with an idea of *C. elegans´* and *B. xylophilus´* behaviour when in presence of amino lipids from *Serratia´*s supernatants (**PRADEL, E. et al., 2007; PUJOL; N. et al., 2001**).
HPLC was performed using an eluent gradient as previously described in **SU, C. et al., 2016** with some modifications. Briefly, eluent A was composed by a 0.05% trifluoracetic acid solution in purified water and eluent B was methanol. Eluent B gradient was 87-92% for 15 min. Flow rate was 1 mL/min and UV detection was read at 215 nm. Methanol was used as control. All the assays regarding i) mortality in *C. elegans* and *B. xylophilus* and ii) attraction of *C. elegans*, were performed as mentioned above in section B. Mortality tests were performed in 96 multi-well plates with 100 µL of extracted amino lipids.

F. Bioinformatic analysis

F1. Serrawettin W1 and W2 cluster analysis

With AntiSMASH software (**BLIN, K. et al., 2019**) we identified a Non-Ribosomal Peptide Synthetase (NRPS) gene cluster, including a core protein and several additional, regulation, transport and other proteins, involved in Serrawettin W1 and W2 lipopeptides biosynthesis.
Every cluster was organized by protein identification in the same order to allow an alignment of all clusters with ClustalW from MegaX software (**KUMAR, S. et al., 2018**). Evolutionary relationship between clusters and between Serrawettin biosynthesis proteins sequences were established using the Neighbor-Joining method, Poisson model (**SAITOU N., & NEI M., 1987**) on MegaX software. Aligned and organized genes were represented in an evolutionary tree according to size and

direction. Functions of common proteins to all clusters were searched on UniProt (**BATEMAN, A., 2019**).

F2. Serrawettin W1 and W2 biosynthesis

Tridimentional models of Serrawettin W1 biosynthesis protein for *Serratia* strain AS13 and of Serrawettin W2 biosynthesis protein for *Serratia marcescens* PWN146 were obtained using I-Tasser software (**YANG, J. et al., 2015**). Protein domains were identified and modulated by Phyre2 software. Binding sites of each domain were predicted by Phyre2 3DLigandSite (**KELLEY, L. et al., 2015**). To obtain better Serrawettins biosynthesis proteins models we used SUPER function on PyMOL software (**VERSION 2.0 SCHRÖDINGER**) to assure optimal configuration of all domains. For Serrawettin W2, due to its large dimension, all 5 modules were independently predicted by I-Tasser software and subsequently aligned in PyMOL in order to build a tridimensional protein model.

Using PyMOL software, biosynthesis proteins predicted by I-Tasser were loaded to determine domain and binding sites.

For Serrawettin W1 biosynthesis, ACP binds to Condensation domain, releasing the 3-D-hydroxydecanoyl to Adenylation domain were Serine will bind. Serratamic acid will be formed in Thiolation domain through L-serine and 3-D-hydroxydecanoyl linkage. Thioesterase domain will bind two serratamic acids and form Serrawettin W1. A 2D structure of ACP (ID: 5490374) was downloaded from PubChem and a 3D structure was predicted by Avogadro software (**HANWELL, M. D. et al., 2012**). 3D structures from 3-D-hydroxydecanoyl (ID: 26612), Serine (ID: 5951), Serratamic acid (ID: 42607281) and Serrawettin W1 (ID: 168994) were retrieved from PubChem and connections with respective binding sites were predicted in PyMOL (**Supplementary data 1**).

For Serrawettin W2, a fatty acid with 10 carbons is released and binds to L-Leucine in the first module. Leucine and fatty acid will bind to Serine in the second module. Those two amino acids and the fatty acid bind to Threonine in third module. Fourth module binds those three amino acids and fatty acid to a Phenylalanine. Fifth module binds those four amino acids and fatty acid to the last amino acid, Isoleucine and forms a connection between the Leucine and Isoleucine, producing Serrawettin W2. 2D structure of Serrawettin W2 (ID: 164436) was downloaded from PubChem and a 3D structure was predicted by Avogadro software. 3D structure of C10 fatty acid (ID: 2969), Leucine (ID: 6106), Serine (ID: 5951), Threonine (ID: 6288), Phenylalanine (ID: 6140) and Isoleucine (ID: 6306) were retrieved from PubChem and connection with respective binding sites were predicted in PyMOL (**Supplementary data 2**).

G. Molecular Dynamics Simulations

In order to perform molecular dynamic simulations and estimate protein-ligand interactions, 3D protein and ligand structures must be obtained. A file with protein 3D information was processed by Groningen Machine for Chemical Simulation (GROMACS version 4.5.4) package (**ABRAHAM, M. J. et al., 2015**) and a file with ligand 3D information was processed by CHARMM36m forcefield (**HUANG, J., & MACKERELL, A. D., 2013**). Systems were created with both protein and ligand, in a volume filled with a water model; temperature and pressure conditions were chosen for molecular dynamic systems to be closely similar to real life events. Two different types of simulations were performed:

1- To understand protein molecular dynamics of Serrawettin W1 biosynthesis protein in water, a simulation was performed, using GROMACS software.
   3D structure of Serrawettin W1 biosynthesis protein was predicted with Phyre2 software (**KELLEY, L. et al., 2015**). Simulation was performed in a cubic box filled with scp216 water model. System was neutralized by ions and energy was minimized by applying a maximum force of 10 kJ/mol to avoid steric hindrance. In order to equilibrate system temperature, a NVT equilibration (constant number of particles, volume, and temperature) was performed at 300.15 K (30 ^{o}C) for 100 ps using Verlet cut-off scheme. System pressure was equilibrated using a NPT equilibration (constant number of particles, pressure, and temperature) performed at 1.0 bar using the V-rescale modified Berendsen thermostat.
   After system equilibration, a Molecular Dynamics (MD) simulation was performed for 1.6 ns. V-rescale thermostat and Parrinello-Rahman system were used during simulations to maintain systems temperature and pressure. Protein simulation commands are described in **Annex 3**.
   Molecular dynamic simulation of Serrawettin W1 biosynthesis protein in water can be seen in **Supplementary data 3 and 4**.
2- Two separated MD simulations were performed to better understand protein-ligand interactions between TOL1 protein from *C. elegans* and Serrawettin W1 and Serrawettin W2. TOL1 protein 3D structure was predicted with I-Tasser software from NCBI protein FASTA (CCD63554.1). 3D structure of Serratomolide Serrawettin W1 was retrieved from PubChem (ID: 168994). Serrawettin W2 2D structure was taken from PubChem (ID: 164436) and a 3D structure was predicted by Avogadro software.
   Simulations were made in GROMACS version 4.5.4. Ligand parameters were generated by CHARMM36m forcefield. Both simulations were prepared in a dodecahedron box filled with TIP3P water model. Systems were neutralized by ions and energy was minimized by applying a maximum force of 10 kJ/mol to avoid steric hindrance. A new group combining protein and ligand atoms was created in order to equilibrate the system and to separate protein atoms from solvent atoms. Ligand restriction was generated and a new topology file was created. In order to equilibrate systems temperature, a NVT equilibration (constant

number of particles, volume, and temperature) was performed at 300.15 K (30 ºC) for 100 ps using Verlet cut-off scheme. Systems pressure was equilibrated using a NPT equilibration (constant number of particles, pressure, and temperature) performed at 1.0 bar using the V-rescale modified Berendsen thermostat.

After system equilibration, molecular dynamics simulation of TOL1 protein with Serrawettin W1 was performed for 3 ns (requiring 4 days of continuous computational processing). Molecular dynamics simulation of TOL1 protein with Serrawettin W2 was performed for 5 ns (requiring 7 days of continuous computational processing). V-rescale thermostat and Parrinello-Rahman system were used during simulations to maintain systems temperature and pressure. To estimate long-range electrostatics, a Particle Mesh Ewald (PME) was used. LINCS algorithm was used to restrain bond lengths. Protein-ligand simulation commands are described in **Annex 4**.

Molecular dynamic simulation of Serrawettin W1 with TOL1 protein can be seen in **Supplementary data 5 and 6** and molecular dynamic simulation of Serrawettin W2 with TOL1 protein can be seen in **Supplementary data 7 and 8**.

All simulations were visualized in VMD program (**HUMPHREY, W. et al., 1996**).

Simulation analysis of interactions between TOL1 and Serrawettin W1 and W2 were performed using GROMACS software (**ABRAHAM, M. J. et al., 2015**). Molecular dynamic simulation results were subject to three different analysis described in **Annex 5**:

1- Root-mean-square deviation (RMSD) analysis was made to measure the scaler distance for backbone atoms from Tol1 protein and Serrawettins throughout the trajectory, using the first frame as origin of comparison. This analysis provides insights of structural conformations and is often used to evaluate simulation quality. RMSD values from 1 to 3 Å indicates a small conformational change and a good quality strong simulation.
2- Root mean square fluctuation (RMSF) analysis was performed to describe local fluctuations in protein and ligand side chain residues throughout both simulations. Peaks higher than 7 Å correspond to residues that fluctuated the most during simulation (**BENSON, N. C., & DAGGETT, V., 2012**).
3- Distance analysis were performed to estimate distances between centres of mass of TOL1 protein and Serrawettins. Protein ligand interactions were established when the distance between two Carbons was less than 5.4 Å.

Results

A. Amplification and purification of *srw1* and *srw2* genes

A1. Bacterial DNA analyses, Polymerase Chain Reaction and Sequencing

The concentrations of bacterial genomic DNA in ng/µL are summarized in **Table 5**. *Serratia* strain A52T1 showed limited growth and its DNA concentration was very low.

Table 5 Bacterial DNA concentrations.

Bacterial strains	ng/µL
M24T3	117.7
A25T1	65.9
A52T1	1.5
A88C3	190.6
A88C4	104.3
A88C6	179.3
Arv-20-4.2	128.8
Arv-22-2.5c	60.6
Arv-22-2.6	181.4
Arv-29-3.11b	106.1
Arv-29-3.9	117.1
M24T3A	51.3
M47C12B1	62.0
M47Tronco1	40.6
M24Tronco5	160.4
A88copa7	172.9
A88copa13	167.6
NBRC 102599^T	39.3
AS13	82.6
Leaf50	53.4

Primers for *swr1* and *srw2* genes were used in order to confirm the presence of Serrawettin genes. PCR products were separated by electrophoresis and bands corresponding to gene-fragments sizes were sequenced. Sequences were compared to Serrawettin genes sequences and BLAST analysis revealed that *Serratia* strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, NBRC 102599^T and AS13 have *srw1* gene and that *Serratia* strains A88copa7 and A88copa13 have *srw2*.
A phylogenetic tree, with all *Serratia* strains presented in this work, based in the 16S rRNA gene sequences, is represented in **Figure 1**: selected strains for laboratory work, strains with *sw1* gene cluster and *srw2* gene cluster and 19 *Serratia* species and 3 *Pseudomonas* species from LPSN (**Parte, A. C., 2014**).

Figure 1. Phylogenetic tree based on 16S rRNA gene of strains used in this study. 16S rRNA genes were aligned using SILVA and an evolutionary tree was established using the Neighbor-Joining method in ARB software. **Bold**: Selected *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T, AS13 and Leaf50; and *Pseudomonas* strain M47Tronco1; **Blue**: *Serratia* strains with Serrawettin W1 cluster ATCC 13880, CDC_813-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EGD-HP20, WW4, FS14, TEL NODE_13, NBRC 102599^T, BXF1, A2, AS13, AS9 and AS12; **Red**: *Serratia* strains with Serrawettin W2 cluster PWN146, SSNIH1, SM39, SmUNAM836, Lr5/4 LG59, RSC-14, AH0650_Sm1 AG2, Db11, SCBI and YD25; 19 *Serratia* species *S. aquatilis, S. oryzae, S. fonticola, S. fonticola DSM 22080, S. liquefaciens, S. grimesii, S. proteamaculans, S. myotis, S. proteamaculans, S. ficaria, S. vespertilionis, S. entomophila, S. odorifera, S. symbiotica, S. nematodiphila, S. marcescens* and *S. ureilytica* and 3 *Pseudomonas* species *P. moorei, P. mohnii and P. aeruginosa*. **Blue arrows** - selected strains with *srw1* gene; **Red arrows** – selected strains with *srw2* gene. Scale bar 0.10 infer nucleotide substitutions per 100 nucleotides.

B. Bacteria-nematode interactions: Mortality, Attraction and Avoidance tests

B1. Mortality tests in *C. elegans*

Mortality tests were performed to assess if bacterial supernatants have nematicidal proprieties. Mortality tests with concentrated supernatants were made in triplicate after one test was performed with non-concentrated supernatant. About 10 *C. elegans* in each test were used in order to evaluate which strains have nematicidal proprieties against them (**PAIVA, G. et al., 2013**). Mortality tests were performed once with bacterial supernatants and repeated three times with concentrated supernatants.

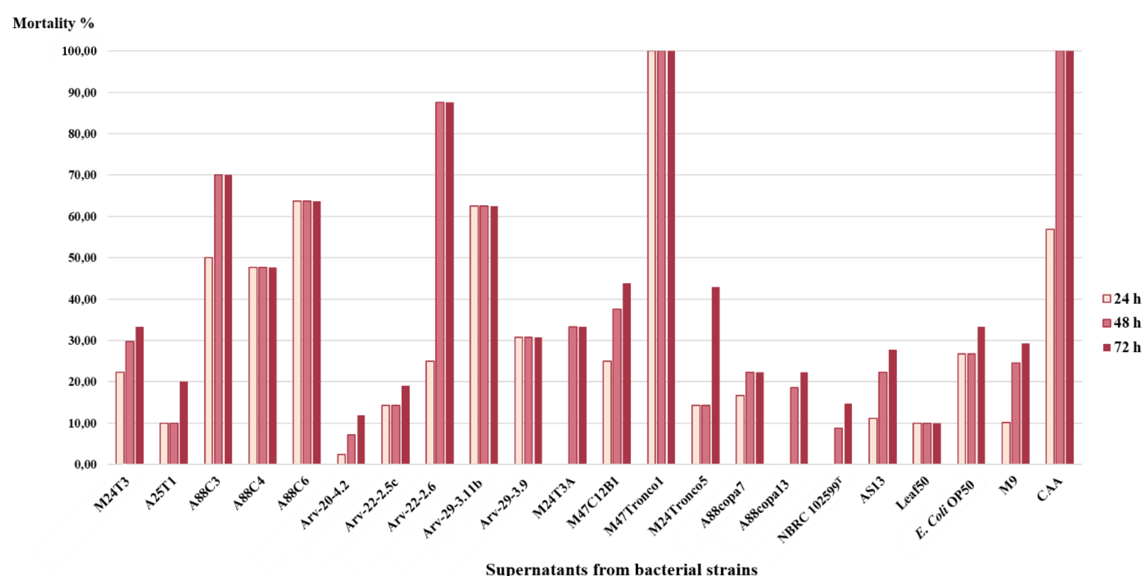

Figure 2 *C. elegans´* mortality tests with supernatants of bacterial strains and M9 and CAA media as control. Supernatants were obtained after a 24 h growth of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50. The number of dead nematodes was assessed at 24 h, 48 h and 72 h after incubation with supernatants. Only one test per supernatant was performed.

Mortality tests with supernatants and *C. elegans* revealed that *Pseudomonas* strain M47Tronco1 was the only able to kill all nematodes in a 24 h period.

Serratia strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, NBRC 102599^T and AS13 have *srw1* gene and, killed 15%, 25%, 31%, 0% and 11% of *C. elegans* after 24 h, respectively.

Serratia strains A88copa7 and A88copa13 have *srw2* gene. *Serratia* strain A88copa7 killed 17% of *C. elegans*. Supernatant from *Serratia* strain A88copa13 did not kill any nematode after 24 h.

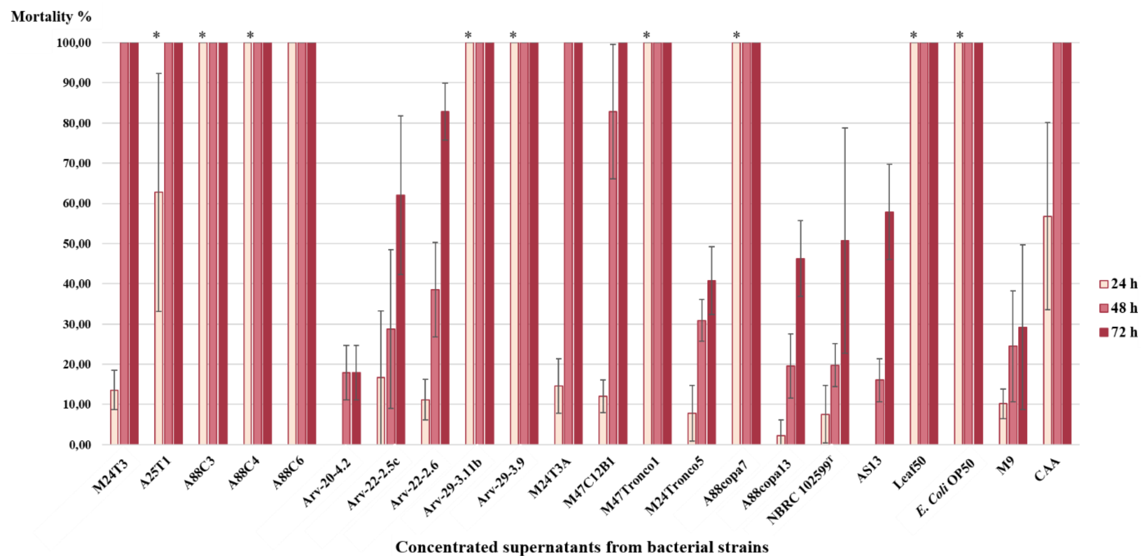

Figure 3 *C. elegans´* mortality tests with concentrated supernatants of bacterial strains and M9 and CAA media as control. Supernatants were concentrated by vacuum and evaporation at low temperatures (-40 ºC) after a 24 h growth of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T , AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50. The number of dead nematodes was assessed at 24 h, 48 h and 72 h after incubation with concentrated supernatants. Each test was performed in triplicate. Results that showed statistical differences on Two-way ANOVA and Tukey Test are marked with (*).

Results from *C. elegans´* mortality tests with concentrated supernatants could not be validated after 48 h and after 72 h because CAA medium, used as control, killed all nematodes after 48 h.
Only concentrated supernatants from *Serratia* strains A88C3, A88C4, A88C6, Arv-29-3.11b, Arv-29-3.9, A88copa7, Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50 were able to kill 100% of *C. elegans* after 24 h, with statistical significance.

B2. Mortality tests in *B. xylophilus*

Mortality tests were performed to ascertain if bacterial supernatants have nematicidal proprieties against PWN. Ten *B. xylophilus* in each test were used in order to evaluate which strains have nematicidal proprieties against this nematode.

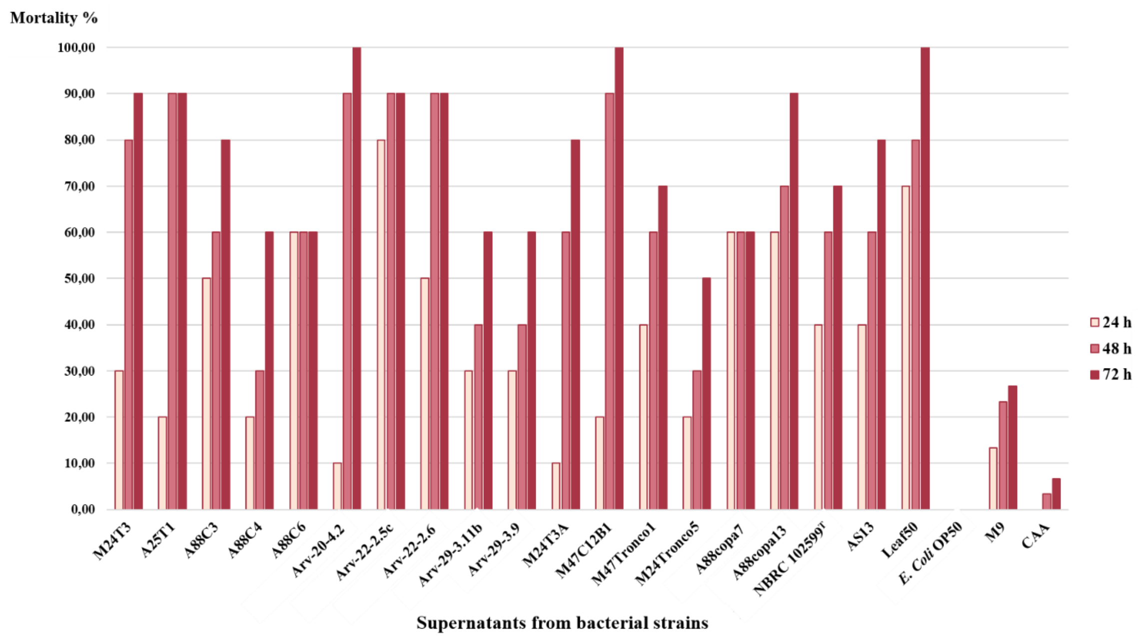

Figure 4 *B. xylophilus´* mortality tests with supernatants from bacteria and M9 and CAA media as control. Supernatants were obtained after a 24 h growth of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50. The number of dead nematodes was assessed at 24 h, 48 h and 72 h after incubation with supernatants. Only one test per supernatant was performed.

Serratia strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, NBRC 102599^T and AS13 have *srw1* gene and were able to kill *B. xylophilus* after a 24 h period.
Serratia strains A88copa7 and A88copa13 have *srw2* gene. *Serratia* strain A88copa7 killed 17% of nematodes and *Serratia* strain A88copa13 did not kill any nematode after 24 h (**Figure 4**).

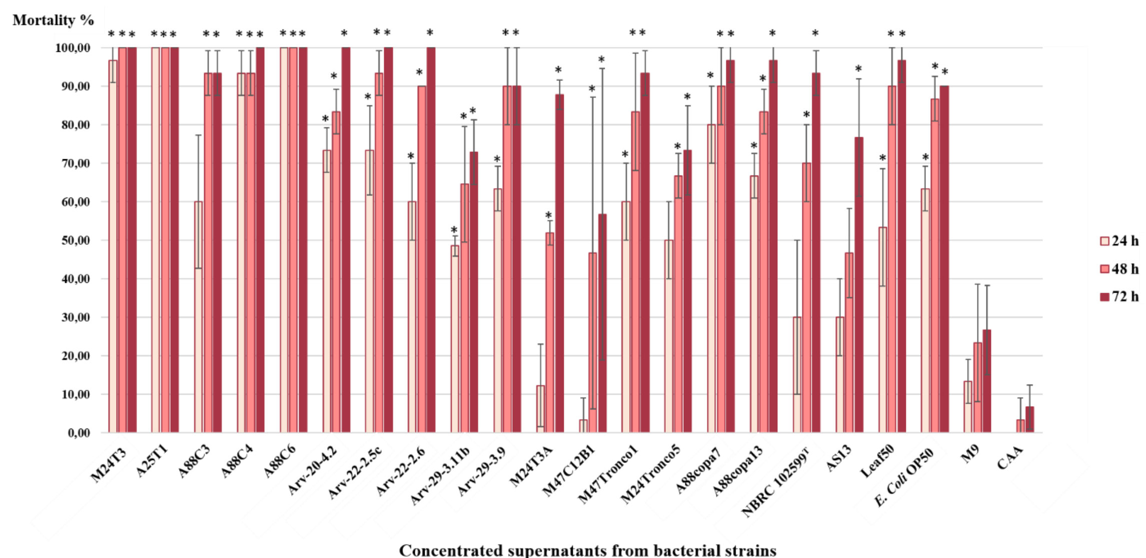

Figure 5 *B. xylophilus´* mortality tests with concentrated supernatants of bacterial strains and M9 and CAA media as control. Supernatants were concentrated by vacuum and evaporation at low temperatures after a 24 h growth of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9,

M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^{T}, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50. The number of dead nematodes was assessed at 24 h, 48 h and 72 h after incubation with concentrated supernatants. Each test was performed in triplicate. Results that showed statistical differences on Two-way ANOVA and Tukey Test are marked with (*).

*B. xylophilus´*mortality tests with concentrated supernatant revealed that this nematode is susceptible to *Serratia* strains. Almost every concentrated supernatant, when compared with control, showed statistical differences after 24 h, namely: concentrated supernatants from *Serratia* strains A25T1, A88C4, A88C6, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, A88copa7, A88copa13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50.
In these mortality tests, strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, A88copa7 and A88copa13, which have Serrawettins genes, were able to kill more than 40% of *B. xylophilus.*
After 48 h, only *Serratia* strain AS13 did not have a statistical difference when compared to control (CAA medium). After 72 h, all concentrated supernatants showed a statistical difference when compared to control: after that period of time, all strains had a nematicidal activity against PWN.
Serratia strains A25T1 and A88C6 were the only ones able to kill all *B. xylophilus* at 24 h (**Figure 5**).

B3. Attraction tests in *C. elegans*

Attraction tests are designed to demonstrate if young starved *C. elegans* are more attracted to bacteria or supernatant of selected strains or to control *E. coli* OP50.
Serratia strains M24T3, A25T1, A88C3, A88copa7 and Leaf50 were able to attract more *C. elegans* than control after 2 h and maintained their attraction effect after 24 h, with statistical significance (**Figure 6**).
Pseudomonas strain M47Tronco1 and *Serratia* strains A88copa13 and AS13 were able to attract more *C. elegans* than control after 2 h but lost attraction effect after 24 h, with statistical significance.
Serratia strains A88C4 and A88C6 were not able to attract *C. elegans* more than control after 2 h, but were able to do it after 24 h, with statistical significance.
Serratia strains Arv-22-2.5c, Arv-29-3.9, M24T3A and M47C12B1 were not able to attract *C. elegans* and, in these cases, nematodes were more attracted to *E. coli* OP50 both after 2 h and after 24 h (**Figure 6**).

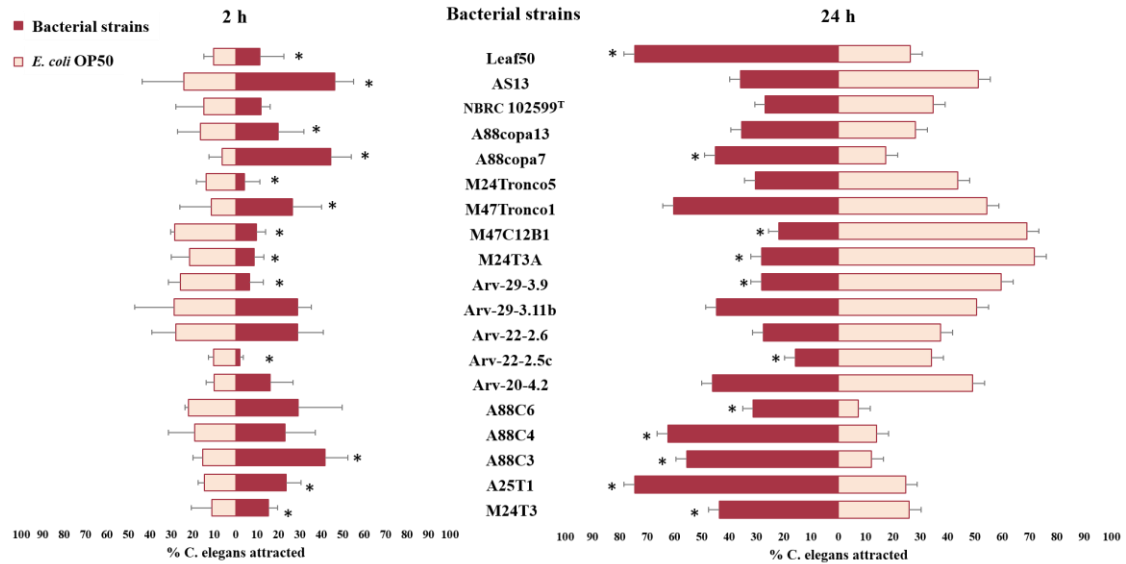

Figure 6 Attraction tests of *C. elegans* by bacterial suspensions versus *E. coli* OP50 as control. After a 24 h growth, bacteria of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50 were used on attraction tests with an OD_{600} of 1.0. The number of attracted nematodes by bacteria and control was counted at 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Results that showed statistical differences on Chi-Squared test are marked with (*).

Supernatants of *Serratia* strains A25T1, A88C3 and A88C6 were able to attract more *C. elegans* than *E. coli* OP50 control after 2 h and after 24 h, with statistical significance.

Serratia strains Arv-22-2.5c and A88copa7 attracted nematodes after 2 h with statistical significance but lost its ability to attract after 24 h.

Nematodes were more attracted to *E. coli* OP50 control than to *Serratia* strains M24T3, Arv-20-4.2, Arv-22-2.6, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa13, AS13 and *Pseudomonas* strain M47Tronco1 after 2 h and after 24 h (**Figure 7**).

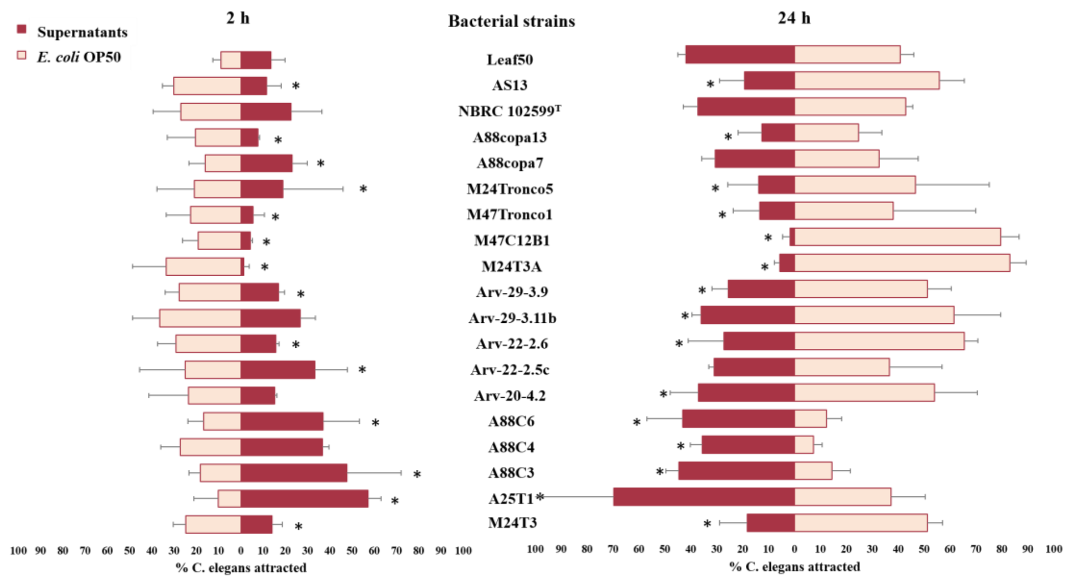

Figure 7 Attraction tests with bacteria's supernatant versus *E. coli* OP50 as control. Supernatants were obtained after a 24 h growth of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^{T}, AS13 and Leaf50 and *Pseudomonas* strain M47Tronco1. *E. coli* OP50 was used as a control with an OD_{600} of 1.0. The number of attracted nematodes by bacteria and control was counted 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Results that showed statistical differences on Chi-Squared test are marked with (*).

All bacteria supernatants were able to attract more *C. elegans* than *E. coli* OP50 supernatant control at 2 h and 24 h with two exceptions: *Serratia* strain M24T3A, for which nematodes were more attracted to *E.coli* OP50 supernatant, and *Serratia* strain M47C12B1 that was able to attract nematodes after 2 h but lost this ability after 24 h (**Figure 8**).

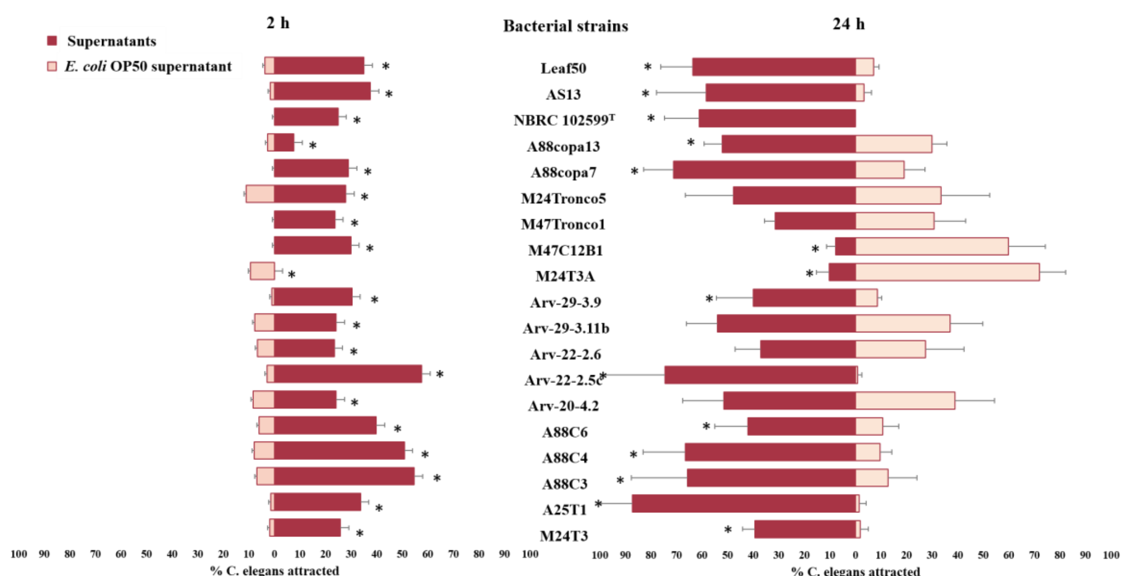

Figure 8 Attraction tests with supernatant from bacteria versus *E. coli* OP50 supernatant as control. Supernatants were obtained after a 24 h growth of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-

22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50 (used as control). The number of attracted nematodes by bacteria and control was counted 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Results that showed statistical differences on Chi-Squared test are marked with (*).

B4. Avoidance tests in *C. elegans*

Avoidance tests with bacteria and *E. coli* OP50 as control revealed that *C. elegans* avoided control on tests with *Serratia* strains Arv-22-2.6 and AS13 after 2 h, with statistical significance.
After 24 h, the nematodes avoided *E. coli* OP50 on tests within the presence of *Serratia* strains A88C3, A88C4, A88C6 and Leaf50, and avoided *Serratia* strain Arv-22-2.6 (**Figure 9**).

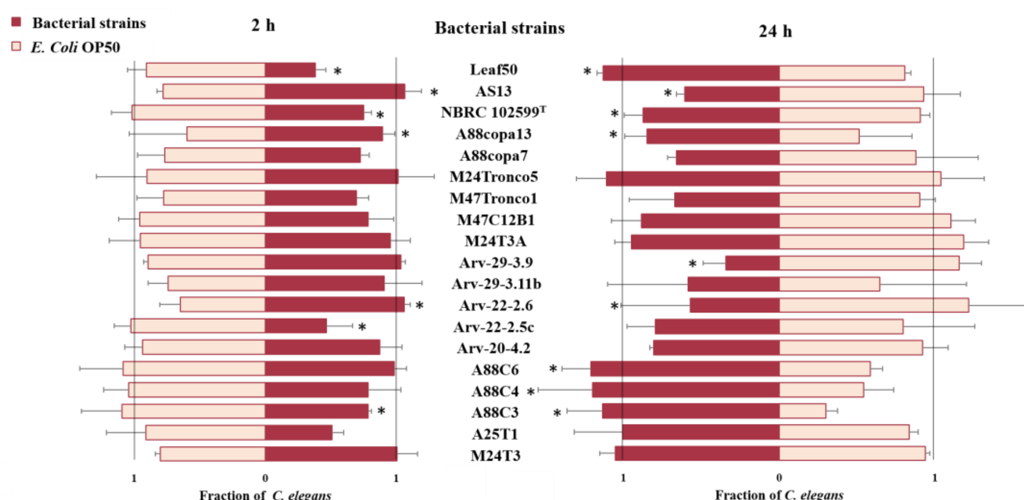

Figure 9 Avoidance tests with bacteria versus *E. coli* OP50 as control. After a 24 h growth, bacteria of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50 were used on avoidance tests with an OD_{600} of 1.0. The number of attracted nematodes by bacteria and control was counted 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Fraction of *C. elegans* in each drop was determined after 2 h and 24 h (number of *C. elegans* after 2 h or 24 h divided by initial number of *C. elegans*). Results that showed statistical differences on Chi-Squared test are marked with (*).

After 2 h, avoidance tests with bacteria´s supernatants versus *E. coli* OP50 as control, showed that *C. elegans* avoided supernatants from *Serratia* strains M24T3, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, NBRC 102599^T and AS13, with statistical significance.
Strains´ supernatants M24T3, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, NBRC 102599^T and AS13 continued to induce a *C. elegans* avoidance response after 24 h with statistical significance. Supernatants from *Serratia* strains A25T1, Arv-22-2.5c, Arv-22-2.6 and Leaf50 were only able to induce an avoidance response after 24 h, with statistical significance (**Figure 10**).

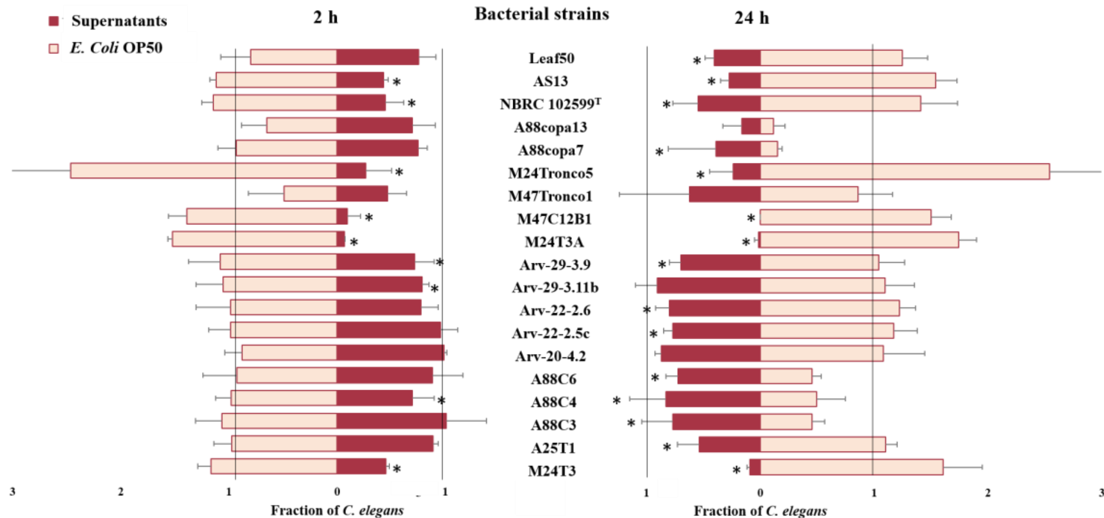

Figure 10 Avoidance tests with supernatants from bacteria versus *E. coli* OP50 as control. Supernatants were obtained after a 24 h growth of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^{T}, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1. *E. coli* OP50 was used as control with an OD_{600} of 1.0. The number of attracted nematodes by bacteria and control was counted 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Fraction of *C. elegans* in each drop was determined after 2 h and 24 h (number of *C. elegans* after 2 h or 24 h divided by initial number of *C. elegans*). Results that showed statistical differences on Chi-Squared test are marked with (*).

As for avoidance tests with bacterial supernatant versus *E. coli* O50 supernatant as control, after 2 h, *C. elegans* avoided *E. coli* OP50 supernatant on tests within presence of *Serratia* strains A88C3, A88C4, Arv-22-2.5c, Arv-29-3.11b and Arv-29-3.9, with statistical significance.

After 24 h, nematodes continued to avoid *E. coli* OP50 supernatant on tests with *Serratia* strains A88C3, A88C4, Arv-22-2.5c, Arv-29-3.11b and Arv-29-3.9 and started avoiding control on tests with *Serratia* strains M24T3, A25T1, Arv-20-4.2, Arv-22-2.6 and Leaf50, with statistical significance.

Serratia strains A88copa7 and M47C12B1 induced an avoidance response on *C. elegans* after 24 h and nematodes moved from test drop to control drop, with statistical significance (**Figure 11**).

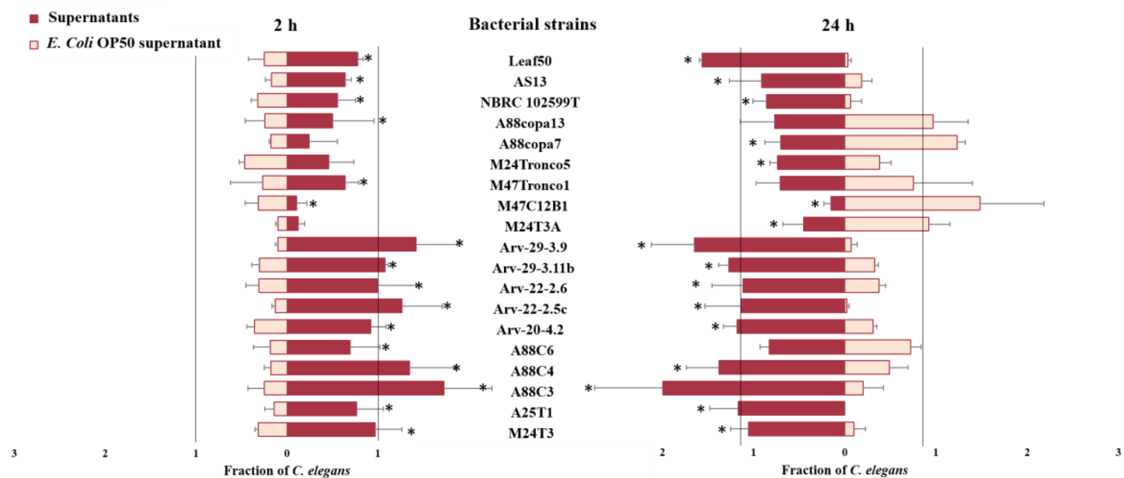

Figure 11 Avoidance tests with supernatant versus *E. coli* OP50 supernatant as control. Supernatants were obtained after a 24 h growth of *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50, used as control. The number of attracted nematodes by bacteria and control was counted 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Fraction of *C. elegans* in each drop was determined after 2 h and 24 h (number of *C. elegans* after 2 h or 24 h divided by initial number of *C. elegans*). Results that showed statistical differences on Chi-Squared test are marked with (*).

B5. Bacterial extracellular proteome by SDS-PAGE

Protein concentration was determined for all supernatants from bacterial growths after 24 h. **Table 6** resumes protein concentrations values (mg/mL).

Table 6 Protein concentration in supernatants. Protein concentration was determined with NanoDrop technology in mg/mL. Concentrated supernatants were obtained by vacuum and evaporation at low temperatures (-40 ºC) from original supernatants.

Bacterial strains	Supernatant (mg/mL)	Concentrated supernatant (mg/mL)
M24T3	0.60	1.74
A25T1	0.59	3.45
A88C3	0.67	7.85
A88C4	0.65	8.68
A88C6	0.50	11.59
Arv-20-4.2	0.03	1.21
Arv-22-2.5c	0.30	0.38
Arv-22-2.6	0.29	0.63
Arv-29-3.11b	0.28	3.59
Arv-29-3.9	0.18	0.61
M24T3A	0.29	0.76
M47C12B1	0.34	0.59
M47Tronco1	1.03	3.14
M24Tronco5	0.79	2.63
A88copa7	0.58	6.21
A88copa13	0.30	1.81
NBRC 102599^T	0.65	2.80
AS13	0.61	2.38
Leaf50	0.36	1.18
E. coli OP50	0.57	4.87

A SDS-PAGE of concentrated supernatants revealed that some *Serratia* strains have a similar protein profile. However, some protein profiles were not clear probably due to lower protein concentration. We observed two common bands that were previously described as metalloproteinase serralysin (50 kDa) and serine protease (70 kDa) (**PAIVA, G. et al., 2013**), from strain A88copa13.

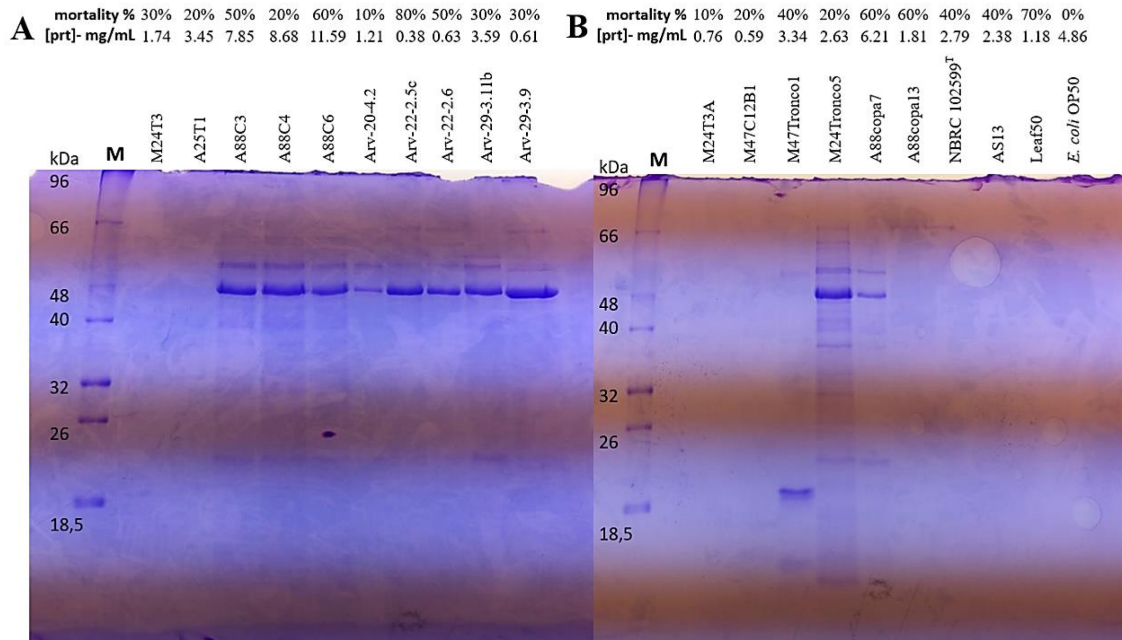

Figure 12 SDS-PAGE of concentrated supernatants. SDS-PAGE with **M** - molecular weight (bp) and concentrated supernatants from **A -** *Serratia* strains M24T3, A25T1, A88C3, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.11b, Arv-29-3.9 and **B -** M24T3A, M47C12B1, M24Tronco5, A88copa7, A88copa13, NBRC 102599^T, AS13 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50. Protein concentration of all concentrated supernatants are exhibited in mg/mL. Mortality percentages of *B. xylophilus* after 24 h are presented for each concentrated supernatant.

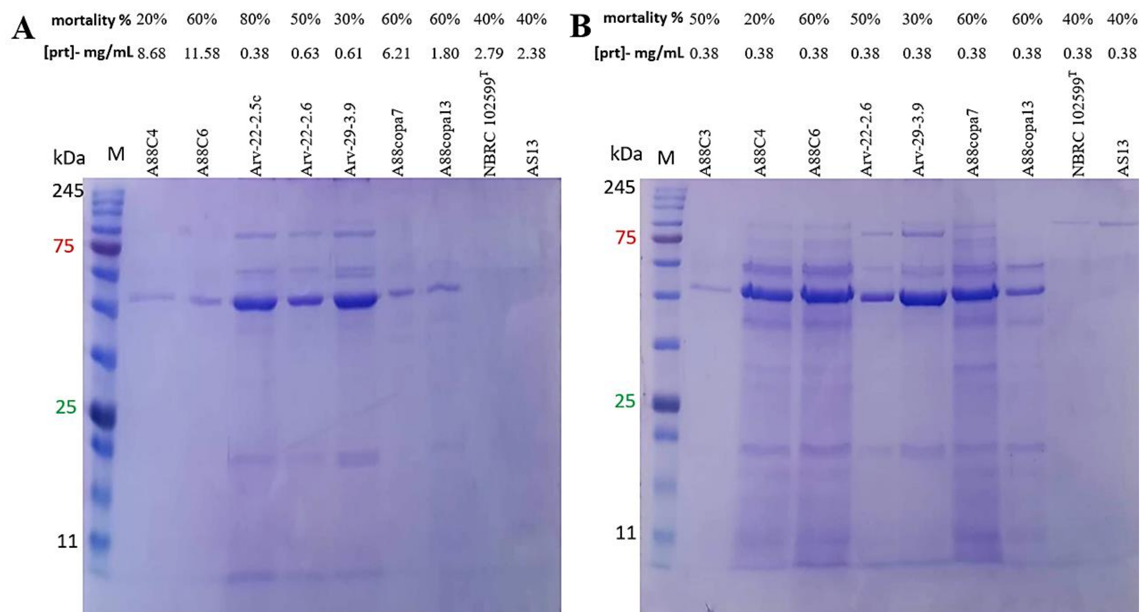

Figure 13 SDS-PAGE of concentrated supernatants before and after normalization. SDS-PAGE with **M** - molecular weight (bp) and concentrated supernatants from *Serratia* strains A88C3, A88C4, A88C6, Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, A88copa7, A88copa13, NBRC 102599^{T} and AS13. **A -** supernatants with original protein concentration**; B -** supernatants with normalized concentrations (0.38 mg/mL). Protein concentration of all concentrated supernatants are exhibited in mg/mL. Mortality percentages of *B. xylophilus* after 24 h are presented for each concentrated supernatant.

C. Disruptive mutants

RAPD showed that *Serratia* strain Arv-29-3.9 produced similar profile to *Serratia* strain Arv-22-2.5c. Remaining strains showed different profiles. Experiments were only performed on Arv-22-2.5c, Arv-22-2.6, A88copa7, A88copa13, NBRC 102599^{T} and AS13.

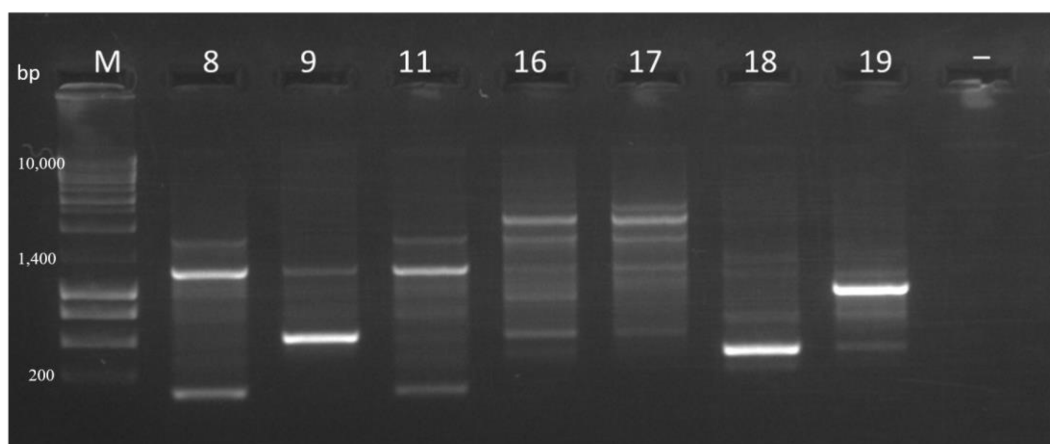

Figure 14 RAPD performed on *Serratia* strains Arv-22-2.5c (8), Arv-22-2.6 (9), Arv-29-3.9 (11), A88copa7 (16), A88copa13 (17), NBRC 102599^{T} (18) and AS13 (19) using OPA 3 primer. **M** - molecular weight (bp); (**-**) - negative control.

Antibiotic resistance studies performed on *Serratia* strains Arv-22-2.5c, Arv-22-2.6, NBRC 102599^{T}, AS13, A88copa7 and A88copa13 revealed that all strains are susceptible to kanamycin antibiotic at 50 mg/mL concentration. After a 24 h incubation period, OD_{600} for LB liquid medium with kanamycin was zero for all *Serratia* strains, proving that kanamycin at 50 mg/mL is able to kill these *Serratia* strains (**Table 7**).

Table 7 Antibiotic resistance studies for *Serratia* strains Arv-22-2.5c, Arv-22-2.6, NBRC 102599^{T} and AS13 with Serrawettin W1 and A88copa7 and A88copa13 with Serrawettin W2. OD_{600} of an overnight growth with LB liquid medium as a control and with LB liquid medium with Kanamycin 50 mg/mL.

***Serratia* strains**	**Arv-22-2.5c**	**Arv-22-2.6**	**NBRC 102599^{T}**	**AS13**	**A88copa7**	**A88copa13**
LB liquid medium	1.01	1.04	0.13	0.86	1.14	1.13
LB liquid medium with 50 mg/mL of kanamycin	0	0	0	0	0	0

Partial Serrawettin genes were amplified with design primers for mutagenesis and DNA was extracted from agarose gel, purified and quantified with NanoDrop Technology (**Table 8**). After restriction, DNA inserts and pK18mob plasmid were extracted and quantified with NanoDrop Technology (**Table 8**).

Table 8 Partial Serrawettin genes DNA quantification from *Serratia* strains Arv-22-2.5c, Arv-22-2.6, A88copa7, A88copa13, NBRC 102599^T and AS13, quantification of pK18mob plasmid and restriction quantification of inserts and pK18mob vector with NanoDrop Technology (ng/µL).

***Serratia* strains**	**Amplification (ng/µL)**	**Restriction (ng/µL)**
Arv-22-2.5c	35.4	14.6
Arv-22-2.6	34.7	18.6
A88copa7	43.1	19.7
A88copa13	45.9	27.8
NBRC 102599^T	23.5	13.5
AS13	24.2	4.7
pK18mob	43.8	16.2

Plasmids were extracted from *E. coli* DH5α competent cells and DNA was quantified with NanoDrop Technology (**Table 9**). Ligation confirmation of extracted plasmids was performed using pK18mob vector primers M13F and M13R.

Table 9 Quantification with NanoDrop Technology (ng/µL) of extracted plasmids from *E. coli* DH5α competent cells after transformation with ligation products. Plasmids with partial Serrawettin W1 as insert from *Serratia* Arv-22-2.5c: 8A, 8B, 8C; from *Serratia* Arv-22-2.6: 9A, 9B, 9C, 9D; from *Serratia* NBRC 102599^T: 18A, 18B, 18C; from *Serratia* AS13: 19A, 19B, 19C. Plasmids with partial Serrawettin W2 as insert from *Serratia* A88copa7: 16A, 16B, 16C; from *Serratia* A88copa13: 17A, 17B, 17C, 17D.

***Serratia* strains**	**Plasmid**	**ng/µL**
Arv-22-2.5c	8A	210
	8B	190.8
	8C	173.7
Arv-22-2.6	9A	165.1
	9B	129.7
	9C	208.9
	9D	106.7
A88copa7	16A	209.1
	16B	179.2
	16C	176
A88copa13	17A	24.8
	17B	28.9
	17C	32.9
	17D	37.9
NBRC 102599^T	18A	207.2
	18B	279.4
	18C	13.5
AS13	19A	447.1
	19B	374.5
	19C	317.3

After electroporation, DNA from transformed *Serratia* strains was extracted. PCR with srw1_1F and srw1_2R primers for *srw1* gene (1,809 bp) and with srw2A_1F and SphIsrw2_R for *srw2* gene (362 bp) were performed to confirm disruptive mutants for Serrawettin genes. Only *Serratia* A88copa13 mutant 17.8 had a band with pK18mob vector size (3,000 bp) (**Figure 16**). Consequently, DNA from *Serratia* A88copa13 mutant 17.8 was extracted by NZY Microbial gDNA Isolation kit. However, PCR did not present any band (**Figure 17**).

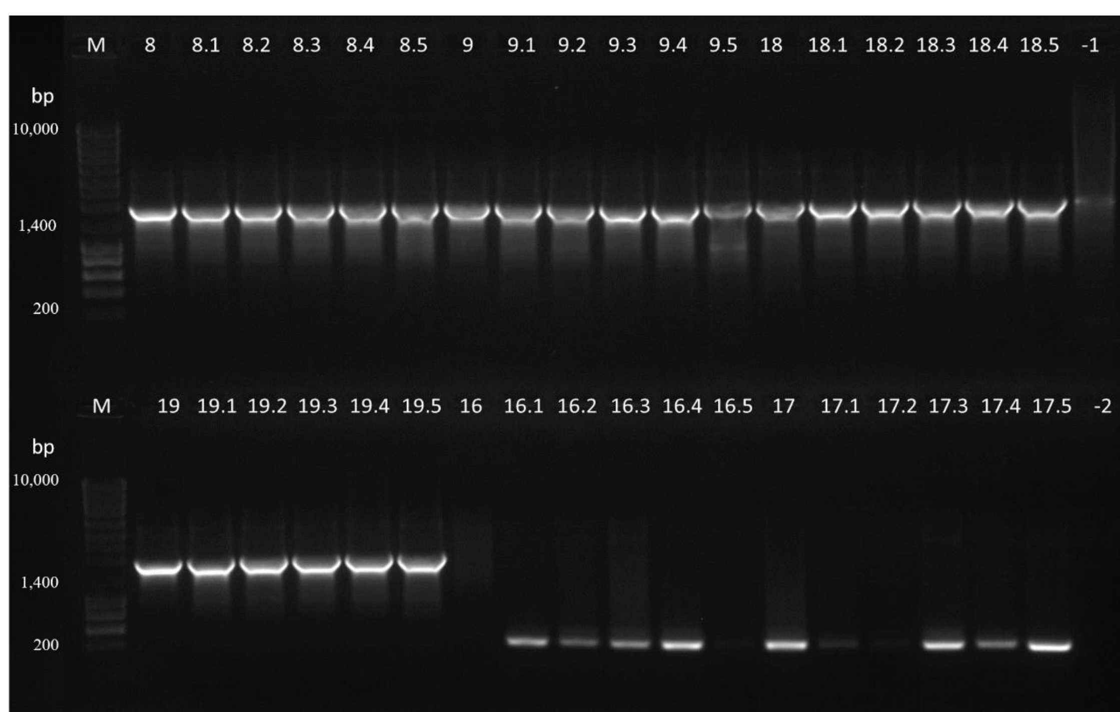

Figure 15 PCR results for DNA extraction of transformed *Serratia* bacteria. srw1_1F and srw1_2R primers were used for *Serratia* strains Arv-22-2.5c (8) and mutants 8.1, 8.2, 8.3, 8.4 and 8.5, Arv-22-2.6 (9) and mutants 9.1, 9.2, 9.3, 9.4 and 9.5, NBRC 102599^T (18) and mutants 18.1, 18.2, 18.3, 18.4 and 18.5 and AS13 (19) and mutants 19.1, 19.2, 19.3, 19.4 and 19.5 with Serrawettin W1 with a total length of 1,809 bp. srw2A_1F and SphIsrw2_R primers were used for *Serratia* strains A88copa7 (16) and mutants 16.1, 16.2, 16.3, 16.4 and 16.5 and A88copa13 (17) and mutants 17.1, 17.2, 17.3, 17.4 and 17.5 with Serrawettin W2 with a total length of 362 bp. **M** - molecular weight (bp); (**-1**) - negative control of Serrawettin W1; (**-2**) - negative control of Serrawettin W2.

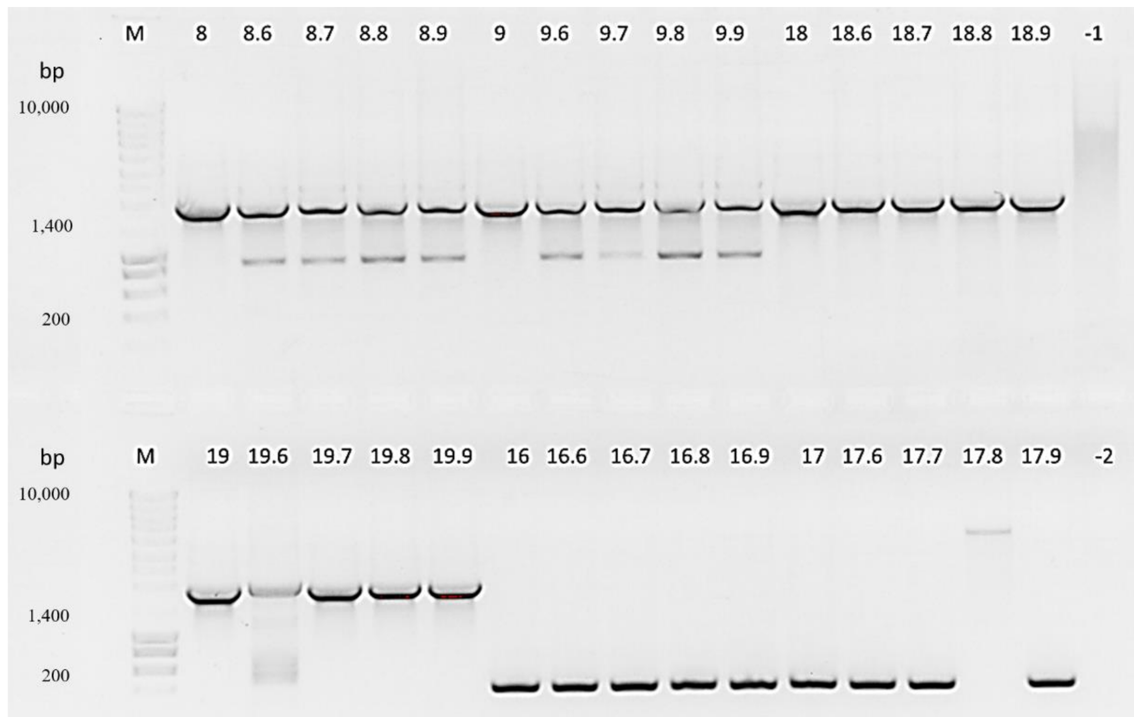

Figure 16 PCR results for DNA extraction of transformed *Serratia* bacteria. srw1_1F and srw1_2R primers were used for *Serratia* strains Arv-22-2.5c (8) and mutants 8.6, 8.7, 8.8 and 8.9, Arv-22-2.6 (9) and mutants 9.6, 9.7, 9.8 and 9.9, NBRC 102599^{T} (18) and mutants 18.6, 18.7, 18.8 and 18.9 and AS13 (19) and mutants 19.6, 19.7, 19.8 and 19.9 with Serrawettin W1 with a total length of 1,809 bp. srw2A_1F and SphIsrw2_R primers were used for *Serratia* strains A88copa7 (16) and mutants 16.6, 16.7, 16.8 and 16.9 and A88copa13 (17) and mutants 17.6, 17.7, 17.8 and 17.9 with Serrawettin W2 with a total length of 362 bp. **M**- molecular weight (bp); (**-1**) - negative control of Serrawettin W1; (**-2**) - negative control of Serrawettin W2.

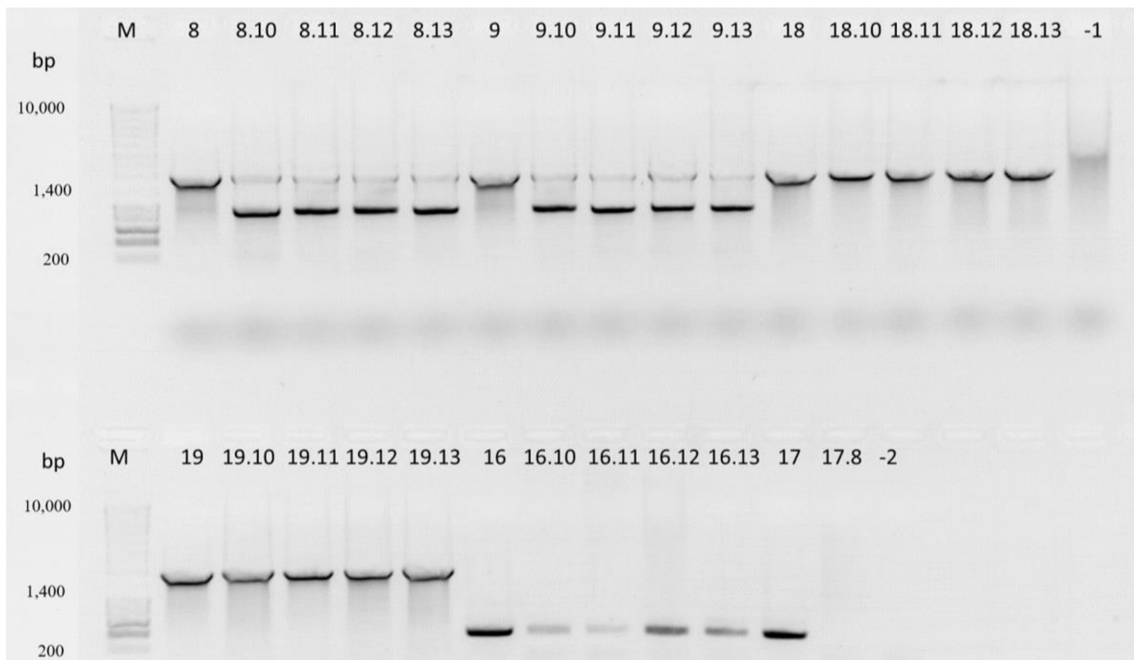

Figure 17 PCR results for DNA extraction of transformed *Serratia* bacteria. srw1_1F and srw1_2R primers were used for *Serratia* strains Arv-22-2.5c (8) and mutants 8.10, 8.11, 8.12 and 8.13, Arv-22-2.6 (9) and mutants 9.10, 9.11, 9.12 and 9.13, NBRC 102599^{T} (18) and mutants 18.10, 18.11, 18.12 and 18.13 and AS13 (19) and mutants 19.10, 19.11, 19.12

and 19.13 with Serrawettin W1 with a total length of 1,809 bp. srw2A_1F and SphIsrw2_R primers were used for *Serratia* strains A88copa7 (16) and mutants 16.10, 16.11, 16.12 and 16.13 and A88copa13 (17) and mutant 17.8 extracted by NZYTech kit with Serrawettin W2 with a total length of 362 bp. **M** - molecular weight (bp); (**-1**) - negative control of Serrawettin W1; (**-2**) - negative control of Serrawettin W2.

D. Heterologous expression in *E. coli*

Serrawettin W1 genes were amplified with design primers for expression (**Figure 18**) and DNA was extracted and quantified with NanoDrop Technology (**Table 11**). After restriction, DNA inserts and pET30a plasmid were extracted and quantified with NanoDrop Technology (**Table 10**).

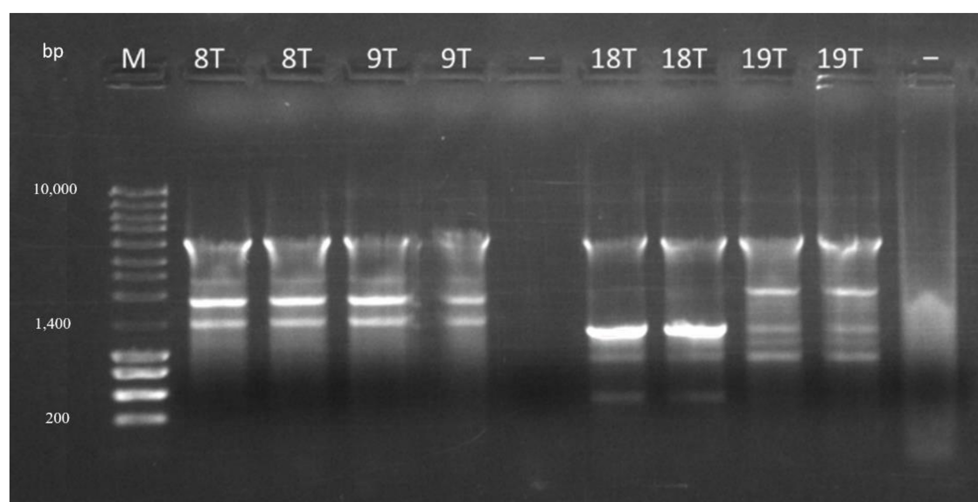

Figure 18 Amplification of Serrawettin W1 genes with expression primers srw1_FT_ALL_SalI and srw1_RT_Arv_NotI for *Serratia* strains Arv-22-2.5c (8T) and Arv-22-2.6 (9T) and srw1_FT_ALL_SalI and srw1_RT_AS13_NBRC_NotI for *Serratia* strains NBRC 102599^T (18T) and AS13 (19T) with a total length of 3,948 bp. **M** - molecular weight (bp); (**-**) – negative controls.

Table 10 DNA quantification with NanoDrop Technology (ng/µL) of Serrawettin W1 genes from *Serratia* strains Arv-22-2.5c, Arv-22-2.6, NBRC 102599^T and AS13, quantification of pET30a plasmid and restriction quantification of inserts and pET30a vector.

***Serratia* strains**	**Amplification (ng/µL)**	**Restriction (ng/µL)**
Arv-22-2.5c	65.9	34.3
Arv-22-2.6	64.8	25.9
NBRC 102599^T	41.1	18.0
AS13	75.8	37.2
pET30a	117.3	36.6

Ligation confirmation was performed with pET30a primer T7promoter and Serrawettin W1 srw1_2R primer (2,062 bp) (**Figure 19**). PCR results revealed a 4,000 bp band in all plasmids extracted. Plasmids 8A, 9A, 18A and 19A were sequenced by StabVida with T7promoter primer. Sequence results revealed that Serrawettin W1 genes were not inserted on pET30a vector.

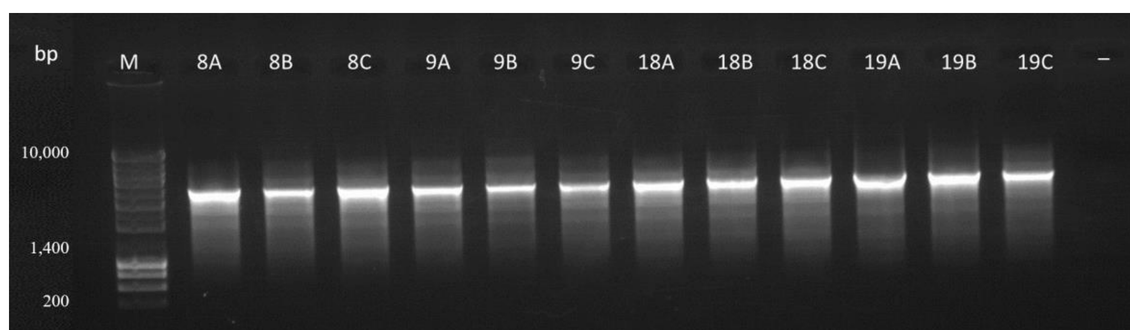

Figure 19 Ligation confirmation of extracted plasmids using pET30a vector primer T7promoter and Serrawettin W1 srw1_2R primer (2,062 bp). Plasmids from ligation with Serrawettin W1 gene of *Serratia* Arv-22-2.5c: 8A, 8B, 8C; *Serratia* Arv-22-2.6: 9A, 9B, 9C; *Serratia* NBRC 102599^{T}: 18A, 18B, 18C; *Serratia* AS13: 19A, 19B, 19C. **M** - molecular weight (bp); (**-**) – Negative control.

Serrawettin W2 genes were not amplified with design primers srw2-FT-NotI and srw2-RT-NotI (**Figure 20**).

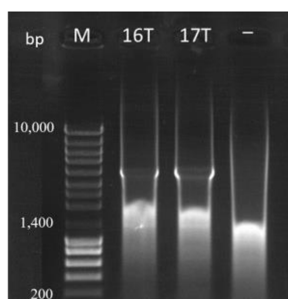

Figure 20 PCR results with expression primers srw2-FT-NotI and srw2-RT-NotI (17,853 bp) for *Serratia* strains A88copa7 (16T) and A88copa13 (17T). **M** - molecular weight (bp); (**-**) – negative control.

E. Lipopeptides extraction and purification

After 24 h growth, *Serratia* strains Arv-22-2.5c, Arv-22-2.6, A88copa7, A88copa13, NBRC 102599 and AS13 OD_{600} was observed (**Table 11**) followed by lipopeptides extraction and purification.

Table 11 Optical density of a 24 h growth of *Serratia* strains Arv-22-2.5c, Arv-22-2.6, A88copa7, A88copa13, NBRC 102599^T and AS13.

***Serratia* strains**	**OD_{600}**
Arv-22-2.5c	1.21
Arv-22-2.6	1.12
A88copa7	0.93
A88copa13	1.73
NBRC 102599^T	0.65
AS13	0.73

TLCs of polar lipids from each *Serratia* strain growth revealed 4 amino lipid spots on *Serratia* strain Arv-22-2.5c (8A, 8B, 8C, 8D), 3 amino lipid spots on *Serratia* stain Arv-22-2.6 (9A, 9B, 9C), 3 amino lipid spots on *Serratia* strain A88copa7 (16A, 16B, 16C), 3 amino lipid spots on *Serratia* strain A88copa13 (17A, 17B, 17C), 7 amino lipid spots on *Serratia* strain NBRC 102599^T (18A, 18B, 18C, 18D, 18E, 18F, 18G) and 5 amino lipid spots on *Serratia* strain AS13 (19A, 19B, 19C, 19D, 19E) (**Figure 21**).

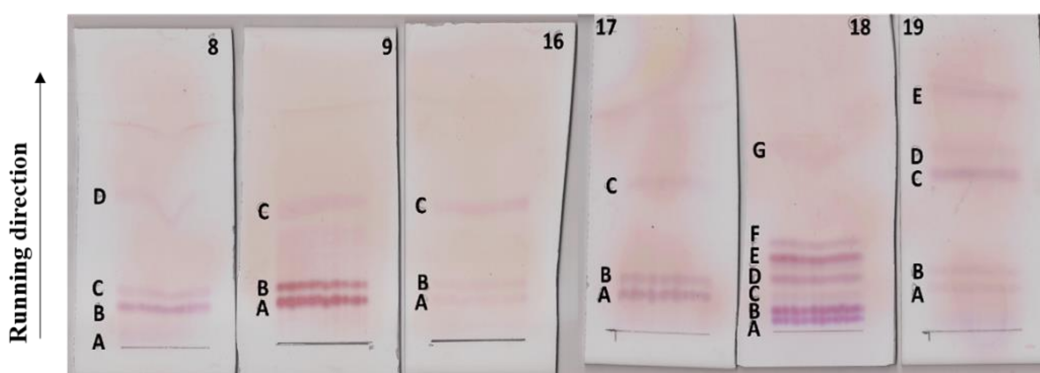

Figure 21 TLCs of polar lipids from supernatants of *Serratia* strains Arv-22-2.5c (8), Arv-22-2.6 (9), A88copa7 (16), A88copa13 (17), NBRC 102599^T (18) and AS13 (19). Positive amino lipids were spotted with ninhydrin solution, coloured in pink. *Serratia* strains Arv-22-2.5c (8) revealed 4 amino lipid spots (8A, 8B, 8C, 8D), 3 amino lipid spots were revealed on *Serratia* stain Arv-22-2.6 (9A, 9B, 9C), 3 amino lipid spots revealed on *Serratia* strain A88copa7 (16A, 16B, 16C), 3 amino lipid spots revealed on *Serratia* strain A88copa13 (17A, 17B, 17C), 7 amino lipid spots revealed on *Serratia* strain NBRC 102599^T (18A, 18B, 18C, 18D, 18E, 18F, 18G) and 5 amino lipid spots revealed on *Serratia* strain AS13 (19A, 19B, 19C, 19D, 19E).

E1. Mortality tests in *C. elegans*

Mortality tests in *C. elegans* with extracted amino lipids revealed no statistical difference when compared to 0.1 M NaCl solution and overall mortality rates were less than 15 % (**Figure 22**). Positive control, *Serratia* strain A88copa13 supernatant, was unable to have a nematicidal effect against *C. elegans*. *E. coli* OP50 with an OD_{600} = 0.4 was able to kill all nematodes in 24 h.

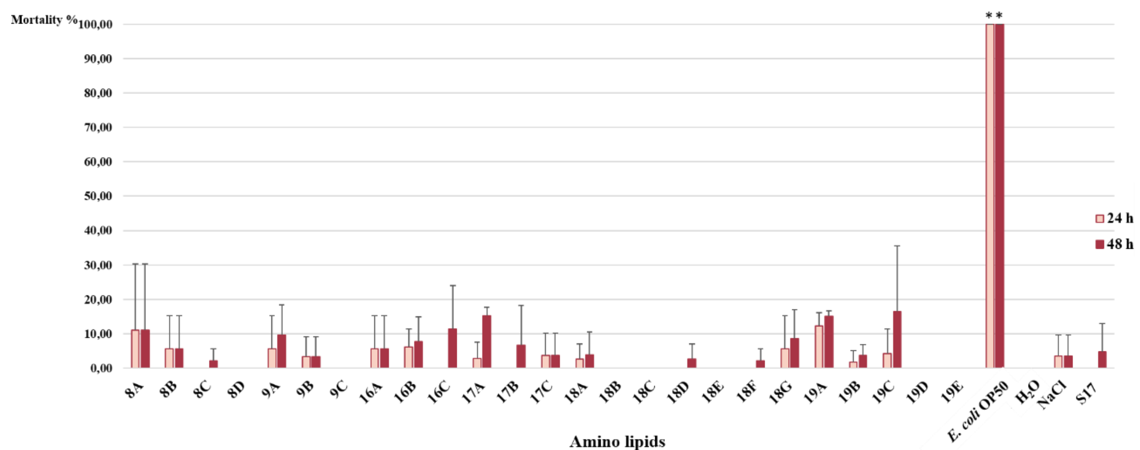

Figure 22 *C. elegans´* mortality tests with amino lipids eluted in 0.1 M NaCl solution and H_2O and 0.1 M NaCl solution as negative control and supernatant of *Serratia* strain A88copa13 (S17) as positive control. Amino lipids were extracted from supernatants of a 24 h growth of *Serratia* strains Arv-22-2.5c (amino lipids 8A, 8B, 8C and 8D), Arv-22-2.6 (amino lipids 9A, 9B and 9C), A88copa7 (amino lipids 16A, 16B and 16C), A88copa13 (amino lipids 17A, 17B and 17C), NBRC 102599^T (amino lipids 18A, 18B, 18C, 18D, 18E, 18F and 18G) and AS13 (amino lipids 19A, 19B, 19C, 19D and 19E). The number of dead nematodes was assessed at 24 h and 48 h after incubation with amino lipids. Each test was performed in triplicate. Results that showed statistical differences on Two-way ANOVA and Tukey Test are marked with (*).

E2. Mortality tests in *B. xylophilus*

Mortality tests performed with extracted amino lipids in *B. xylophilus* (**Figure 23**) revealed higher mortality rates when compared to *C. elegans* (**Figure 22**). When compared with 0.1 M NaCl solution, amino lipids 8D from *Serratia* strain Arv-22-2.5c, 9C from *Serratia* strain Arv-22-2.6, 17C from *Serratia* strain A88copa13, 18F and 18G from *Serratia* strain NBRC 102599, 19D and 19E from *Serratia* strain AS13 showed statistical significance, killing more than 55% of *B. xylophilus* after 24 h (**Figure 23**). Amino lipids 8C and 8D from *Serratia* strain Arv-22-2.5c, 9C from *Serratia* strain Arv-22-2.6, 17A, 17B and 17C from *Serratia* strain A88copa13, 18E, 18F and 18G from *Serratia* strain NBRC 102599, 19D and 19E from *Serratia* strain AS13 were able to kill *B. xylophilus* after 48 h, with statistical significance. Negative controls 0.1 M NaCl solution and H_2O were unable to kill more than 15% of *B. xylophilus.* Positive control, *Serratia* strain A88copa13 supernatant, was unable to kill more than 60% of nematodes.

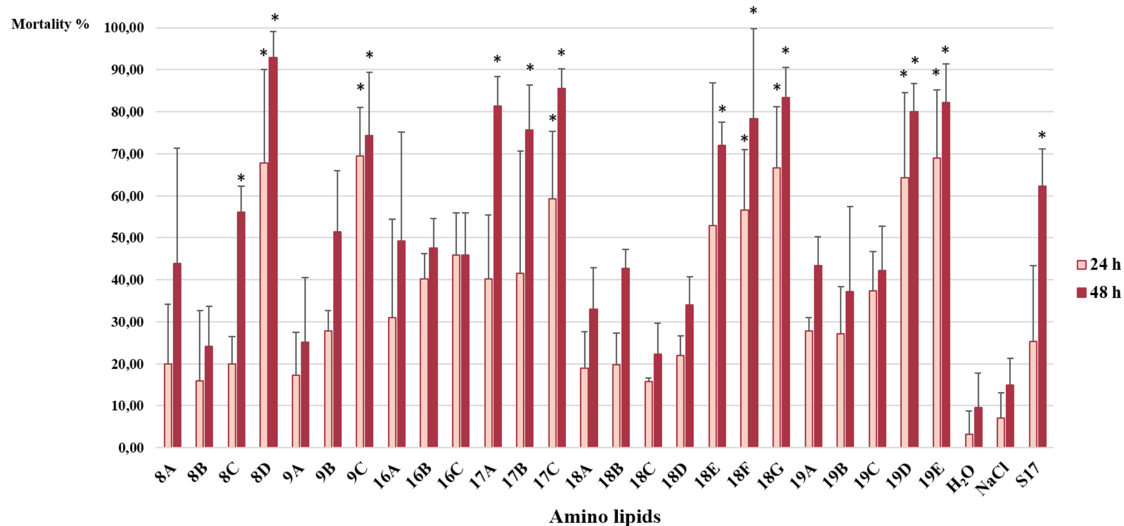

Figure 23 *B. xylophilus´* mortality tests with amino lipids eluted in 0.1 M NaCl solution and H_2O and 0.1 M NaCl solution as negative control and supernatant of *Serratia* strain A88copa13 (S17) as positive control. Amino lipids were extracted from supernatants of a 24 h growth of *Serratia* strains Arv-22-2.5c (amino lipids 8A, 8B, 8C and 8D), Arv-22-2.6 (amino lipids 9A, 9B and 9C), A88copa7 (amino lipids 16A, 16B and 16C), A88copa13 (amino lipids 17A, 17B and 17C), NBRC 102599^T (amino lipids 18A, 18B, 18C, 18D, 18E, 18F and 18G) and AS13 (amino lipids 19A, 19B, 19C, 19D and 19E). The number of dead nematodes was assessed at 24 h and 48 h after incubation with amino lipids. Each test was performed in triplicate. Results that showed statistical differences on Two-way ANOVA and Tukey Test are marked with (*).

E3. Attraction tests in *C. elegans*

Attraction tests with extracted amino lipids against 0.1 M NaCl solution revealed a very low percentage of attraction (**Figure 24**). After 2 h, only amino lipids 9C from *Serratia* strain Arv-22-2.6, 16C from *Serratia* strain A88copa7, 17A and 17C from *Serratia* strain A88copa13, 18E and 18G from *Serratia* strain NBRC 102599 and 19D from *Serratia* strain AS13 were able to attract more nematodes than control, with statistical significance. In attraction tests with amino lipids 8A and 8B from *Serratia* strain Arv-22-2.5c, 18B from *Serratia* strain NBRC 102599 and 19B from *Serratia* strain AS13, nematodes preferred the NaCl control solution. After 24 h, amino lipids 8A and 8B from *Serratia* strain Arv-22-2.5c and 18C from *Serratia* strain NBRC 102599 were able to attract more nematodes than 0.1 M NaCl solution, with statistical significance. In attraction tests performed with amino lipids 18B and 18G from *Serratia* strain NBRC 102599 and 19A from *Serratia* strain AS13, nematodes preferred the control solution.

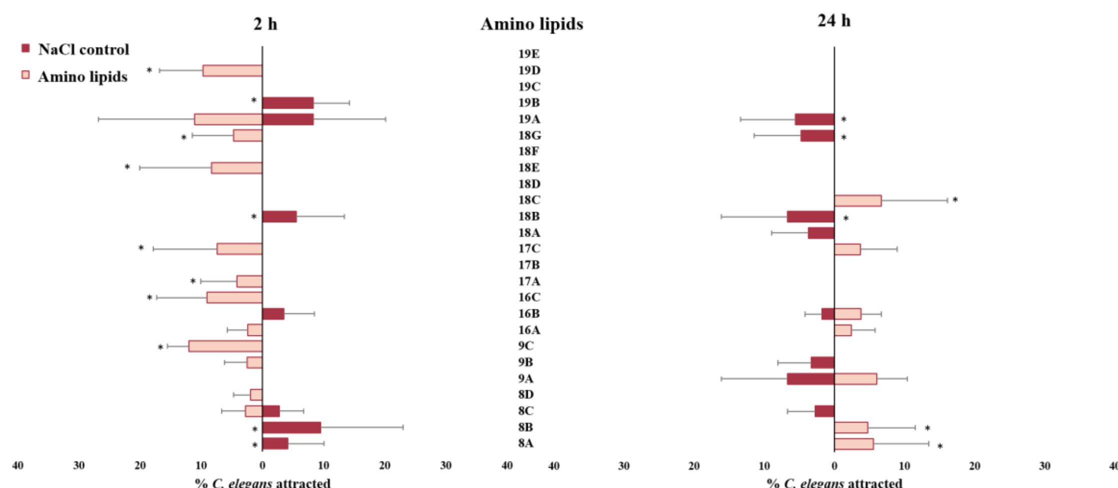

Figure 24 Attraction tests with amino lipids eluted in 0.1 M NaCl versus 0.1 M NaCl solution as control. Amino lipids were extracted from supernatants of a 24 h growth of *Serratia* strains Arv-22-2.5c (amino lipids 8A, 8B, 8C and 8D), Arv-22-2.6 (amino lipids 9A, 9B and 9C), A88copa7 (amino lipids 16A, 16B and 16C), A88copa13 (amino lipids 17A, 17B and 17C), NBRC 102599^T (amino lipids 18A, 18B, 18C, 18D, 18E, 18F and 18G) and AS13 (amino lipids 19A, 19B, 19C, 19D and 19E). The number of attracted nematodes by amino lipids and control was counted 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Results that showed statistical differences on Chi-Squared test are marked with (*).

Attraction tests performed with extracted amino lipids against supernatants revealed a poor attraction by amino lipid (**Figure 25**). After 2 h, only amino lipids 8B from *Serratia* strain Arv-22-2.5c and 18C from *Serratia* strain NBRC 102599 were able to attract more nematodes than respective supernatants, with statistical significance. In attraction test with amino lipids 8A from *Serratia* strain Arv-22-2.5c, 16B from *Serratia* strain A88copa7,17B and 17C from *Serratia* strain A88copa13, 18B and 18F from *Serratia* strain NBRC 102599, nematodes preferred supernatants. After 24 h, almost all nematodes were attracted to supernatants except for attraction tests with amino lipids 8C from *Serratia* strain Arv-22-2.5c, 18A and 18C from *Serratia* strain NBRC 102599, and 19B from *Serratia* strain AS13, where most nematodes were dispersed over NGM plate.

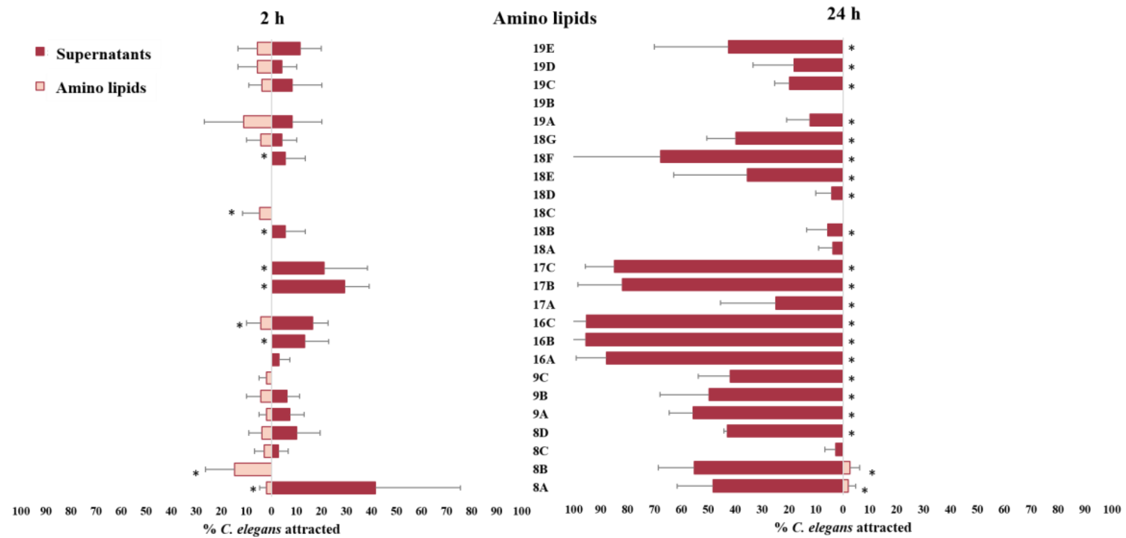

Figure 25 Attraction tests with amino lipids eluted in 0.1 M NaCl versus supernatants. Amino lipids were extracted from supernatants of a 24 h growth of *Serratia* strains Arv-22-2.5c (amino lipids 8A, 8B, 8C and 8D), Arv-22-2.6 (amino lipids 9A, 9B and 9C), A88copa7 (amino lipids 16A, 16B and 16C), A88copa13 (amino lipids 17A, 17B and 17C), NBRC 102599^T (amino lipids 18A, 18B, 18C, 18D, 18E, 18F and 18G) and AS13 (amino lipids 19A, 19B, 19C, 19D and 19E). The number of attracted nematodes by amino lipids and supernatants was counted 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Results that showed statistical differences on Chi-Squared test are marked with (*).

Attraction tests performed with extracted amino lipids against *E. coli* OP50 revealed that starved *C. elegans* preferred the food source over the amino lipids (**Figure 26**). After 2 h, only amino lipid 17A from *Serratia* strain A88copa13 was able to attract more nematodes than *E. coli* OP50, with statistical significance. After 24h, nematodes were attracted by food source in all tests.

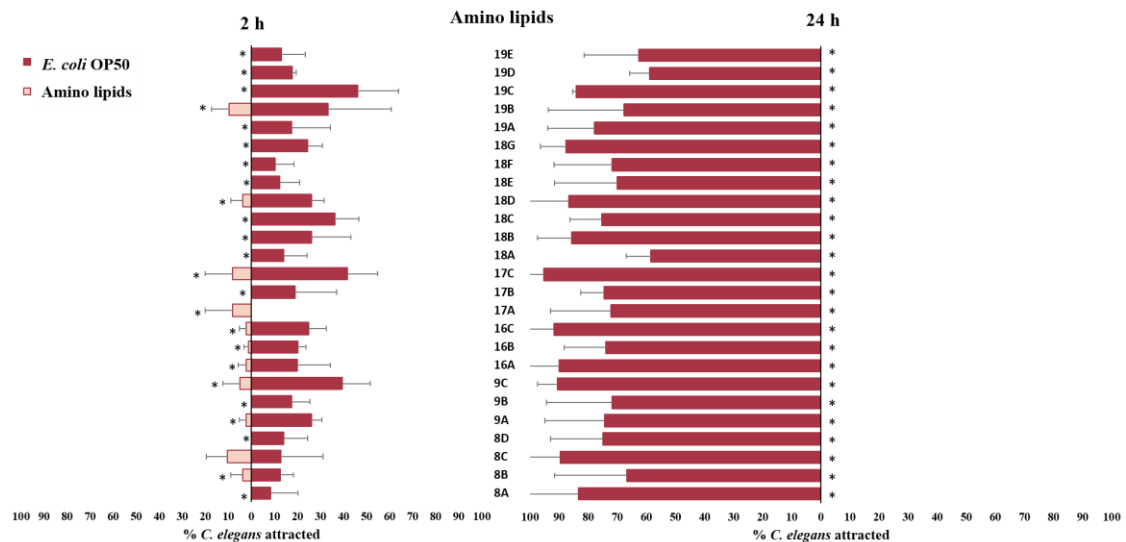

Figure 26 Attraction tests with amino lipids eluted in 0.1 M NaCl versus *E. coli* OP50. Amino lipids were extracted from supernatants of a 24 h growth of *Serratia* strains Arv-22-2.5c (amino lipids 8A, 8B, 8C and 8D), Arv-22-2.6 (amino lipids 9A, 9B and 9C), A88copa7 (amino lipids 16A, 16B and 16C), A88copa13 (amino lipids 17A, 17B and 17C), NBRC 102599^T (amino lipids 18A, 18B, 18C, 18D, 18E, 18F and 18G) and AS13 (amino lipids 19A, 19B, 19C, 19D and 19E).

The number of attracted nematodes by amino lipids and *E. coli* OP50 was counted 2 h and 24 h after *C. elegans* were added. Each test was performed in triplicate. Results that showed statistical differences on Chi-Squared test are marked with (*).

E4. HPLC analysis

Eight extracted amino lipids were chosen for HPLC analysis because they showed a better nematicidal activity against *B. xylophilus* when compared to other amino lipids. Amino lipids 8D from *Serratia* strain Arv-22-2.5c, 9C from *Serratia* strain Arv-22-2.6, 16C from *Serratia* strain A88copa7, 17C from *Serratia* strain A88copa13, 18F and 18G from *Serratia* strain NBRC 102599 and 19D and 19E from *Serratia* strain AS13. These amino lipids were able to kill more than 70% of *B. xylophilus* after 24 h, except amino lipid 16C from *Serratia* strain A88copa7 that was able to kill 46%. Amino lipids 9C from *Serratia* strain Arv-22-2.6, 16C *Serratia* strain A88copa7, 17C from *Serratia* strain A88copa13, 18G from *Serratia* strain NBRC 102599 and 19D from *Serratia* strain AS13 were able to attract more *C. elegans* than 0.1 M NaCl solution after 2 h, with statistical significance.

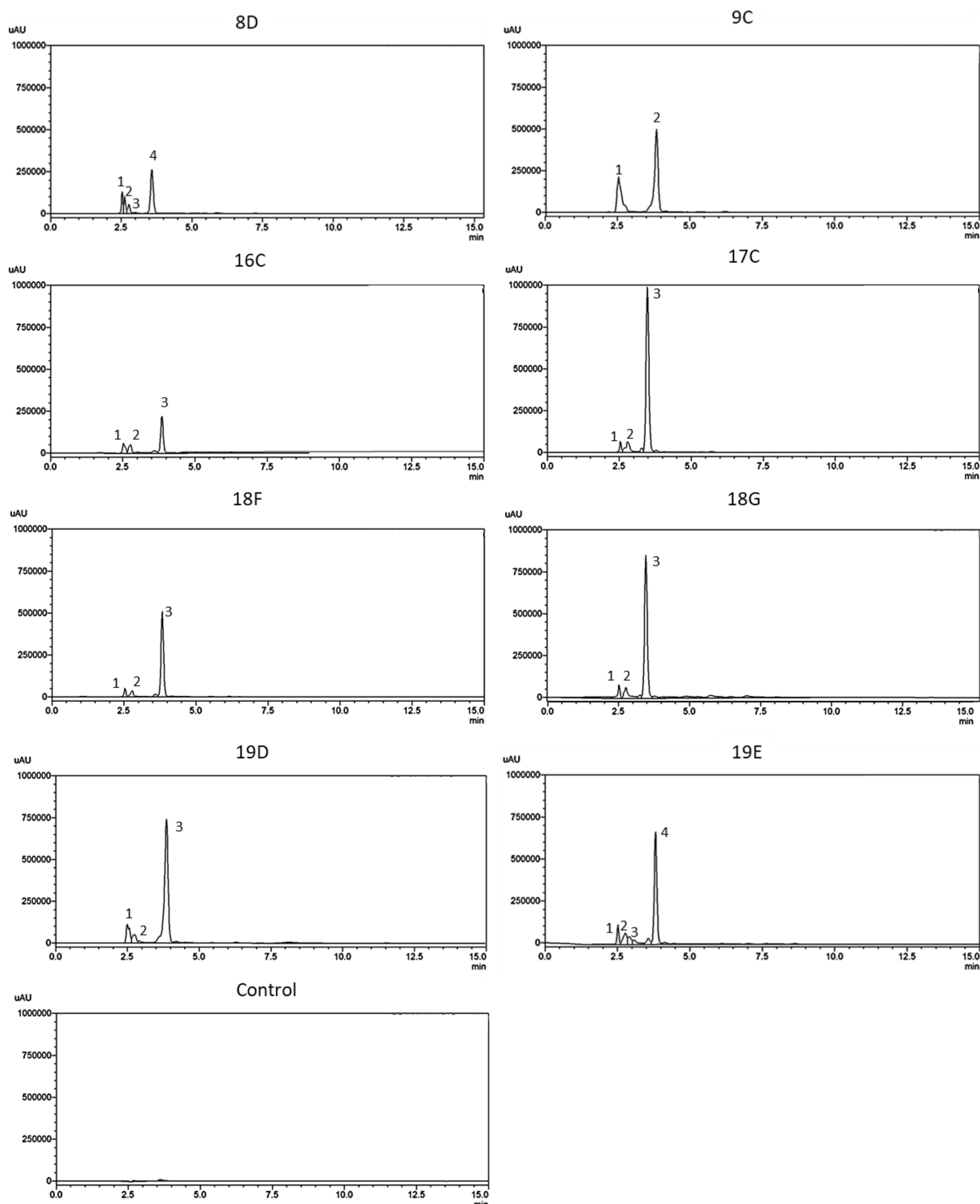

Figure 27 HPLC analysis of amino lipids samples 8D from *Serratia* strain Arv-22-2.5c, 9C from *Serratia* strain Arv-22-2.6, 16C from *Serratia* strain A88copa7, 17C from *Serratia* strain A88copa13, 18F and 18G from *Serratia* strain NBRC 102599^{T} and 19D and 19E from *Serratia* strain AS13 and methanol control. HPLC was performed for 15 min with an eluent gradient of 87-92% of methanol. UV was detected at 215 nm.

HPLC analysis showed similar peaks in all amino lipids (**Figure 27**) except in methanol control. All samples showed a peak between 3.48 and 3.86 min of retention time (**Table 12**).

Amino lipid 8D from *Serratia* strain Arv-22-2.5c showed 4 peaks: the fourth peak, at 3.59 min of retention time, was the most abundant with an area percentage of 34.45. Amino lipid 9C from *Serratia* strain Arv-22-2.6 had 2 peaks: the second peak, at 3.83 min of retention time, was the most

abundant with 56.59 of area percentage. Amino lipid 16C from *Serratia* strain A88copa7 had 3 peaks: the third peak, at 3.86 min of retention time, was the most abundant with an area percentage of 34.93. Amino lipid 17C from *Serratia* strain A88copa13 had 3 peaks: the third, at 3.48 min of retention time, was the most abundant with 80.28 of area percentage. Amino lipid 18F from *Serratia* strain NBRC 102599^T had 3 peaks: the third peak, at 3.48 min of retention time, was the most abundant with an area percentage of 60.57. Amino lipid 18G from *Serratia* strain NBRC 102599^T had 3 peaks: the third peak, at 3.48 min of retention time, was the most abundant with 76.10 of area percentage. Amino lipid 19D from *Serratia* strain AS13 had 3 peaks: the third peak, at 3.86 min of retention time, was the most abundant with an area percentage of 79.54. Amino lipid 19E from *Serratia* strain AS13 had 6 peaks: the sixth peak, at 3.82 min of retention time, was the most abundant with 66.21 of area percentage (**Table 12**).

Control with methanol reagent had no relevant peaks (**Figure 27**).

High nematicidal activity of these extracted amino lipids may be associated with higher peaks.

Table 12 HPLC analysis and peaks information from amino lipids extracted from *Serratia* supernatants with nematicidal activity. Retention time and area percentage of HPLC peaks of amino lipid samples 8D from *Serratia* strain Arv-22-2.5c, 9C from *Serratia* strain Arv-22-2.6, 16C from *Serratia* strain A88copa7, 17C from *Serratia* strain A88copa13, 18F and 18G from *Serratia* strain NBRC 102599^T and 19D and 19E from *Serratia* strain AS13.

Amino lipid	Peak	Retention time	Area %
8D	1	2.54	14.22
	2	2.63	11.18
	3	2.78	8.89
	4	**3.59**	**34.45**
9C	1	2.52	32.65
	2	**3.83**	**56.59**
16C	1	2.53	13.99
	2	2.77	13.01
	3	**3.86**	**34.93**
17C	1	2.54	3.63
	2	2.79	8.56
	3	**3.48**	**80.28**
18F	1	2.53	6.04
	2	2.79	8.15
	3	**3.48**	**60.57**
18G	1	2.54	5.07
	2	2.78	7.68
	3	**3.48**	**76.10**
19D	1	2.49	10.49
	2	2.75	5.45
	3	**3.86**	**79.54**
19E	1	2.52	7.17
	2	2.78	8.29
	3	2.91	5.14
	4	3.07	4.24
	5	3.57	4.31
	6	**3.82**	**66.21**

F. Bioinformatic analysis

F1. Serrawettin W1 gene cluster analysis

Bioinformatic analysis through AntiSMASH software (**BLIN, K. et al., 2019**) demonstrated that Serrawettin W1 cluster is present in seventeen *Serratia* genomes: *Serratia* strains ATCC 13880, CDC_813-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EGD-HP20, WW4, FS14, BIDMC81, TEL NODE_13, NBRC 102599^T, BXF1, A2, AS13, AS9 and AS12 (**Table 13**).

Table 13 *Serratia* genomes with Serrawettin W1 cluster identified by AntiSMASH software and NCBI accession numbers.

***Serratia* strains**	**Accession number**
Serratia marcescens subsp. marcescens ATCC 13880	JMPQ01000033.1
Serratia marcescens strain CDC_813-60 DP21	JOVM01000004.1
Serratia marcescens strain UMH8	CP018927.1
Serratia marcescens IOMTU 115	AB894481.1
Serratia nematodiphila DZ0503SBS1 strain DSM 21420	JPUX00000000.1
Serratia marcescens VGH107	AORJ00000000.1
Serratia marcescens EGD-HP20	AVSR00000000.1
Serratia marcescens WW4	CP003959.1
Serratia strain FS14	CP005927.1
Serratia marcescens BIDMC 81	JJMZ01000006.1
Serratia strain TEL NODE_13	LDEG01000018.1
Serratia plymuthica NBRC 102599^T	BCTU00000000.1
Serratia grimesii isolate BXF1	LT883155.1
Serratia grimesii strain A2	JGVP00000000.1
Serratia strain AS13	CP002775.1
Serratia plymuthica AS9	CP002773.1
Serratia strain AS12	CP002774.1

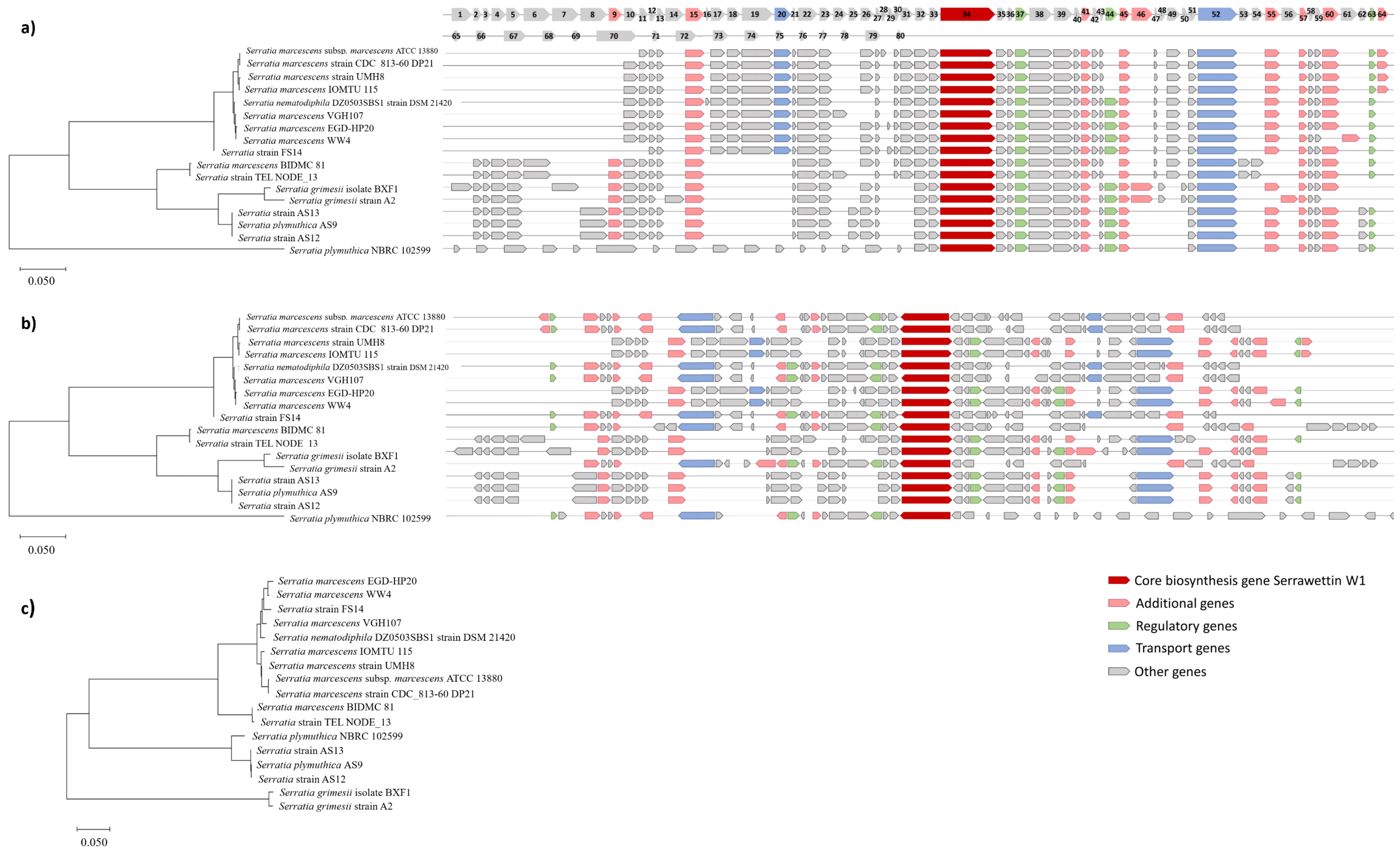

Figure 28 Serrawettin W1 gene cluster analysis. The phylogenetic relationship was established with Mega X software by Neighbor-Joining method on aligned Serrawettin W1 gene clusters of *Serratia* strains ATCC 13880, CDC_813-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EGD-HP20, WW4, FS14, BIDMC81, TEL NODE_13, NBRC 102599, BXF1, A2, AS13, AS9 and AS12. **a)** Phylogenetic tree of Serrawettin W1 gene cluster with align genes. **b)** Evolutionary tree of Serrawettin W1 cluster with organized genes. **c)** Phylogenetic tree of Serrawettin W1 biosyntesis protein. Scale bar 0.050 infer nucleotide substitutions per 100 nucleotides.

AntiSMASH software predicted a Condensation (C), Adenylation (A), Thiolation (T) and Thioesterase (TE) domain on Serrawettin W1 biosynthesis protein.

Eighty different cluster proteins were identified by AntiSMASH and NCBI BlastP softwares. Thirteen proteins are common to all seventeen clusters (**BATEMAN, A., 2019**) (**Figure 28 and Annexes 6, 7 and 8**)**:**

Proteins 65 to 80 are exclusive to *S. plymuthica* NBRC 102599^{T}´s cluster (**Figure 28 and Annexes 7 and 9**). Seven proteins were present in every clusters except NRBC cluster (**Figure 28 and Annexes 6, 7 and 10**).

AntiSMASH cluster prediction identified 4 additional proteins involved on PKS-NRPS (polyketide synthases non-ribosomal peptide synthetases): protein **9-** quinone oxidoreductase (non-present in all clusters), protein **45-** 3-oxoacyl-(acyl-carrier-protein) reductase, protein **55-** Mannitol dehydrogenase and protein **60-** Aromatic amino acid aminotransferase.

Accession numbers of identified proteins are showed on **Annexes 6 and 7.**

F2. Serrawettin W2 cluster gene analysis

Genome analysis by AntiSMASH software (**Blin, K. et al., 2019**) showed that Serrawettin W2 gene cluster is present in eleven *Serratia* strains: PWN146, SSNIH1, SM39, SmUNAM836, BIDMC 44, Lr5/4 LG59, RSC-14, AH0650_Sm1 AG2, Db11, SCBI and YD25 (**Table 14**).

Table 14 *Serratia* genomes with Serrawettin W2 cluster identified by AntiSMASH software and NCBI accession numbers.

***Serratia* strains**	**Accession number**
Serratia marcescens PWN146	LT575490.1
Serratia sp. SSNIH1	CP026383.1
Serratia marcescens SM39	AP013063.1
Serratia marcescens SmUNAM836	CP012685.1
Serratia marcescens BIDMC 44	JAPD01000005.1
Serratia ureilytica Lr5/4 LG59	JSFB01000001.1
Serratia marcescens RSC-14	CP012639.1
Serratia marcescens subsp. *marcescens* AH0650_Sm1 AG2	LFJS01000014.1
Serratia marcescens subsp. *marcescens* Db11	HG326223.1
Serratia sp. SCBI	CP003424.1
Serratia sp. YD25	CP016948.1

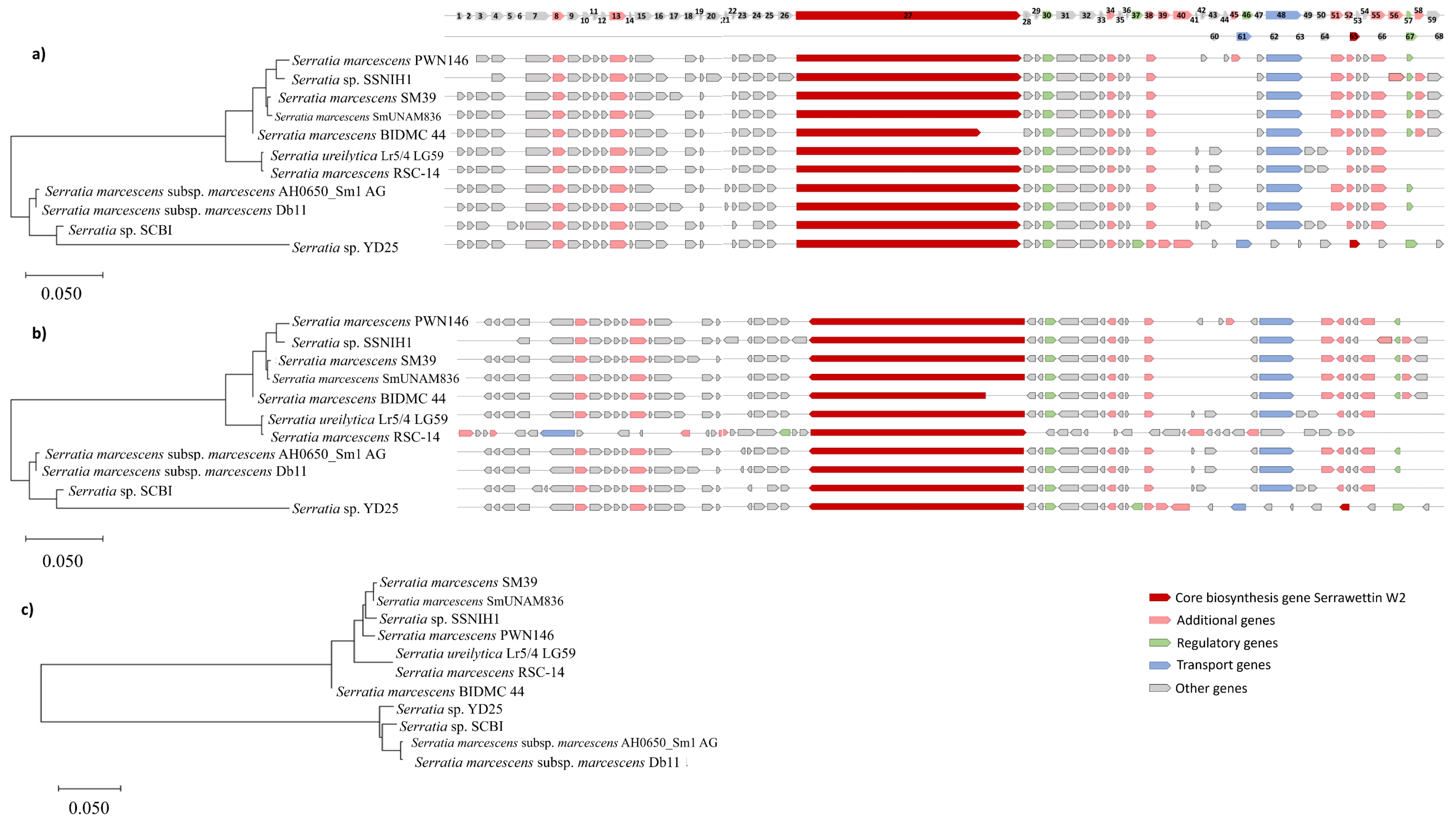

Figure 29 Serrawettin W2 cluster analysis. The evolutionary relationship was established with Mega X software by Neighbor-Joining method on Serrawettin W2 aligned clusters of *Serratia* strains PWN146, SSNIH1, SM39, SmUNAm836, BIDMC44, Lr5/4 LG59, RSC-14, AH0650, Db11, SCBI and YD25. **a)** Phylogenetic tree of Serrawettin W2 gene cluster with align genes. **b)** Evolutionary tree of Serrawettin W2 cluster with organized genes. **c)** Phylogenetic tree of Serrawettin W2 biosyntesis protein. Scale bar 0.050 infer nucleotide substitutions per 100 nucleotides.

AntiSMASH software predicted 5 modules, each with a Condensation (C1, C2, C3, C4 and C5), Adenylation (A1, A2, A3, A4 and A5) and Thiolation (T1, T2, T3, T4 and T5) domains on Serrawettin W2 biosynthesis protein. Module 5 has an additional Thioesterase (TE) domain, as previously described in **C. SU, et al. 2016**.

Sixty-eight proteins were identified in Serrawettin W2 clusters.

Twenty-four were present in all eleven clusters (**Figure 29 and Annexes 11, 12 and 13**).

Proteins 60 to 68 are exclusive to *Serratia* sp. YD25 ´s cluster (**Figure 29 and Annexes 12 and 14**).

Accession number of identified proteins are showed on **Annexes 11 and 12**.

G. Binding sites of Serrawettin W1 and W2 biosynthesis proteins

Using the intensive mode of Phyre2 software, a tridimensional model of Serrawettin W1 biosynthesis protein was established for *Serratia* strain AS13 with 100% coverage and 1,286 residues (98%) modelled at > 90% accuracy. This *Serratia* strain was chosen because it was one of initial selected strains and its *srw1* gene and cluster were similar to most other strains. This Serrawettin W1 biosynthesis gene has 3942 base pairs and its protein has 1,313 residues.

Binding sites of all domains from Serrawettin W1 biosythesis protein were predicted by Phyre2 3DLigandSite with a confidence score of 100. Condensation domain has 297 residues, of which 6 residues were predicted as possible binding sites. Adenylation domain has 405 residues, sixteen predicted as binding sites. Two of the 66 residues of Thiolation domain were identified as binding sites. Thioesterase (TE) domain, with 204 residues, had only one residue identified as binding site (**Table 15**). A video with Serrawettin W1 biosynthesis protein domains, binding sites and respective ligand connections was made using PyMOL software (**Supplementary data 1**).

Table 15 Suggested binding sites of Serrawettin W1 protein domains from *Serratia* strain AS13. Phyre2 3DLigandSite prediction on Condensation (C), Adenylation (A), Thiolation (T) and Thioesterase (TE) domains, showing predicted binding-site residues, number of ligands they contact with, average of predicted distance between each residue and ligands.

Domain	Residue	Amino acid	Ligand contacts	Average distance
C	138	ASP	2	0.00
	140	THR	2	0.61
	243	SER	3	0.00
	245	MET	3	0.00
	271	VAL	2	0.52
	272	HIS	3	0.00
A	151	THR	8	0.18
	196	ASP	12	0.23
	266	SER	11	0.35
	267	GLY	16	0.00
	268	GLU	16	0.18
	269	ALA	15	0.41

Table 15 (continuation)

Domain	Residue	Amino acid	Ligand contacts	Average distance
A	289	ASN	16	0.13
	290	LEU	16	0.00
	291	TYR	16	0.06
	292	GLY	16	0.11
	293	PRO	16	0.20
	294	THR	16	0.00
	383	ASP	16	0.18
	395	TYR	12	0.42
	398	ARG	16	0.00
	404	LYS	16	0.00
T	37	LEU	3	0.17
	41	LYS	3	0.26
TE	62	HIS	3	0.03

Using the intensive mode of Phyre2 software, a tridimensional model of Serrawettin W2 was established for *Serratia marcescens* PWN146. This strain was chosen due to its similarity to selected *Serratia* strain A88copa13. The five modules were predicted with 100% coverage: module 1 was modelled at > 76% accuracy, module 2 at > 99% accuracy, module 3 at > 96% accuracy, module 4 at 100% accuracy and module 5 at 96% accuracy. Each module has a Condensation, Adenylation and a Thiolation domain and module 5 also has a Thioesterase domain. Every domain was predicted with 100% coverage and with >90% accuracy. This protein has 5,929 residues, module 1 has 1,421 residues, module 2 has 1,051 residues, module 3 has 1,075 residues, module 4 has 1,045 residues and module 5 has 1,337 residues.

Binding sites for all domains from Serrawettin W2 biosythesis protein were predicted by Phyre2 3DLigandSite with a confidence score of 100. Condensation domain 1 (C1) has 288 residues, 10 were predicted as binding sites. Adenylation domain 1 (A1) has 402 residues, 18 were predicted as binding sites. Thiolation domain 1 (T1) has 66 residues, 4 predicted as binding sites. Condensation domain 2 (C2) has 296 residues, 7 were predicted as binding sites. Adenylation domain 2 (A2) has 398 residues, 17 were predicted as binding sites. Thiolation domain 2 (T2) has 67 residues, 3 predicted as binding sites. Condensation domain 3 (C3) has 229 residues, 6 were predicted as binding sites. Adenylation domain 3 (A3) has 398 residues, 16 were predicted as binding sites. Thiolation domain 3 (T3) has 68 residues, 2 predicted as binding sites. Condensation domain 4 (C4) has 299 residues, 13 were predicted as binding sites. Adenylation domain 4 (A4) has 392 residues, 17 were predicted as binding sites. Thiolation domain 4 (T4) has 66 residues, 3 predicted as binding sites. Condensation domain 5 (C5) has 294 residues, 6 were predicted as binding sites. Adenylation domain 5 (A5) has 391 residues, 16 were predicted as binding sites. Thiolation domain 5 (T5) has 68 residues, 4 predicted as binding sites. Thioesterase domain has 248 residues, 1 predicted as binding site (**Table 16**). A video with Serrawettin W2 biosynthesis protein domains, binding sites and respective ligand connections was made using PyMOL software (**Supplementary data 2**).

Table 16 Suggested binding sites of Serrawettin W2 protein domains from *Serratia* strain PWN146. Phyre2 3DLigandSite prediction on Condensation domains 1, 2, 3, 4 and 5 (C1, C2, C3, C4 and C5), Adenylation domains 1, 2, 3, 4 and 5 (A1, A2, A3, A4 and A5), Thiolation domains 1, 2, 3, 4 and 5 (T1, T2, T3, T4 and T5) and Thioesterase domain (TE). Predicted binding-site residues and number of ligands they contact with and average distances between the residue.

Domain	Residue	Amino acid	Ligand contact	Average distance
C1	131	HIS	2	0.39
	135	ASP	3	0.22
	136	GLY	3	0.22
	137	ARG	4	0.52
	238	HIS	4	0.00
	240	ALA	4	0.62
	265	PRO	3	0.58
	266	VAL	4	0.46
	267	THR	4	0.00
	279	SER	4	0.04
C2	135	ASP	2	0.02
	136	GLY	2	0.39
	244	LEU	2	0.67
	270	PRO	3	0.45
	271	GLU	3	0.10
	272	ALA	3	0.00
	287	VAL	3	0.00
C3	239	SER	3	0.00
	241	ALA	3	0.39
	266	VAL	3	0.00
	267	LEU	3	0.33
	268	LEU	3	0.00
	285	ILE	3	0.00
C4	134	HIS	4	0.00
	138	ASP	5	0.08
	139	GLY	4	0.08
	241	ARG	2	0.44
	245	GLY	2	0.42
	246	THR	3	0.18
	273	PRO	6	0.40
	274	GLU	6	0.00
	275	ALA	6	0.00
	276	ASN	3	0.00
	288	PHE	4	0.08
	289	PHE	3	0.47
	290	VAL	6	0.00
C5	140	ASP	2	0.33
	239	SER	2	0.39
	266	VAL	2	0.35
	267	LEU	3	0.20
	268	LEU	3	0.00
	285	ILE	3	0.00
A1	143	THR	8	0.22
	188	ASP	14	0.19
	189	PHE	10	0.29
	259	GLY	15	0.33
	260	GLY	16	0.00
	261	GLU	16	0.01
	262	MET	15	0.32
	284	ASN	16	0.05
	285	MET	16	0.00
	286	TYR	16	0.17
	287	GLY	16	0.00
	288	ILE	16	0.02
	289	THR	16	0.00
	379	ASP	16	0.14
	391	TYR	11	0.40
	394	ARG	16	0.06
	398	GLN	8	0.21
	400	LYS	16	0.00
A2	144	THR	11	0.40
	189	ASP	10	0.24
	260	SER	14	0.35
	261	GLY	15	0.00
	262	GLU	15	0.00

Table 16 (continuation)

Domain	Residue	Amino acid	Ligand contacts	Average distance
A2	263	ALA	15	0.10
	283	ASN	15	0.27
	284	LEU	15	0.00
	285	TYR	15	0.09
	286	GLY	15	0.21
	287	PRO	15	0.23
	288	THR	15	0.00
	375	ASP	15	0.06
	390	ARG	15	0.04
	394	GLN	7	0.26
	396	LYS	9	0.46
A3	144	THR	11	0.40
	189	ASP	10	0.24
	260	SER	14	0.35
	261	GLY	15	0.00
	262	GLU	15	0.00
	263	ALA	15	0.10
	283	ASN	15	0.27
	284	LEU	15	0.00
	285	TYR	15	0.09
	286	GLY	15	0.21
	287	PRO	15	0.23
	288	THR	15	0.00
	375	ASP	15	0.06
	390	ARG	15	0.04
	394	GLN	7	0.26
	396	LYS	9	0.46
A4	141	THR	7	0.13
	185	ASP	12	0.21
	254	GLY	12	0.27
	255	GLY	16	0.00
	256	GLU	16	0.02
	257	ALA	14	0.34
	277	ASN	16	0.07
	278	GLY	16	0.00
	279	TYR	16	0.12
	280	GLY	16	0.06
	281	PRO	16	0.18
	282	THR	16	0.00
	369	ASP	16	0.13
	381	TYR	14	0.45
	384	ARG	16	0.25
	388	GLN	7	0.34
	390	LYS	16	0.00
A5	138	THR	8	0.18
	182	ASP	12	0.18
	251	GLY	14	0.31
	252	GLY	16	0.00
	253	GLU	16	0.01
	254	LYS	15	0.31
	274	HIS	16	0.00
	275	ALA	16	0.00
	276	TYR	16	0.09
	277	GLY	16	0.06
	278	PRO	16	0.18
	279	THR	16	0.00
	284	PHE	9	0.49
	366	THR	14	0.47
	368	ASP	16	0.13
	383	ARG	16	0.08
T1	46	GLN	3	0.24
	47	LEU	4	0.46
	48	PRO	3	0.11
	49	LEU	3	0.19
T2	37	MET	3	0.05
	41	ARG	4	0.00
	49	VAL	3	0.00
T3	41	GLN	4	0.03
T4	37	MET	6	0.26
	47	ALA	3	0.74
	49	VAL	3	0.15

Table 16 (continuation)

Domain	Residue	Amino acid	Ligand contacts	Average distance
T5	46	THR	3	0.00
	47	LEU	3	0.76
	48	PRO	3	0.53
	49	VAL	3	0.28
TE	225	ASP	3	0.00

H. Molecular Dynamics Simulations

H1. TOL1 protein and Serrawettin W1 simulation

GROMACS simulation between *C. elegans* TOL1 protein and Serrawettin W1 was performed to better understand the interaction between protein and ligand (**Figure 34**). Root mean square deviation (RMSD) analysis of TOL1 protein showed that scaler distance for backbone atoms throughout the trajectory passes 10 Å. This result reveals that the simulation was not long enough for TOL1 protein stabilize and indicates a large conformational change. RMSD analysis of Serrawettin W1 was below 3 Å showing a small ligand conformational change (**Figure 30**).

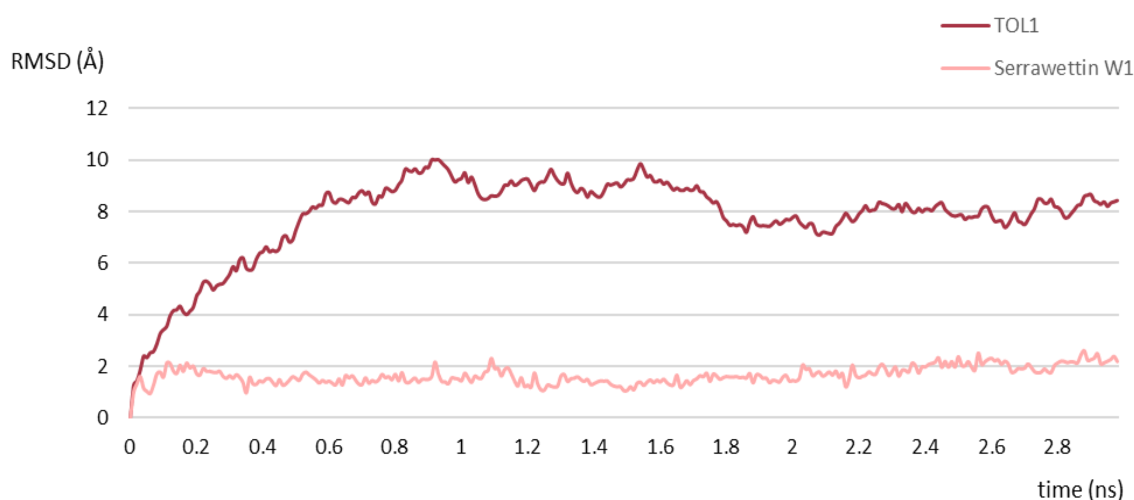

Figure 30 RMSD analysis of TOL1 and Serrawettin W1.

Root mean square fluctuation (RMSF) analysis for TOL1 protein in the simulation with Serrawettin W1 showed many local fluctuations throughout the simulation. All side chain residues had a fluctuation higher than 7 Å, demonstrating that all residues of side chain are able to contact with ligands. These results are in agreement with the high values of RSMD as the protein did not had time to stabilize. Residues 8,955 to 9,332 of TOL1 side chain had higher RMSF values, fluctuating more than 40 Å throughout the simulation (**Figure 31**).

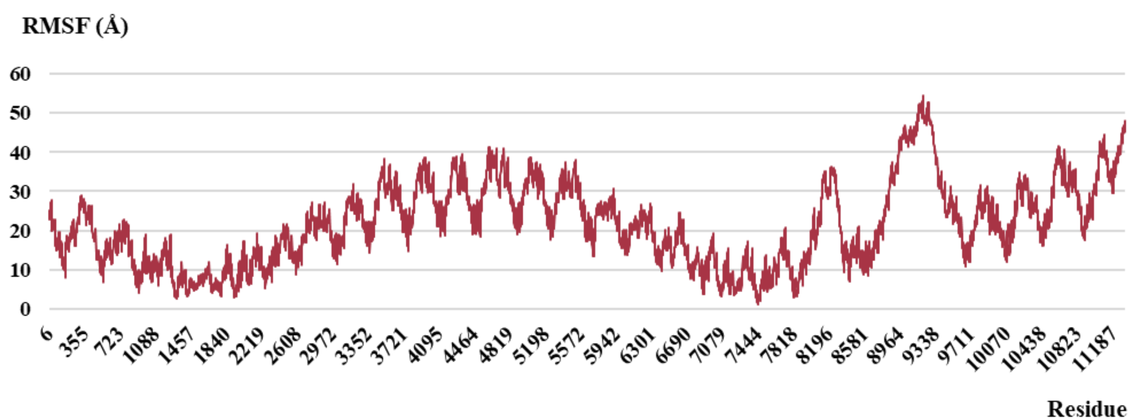

Figure 31 RMSF analysis of side chain of TOL1 protein fluctuation.

RMSF analysis for Serrawettin W1 ligand showed no significant local fluctuations throughout the simulation. All side chain residues had a fluctuation lower than 7 Å. Serrawettin W1 residues 75 to 77 fluctuated more than 4 Å throughout the simulation and are good candidates for protein ligand interactions (**Figure 32**).

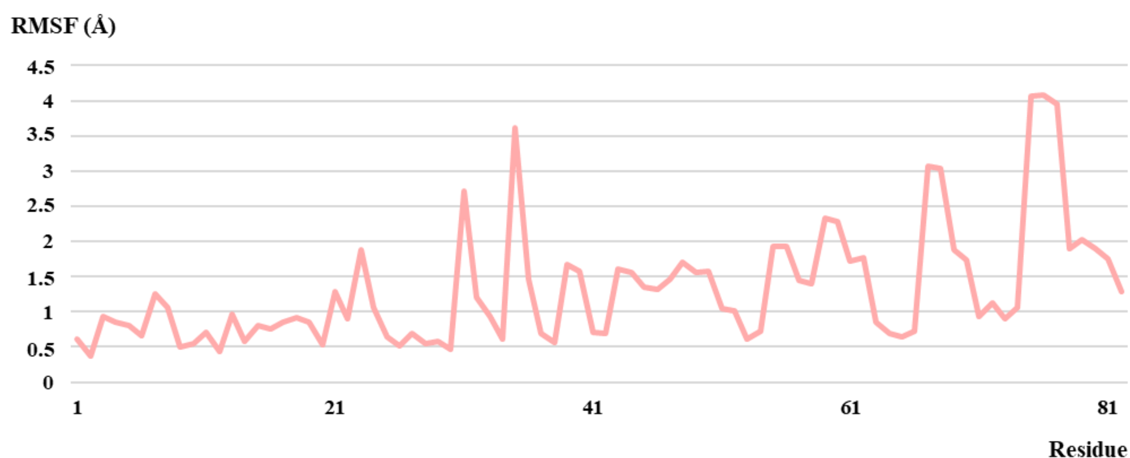

Figure 32 RMSF analysis of Serrawettin W1 fluctuation.

Distance analysis between centres of mass of TOL1 protein and Serrawettin W1 showed a stable distance around 35 Å. No protein ligand interactions occurred throughout the simulation (**Figure 33**).

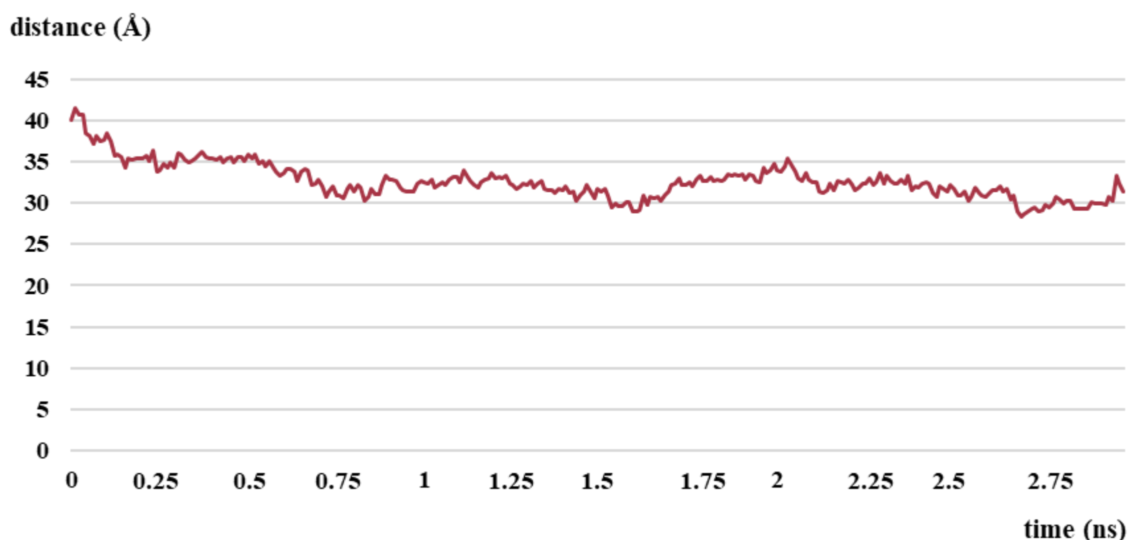

Figure 33 Distance analysis between TOL1 protein and Serrawettin W1.

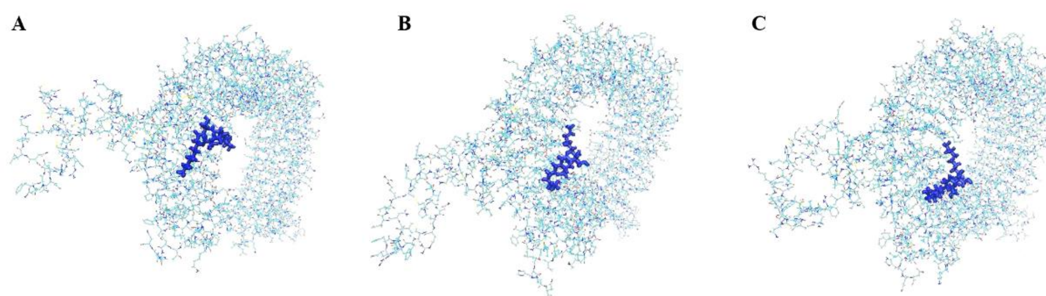

Figure 34 MD simulation of TOL1 protein and Serrawettin W1. **A -** initial simulation positions; **B -** middle simulation positions; **C -** terminal simulation positions.

H2. TOL1 protein and Serrawettin W2 simulation

GROMACS simulation between *C. elegans* TOL1 protein and Serrawettin W2 was performed to better understand the interaction between protein and ligand (**Figure 39**). Root mean square deviation (RMSD) analysis of TOL1 protein showed that scaler distance for backbone atoms throughout the trajectory passes 16 Å. This result reveals that the simulation was not long enough for TOL1 protein stabilize and indicates a large conformational change. RMSD analysis of Serrawettin W2 was below 3 Å showing a small ligand conformational change (**Figure 35**).

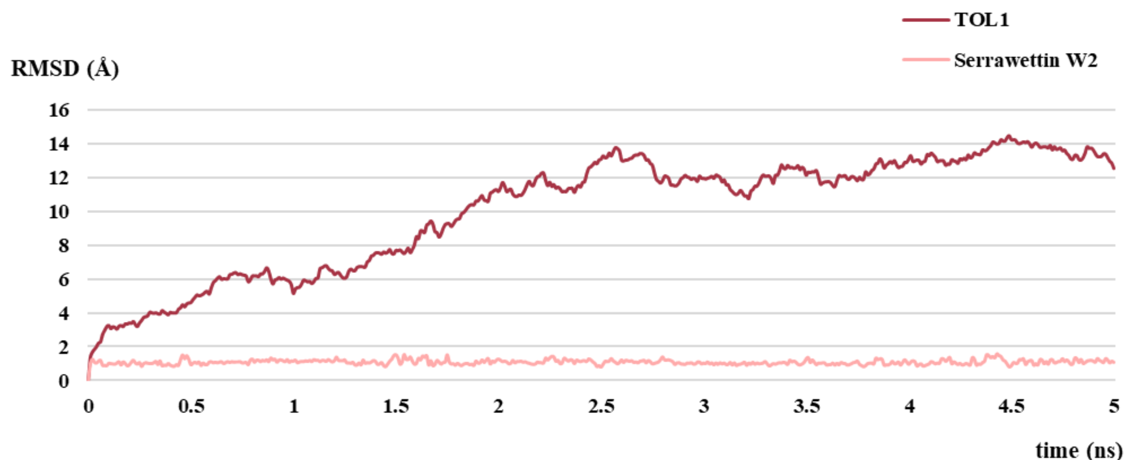

Figure 35 RMSD analysis of TOL1 protein and Serrawettin W2.

Root mean square fluctuation (RMSF) analysis for TOL1 protein in the simulation with Serrawettin W2 showed many local fluctuations throughout the simulation. All side chain residues had a fluctuation higher than 7 Å, demonstrating that all residues of side chain are able to contact with ligands. These results are in agreement with the high values of RSMD as the protein didn´t had time to stabilize. Residues 9,013 to 9,304 of TOL1 side chain had higher RMSF values, fluctuating more than 40 Å throughout the simulation (**Figure 36**).

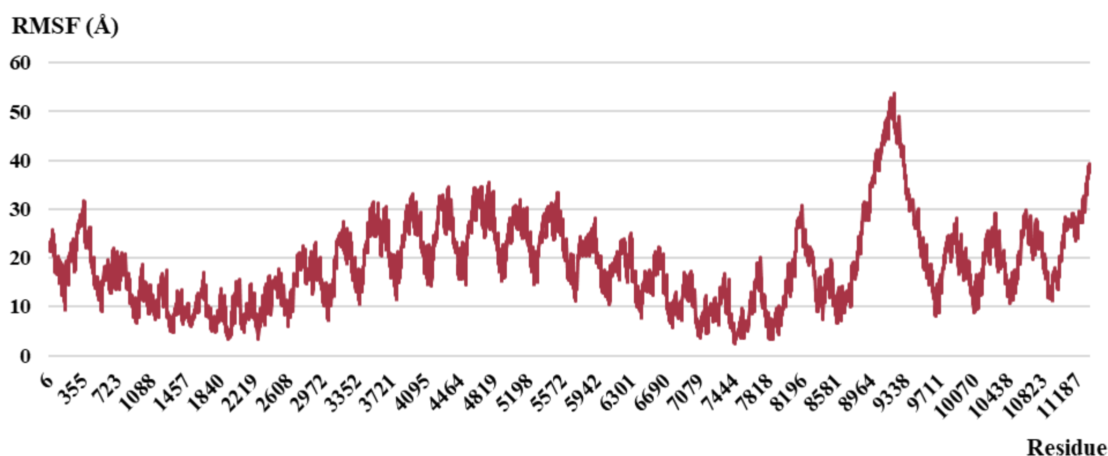

Figure 36 RMSF analysis of TOL1 protein fluctuation.

RMSF analysis for Serrawettin W2 ligand showed no significant local fluctuations throughout the simulation. All side chain residues had a fluctuation lower than 7 Å. Serrawettin W1 residues 99 to 101 fluctuated more than 2.5 Å throughout the simulation and are good candidates for protein ligand interactions (**Figure 37**).

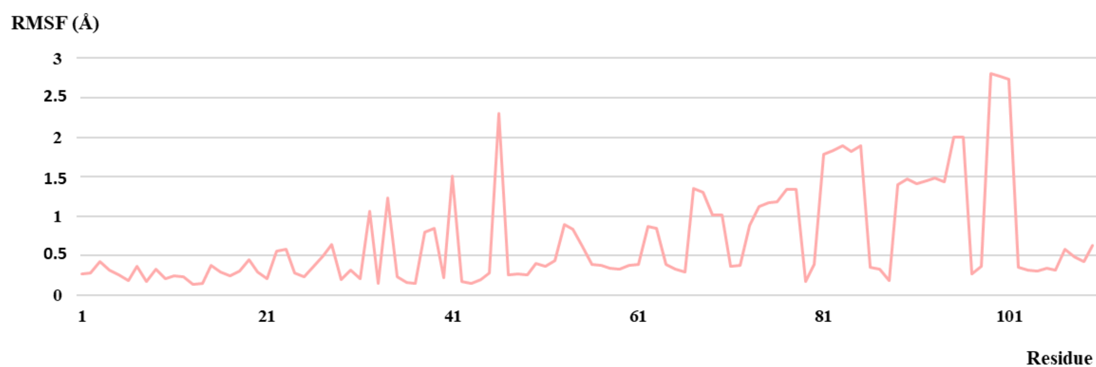

Figure 37 RMSF analysis of Serrawettin W2 fluctuation.

Distance analysis between centres of mass of TOL1 protein and Serrawettin W2 showed an ascending distance between protein and ligand, higher than 40 Å. No protein ligand interactions occurred throughout the simulation (**Figure 38**).

Figure 38 Distance analysis between TOL1 protein and Serrawettin W2.

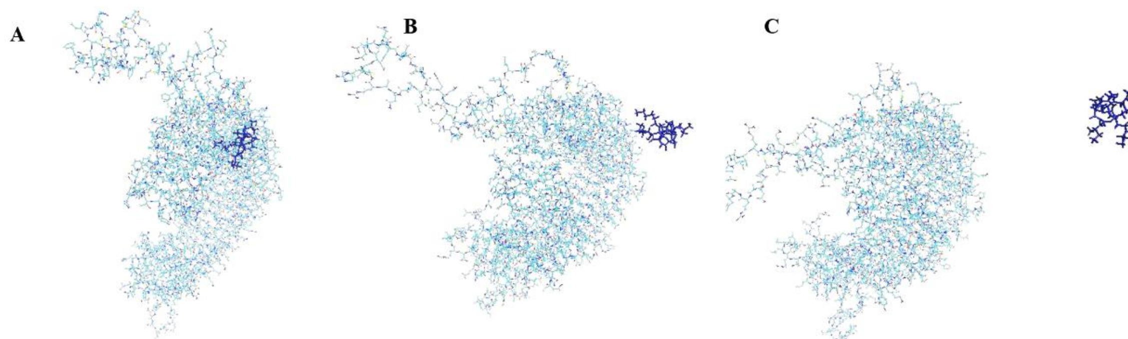

Figure 39 MD simulation of TOL1 protein and Serrawettin W2. **A -** initial simulation positions; **B -** middle simulation positions; **C -** terminal simulation positions.

Discussion

PWN, the known pathogenic agent of PWD, threatens pine and conifer forests all over the world. As global climate changes, a new scenario of PWN distribution evolves (**HIRATA, A., 2017**). In order to prevent disease spread, interactions between PWN and host trees, insect vectors and bacterial community must be understood.

Serratia strains, previously described as part of pine endophytic community (**PROENÇA, D. N. et al., 2017b**) and associated with PWN (**PROENÇA, D. N. et al., 2010; PROENÇA, D. N. et al., 2014**), were chosen to study their nematicidal potential.

This work intends to better understand the role of Serratomolides, produced by *Serratia* strains, in PWD and, especially, to assess if they are effective against PWN. Seven specific objectives were determined.

First, we screened for the presence of Serrawettin genes in selected *Serratia* strains. Serrawettin genes have already been found in several *Serratia* strains (**MATSUYAMA, T. et al., 1992; MATSUYAMA, T. et al., 1996; LI, H. et al., 2005; PRADEL, E. et al., 2007; ROBERTS, D. P. et al., 2007; ZHANG, L. et al., 2010; SU, C. et al., 2017; NASCIMENTO, F. et al., 2018**)

Serrawettin genes were present in seven of nineteen selected strains: *srw1* gene was present in *Serratia* strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, NBRC 102599^T and AS13 and *srw2* gene was present in *Serratia* strains A88copa7 and A88copa13. Serrawettin W1 gene was present in more strains than Serrawettin W2 gene. This result suggests that *srw1* might be more frequent in *Serratia* genus than *srw2* gene and might explain the lower number of studies published about Serrawettin W2. Three selected strains with *srw1* were carried by PWN in USA (*Serratia* strains Arv-22-2.5c, Arv-22-2.6 and Arv-22-3.9) (**PROENÇA, D. N. et al., 2014**) and two strains are similar to *Serratia plymuthica* (*Serratia plymuthica* NBRC 102599^T and *Serratia* strain AS13). Selected strains with *srw2* gene were carried by PWN in Portugal areas (**PROENÇA, D. N. et al., 2010**).

Serratia strains have been shown to produce high quantities of Serrawettin, up to 17% of their mass dried weight (**MATSUYAMA, T. et al., 1992**), suggesting that these amino lipids play an important role. Some *Serratia* strains were already described as nematicidal (**PAIVA, G. et al., 2013; PROENÇA, D. N. et al., 2019b; RAHUL, S. et al., 2014**), and this study aims to determine Serrawettins nematicidal activity.

No selected *Serratia* strain presented both genes, confirming previous findings (**SU, C. et al., 2017**).

Second, we studied bacteria-nematode interactions by way of three different tests.

<u>Mortality tests:</u> overall nematicidal effect of supernatants from selected strains was higher against *B. xylophilus* than against *C. elegans*. All supernatants were able to kill more than 30% of *B. xylophilus* after 48 h and more than 50% after 72 h (**Figure 4**), suggesting that this nematode is more susceptible to *Serratia* strains when compared to *C. elegans*.

Nevertheless, our results confirm previous studies that identified some *Serratia* strains with nematicidal activity against *C. elegans* (**PRADEL, E. et al., 2007; GLATER, E. E. et al., 2014**; **GIBSON, A. K., & MORRAN, L. T., 2017**).

Our findings confirmed nematicidal activity of *Serratia* strains against *B. xylophilus,* previously described in **PAIVA, G. et al., 2013,** increasing our knowledge about nematicidal activity of *Serratia* strains collected in different sampling sites, from USA (**PROENÇA, D. N. et al., 2014**) and Portugal (**PROENÇA, D. N. et al., 2010**), and endophytic isolates (**PROENÇA, D. N. et al., 2017b**).

Concentrated supernatants presented better nematicidal results than non-concentrated supernatants and were able to kill more than 57% of *B. xylophilus* after 72 h (**Figure 5**). These results suggest that nematicidal activity increases, as expected, when supernatant components are more concentrated. Concentrated supernatants from endophytic bacteria (*Serratia* strains A25T1, A88C4, A88C6), bacteria carried by PWN from Portugal (*Serratia* strain M24T3) and bacteria carried by PWN from USA (*Serratia* strains Arv-20-4.2, Arv-22-2.5c, Arv-22-2.6) were able to kill all *B. xylophilus* after 72 h. These results suggest that nematicidal activity is independent of where the sample was isolated (sample site).

Mortality tests with concentrated supernatants and *C. elegans* revealed that *Serratia* strains A88C3, A88C4, A88C6, Arv-29-3.11b, Arv-29-3.9, A88copa7 and Leaf50, *Pseudomonas* strain M47Tronco1 and *E. coli* OP50 were able to kill all nematodes in 24 h (**Figure 3**).

All concentrated supernatants showed a significant increase of nematicidal activity compared to non-concentrated supernatants, suggesting that some extracellular components in *Serratia* supernatants possess strong nematicidal activity against both nematodes (**Figures 3 and 5**).

Even those strains without *srw1* and *srw2* genes were able to kill nematodes, although showing diverse mortality rates, suggesting that nematicidal potential is not exclusively dependent of Serratomolide presence. A previous study (**PAIVA, G. et al., 2013**) had already concluded that some extracellular proteases, like serine protease and serralysin, produced by *Serratia* strains, have nematicidal activity against *B. xylophilus*. Other nematicidal agents were identified in endophytic strain *Raoultella ornithinolytica* (**SHANMUGAM, G. et al., 2018**). Natural halogenated indoles have been reported as nematicidal against PWN, increasing nematode mortality in different stages (**SEO, S. M. et al., 2017; RAJASEKHARAN, S. K. et al., 2017, RAJASEKHARAN, S. K. et al., 2019**)

<u>Attraction tests:</u> we consider results after 2 h more informative as they probably better reflect immediate attraction; results after 24 h are probably more influenced by random nematode movements.

Serratia strains M24T3, A25T1, A88C3, A88copa7 and Leaf50 were able to attract more nematodes than *E. coli* OP50 after 2 h and 24 h (**Figure 6**). Since *C. elegans* are starved, the preference for bacteria other than their usual food source, *E. coli* OP50 (**STIERNAGLE, T., 2006**), reveals a strong attraction ability. Our findings confirm that *Serratia* strains have the ability to

attract nematodes, as previously described in **PUJOL, N. et al., 2001**, and that *C. elegans* is more attracted to some *Serratia* strains than to *E. coli*, as described in **WORTHY, S. E. et al., 2018**.

Supernatants of *Serratia* strains A25T1, A88C3, A88C4 and A88C6 were able to attract more nematodes than *E. coli* OP50 after 2 h and 24 h (**Figure 7**). None of these supernatants have Serratomolides, suggesting that another component is responsible for attraction by *Serratia* strains. The presence of some metabolites in supernatants (like amino acids, odours or autoinducers) is able to attract *C. elegans* and may induce a food seeking behaviour (**BARGMANN, C. I., 2006**; **PRADEL, E. et al., 2007**)

Almost all *Serratia* supernatants were able to attract more *C. elegans* than *E. coli* OP50 supernatant (**Figure 8**). *E. coli* OP50 supernatant had poor attraction ability, suggesting that *C. elegans* may be attracted by some other *E. coli* OP50 cellular structure or component, that is absent or in low concentration in its respective supernatant, and/or that *C. elegans* preferred *Serratia* supernatant odours over *E. coli* OP50 odours, as previously described in **WORTHY, S. E. et al., 2018**.

<u>Avoidance tests:</u> *C. elegans* revealed low avoidance behaviour towards *Serratia* strains (**Figure 9**), suggesting that these strains can be perceived as food sources.

C. elegans avoided supernatants from *Serratia* strains M24T3, Arv-29-3.9, M24T3A, M47C12B1, M24Tronco5, NBRC 102599^T and AS13, both at 2 h and 24 h (**Figure 10**), but these results are not surprising as nematodes were starved and preferred their usual food source.

A higher percentage of nematodes avoided *E. coli* OP50 supernatant over *Serratia* supernatants (**Figure 11**). These results suggest that *E. coli* OP50 supernatant is not recognized as a proper food source by *C. elegans* and that some components of *Serratia* supernatants may have an attracting effect over *C. elegans* (**WORTHY, S. E. et al., 2018**).

Our findings did not confirm an avoidance response of *C. elegans* to *Serratia* strains, previously described in **PUJOL, N. et al., 2001** and **PRADEL, E. et al., 2007**.

SDS-PAGE was performed to better understand concentrated supernatants results and revealed that *Serratia* strains had a similar protein profile (**Figures 12 and 13**). There were two common bands previously described as metalloproteinase serralysin (50 kDa) and serine protease (70 kDa) (**PAIVA, G. et al., 2013**). Serine protease has a confirmed nematicidal activity (**PAIVA, G. et al., 2013**) and might be one of the compounds associated to supernatants with nematicidal activity.

Some other biosurfactants like surfactin where described as nematicidal and also as capable of inducing an avoidance response towards *C. elegans* (**PRADEL, E. et al., 2007**; **CHOWDHURY, S. P. et al., 2015; DEVARAJ, S. et al., 2018**).

As we intend to study Serrawettins, we discuss with detail (**Table 17**) results from those strains with *srw1* gene (*Serratia* strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, NBRC 102599^T and AS13) and with *srw2* gene (*Serratia* strains A88copa7 and A88copa13).

Table 17 Integrated results of mortality tests against *C. elegans* and *B. xylophilus* and attraction tests with *C. elegans* of selected strains with *srw1* and *srw2* genes, with information about serralysin and serine protease presence.

Genes	Strains	Mortality test		Attraction test		Serralysin/ Serine protease
		Concentrated supernatants		Bacteria	Supernatant	
		C. elegans	*B. xylophilus*	*C. elegans*	*C. elegans*	
		24 h	**72h**	**2 h**	**2 h**	
srw1	Arv-22-2.5c	17%	100%	2%	33%	YES
srw1	Arv-22-2.6	11%	100%	28%	16%	YES
srw1	Arv-29-3.9	100%	90%	6%	17%	YES
srw1	NBRC 102599^T	8%	93%	12%	22%	NO
srw1	AS13	0%	77%	46%	12%	NO
srw2	A88copa7	100%	97%	45%	23%	YES
srw2	A88copa13	2%	97%	20%	8%	YES

Presence of *srw1* and *srw2* genes did not assure a strong nematicidal activity of concentrated supernatants against *C. elegans* (for example, *Serratia* strains AS13 and A88copa13).
However, against *B. xylophilus,* concentrated supernatants from *Serratia* strains with *srw1* and *srw2* genes showed higher nematicidal activity (more than 77% overall), suggesting that PWN may be more susceptible than *C. elegans* when exposed to the same concentrated supernatants, with exception of concentrated supernatants of *Serratia* strains Arv-29-3.9 and A88copa7 which showed a marginally higher nematicidal activity against *C. elegans*.
C. elegans showed an attraction response to some supernatants of *Serratia* strains with *srw1* and *srw2* genes, however these results are insufficient to relate such response to Serratomolides presence.
Concentrated supernatant of *Serratia* strain A88copa13 presented serralysin and serine protease bands in SDS-PAGE, as previously described in **PAIVA, G. et al., 2013**, and showed a high nematicidal activity against PWN.
Concentrated supernatants of *Serratia* strains NBRC 102599^T and AS13 did not present serralysin and serine protease bands but demonstrated a strong nematicidal activity against PWN. These results suggest that Serrawettin W1 or other molecules may have, by themselves, a significant nematicidal activity against PWN, even in strains that do not express serralysin and serine protease.
Concentrated supernatants of *Serratia* strains with both Serrawettins genes and serralysin/serine protease bands demonstrated a higher nematicidal activity against PWN, suggesting a synergic effect resulting from concomitant nematicidal activity of all components.
Despite our best efforts, we were unable to successfully mutate Serrawettin genes (third specific goal) and clone *srw1* and *srw2* genes into *E. coli* DH5α (fourth specific goal).
Probably, amplificated gene sequences chosen to create disruptive mutants were too small to become incorporated in *Serratia* genomes, as suggested by **ZHANG, L. et al., 2010** that used a sequence with more than 1,200 pb to successfully achieve disruptive mutation of Serrawettin W1.
Serrawettin W1 was successfully cloned and produced in *E. coli*, as described by **THIES, S. et al., 2014.** We were able to amplify the complete sequence of Serrawettin W1 genes from four *Serratia* strains, but we were unable to confirm gene ligation to vector pET30a. Complete Serrawettin W2 gene sequence amplicons proved to be extremely long, approximately 18,000 bp, and very complex to amplify.

Fifth, we extracted amino lipids produced by *Serratia* strains with Serrawettin genes and studied amino lipid-nematode interactions by way of two different tests.

A total of 25 amino lipids from *Serratia* strains with *srw1* and *srw2* genes were extracted and purified from supernatants (**Figure 21**).

Extracted amino lipids overall mortality rates against *C. elegans* were very low (**Figure 22**), and identical to control. These results suggest that these extracted amino lipids are unable to cause *C. elegans* mortality. Positive control (fresh supernatant of *Serratia* strain A88copa13), was also unable to have a significant nematicidal activity against *C. elegans*.

Mortality rates showed that *B. xylophilus* was more susceptible to these amino lipids than *C. elegans* (**Figure 23**). These results suggest that *B. xylophilus* may have more target proteins (**SHANMUGAM, G. et al., 2018**) susceptible to amino lipids than *C. elegans*.

Attraction tests with *C. elegans* revealed a poor attraction response to amino lipids (**Figures 24, 25 and 26**) and, overall, nematodes were more attracted to *Serratia* supernatants and food source *E. coli* OP50. As *C. elegans* were starved, these results suggest that amino lipids are not recognize as potential food source. These results are consistent with amino lipids absence in nutritional requirements of *C. elegans* (**ZEČIĆ, A. et al., 2019**).

As we intend to study Serrawettins, we discuss in detail results of amino lipids extracted from *Serratia* strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, NBRC 102599^T, AS13 A88copa7 and A88copa13.

Amino lipids 8D, 9C, 16C, 17C, 18F, 18G, 19D and 19E had a higher nematicidal activity against *B. xylophilus* than other extracted amino lipids from respective supernatants (**Table 18**).

Table 18 Integrated results of mortality tests against *B. xylophilus* after 48 h with selected amino lipids extracted from supernatants of *Serratia* strains with *srw1* and *srw2* genes.

Genes	Strain	Amino lipid	Peak	Retention time	Area %	Mortality
srw1	Arv-22-2.5c	**8D**	4	3.59	34.45	93%
srw1	Arv-22-2.6	**9C**	2	3.83	56.59	74%
srw1	NBRC 102599^T	**18F**	3	3.48	60.57	78%
srw1	NBRC 102599^T	**18G**	3	3.48	76.10	83%
srw1	AS13	**19D**	3	3.86	79.54	80%
srw1	AS13	**19E**	6	3.82	66.21	82%
srw2	A88copa7	**16C**	3	3.86	34.93	46%
srw2	A88copa13	**17C**	3	3.48	80.28	86%

Subsequent HPLC analysis of these amino lipids extracted from *Serratia* strains with *srw1* and with *srw2* genes showed similar retention times on higher peaks, from 3.48 – 3.86 min. These results suggest that amino lipids, other than Serrawettin W1 and W2, for example, other biosurfactants or siderophores, may also contribute to *Serratia* nematicidal activity (**PAIVA, G. et al., 2013**).

Amino lipid higher area percentage corresponded with highest mortality in *Serratia* strains with *srw2* gene. In *Serratia* strains with *srw1* gene this correspondence was not as evident, for example,

amino lipid 8D, from *Serratia* strain Arv-22-2.5c, had the lowest area percentage but the highest mortality rate.

Sixth, Serrawettin W1 and W2 gene clusters were analysed to better understand Serrawettins biosynthesis process.

Gene cluster analysis of *srw1* genes from 17 *Serratia* genomes (**Table 13**), confirmed 4 Serrawettin W1 biosynthesis protein domains, as previously described in **LI, H. et al., 2005**. From *srw1* gene clusters, a total of 80 different proteins were identified, 13 common to all gene clusters and 15 exclusive to *Serratia* strain NBRC 102599^{T} gene cluster (**Figure 28 and Annexes 6 and 7**). Proteins present in all 17 clusters may play an important role in Serrawettin W1 biosynthesis process.

Analysis of *srw2* gene clusters from 11 *Serratia* genomes (**Table 14**) demonstrated complex Serrawettin W2 biosynthesis protein domains: 5 Condensation domains, 5 Adenylation domains, 5 Thiolation domains and 1 Thioesterase domain, as previously described in **C. SU, et al. 2016**. From *srw2* gene clusters, a total of 68 different proteins were identified, 24 common to all clusters and 8 exclusive to *Serratia* sp. YD25 cluster (**Figure 29 and Annexes 11 and 12**). Proteins present in all 11 clusters may play an important role in Serrawettin W2 biosynthesis process.

No studies regarding *srw1* and *srw2* genes clusters were found in researched scientific databases, however **C. SU, et al. 2016** identified three proteins involved in PKS-NRPS hybrid polyketide synthase (acyltransferase, ketosynthase and ketoreductase) involved in Serrawettin W2 biosynthesis process. Our results of Serrawettin W2 gene clusters revealed proteins with similar functions to those already described: protein 24 probably corresponds to an acyltransferase and protein 38 to a ketoredutase. Serrawettin W1 gene clusters results also revealed proteins with similar functions: protein 57 is an acetyltransferase and protein 45 is a redutase. Four proteins were identified as part of a PKS-NRPS hybrid polyketide synthase in Serrawettin W1 cluster. This result suggests a similar gene organization between Serrawettin W1 and W2 cluster.

Proteins common to all *srw1* and *srw2* gene clusters are presented in **Table 19** and probably are very important in Serrawettins biosynthesis process.

Table 19 Common proteins to *srw1* and *srw2* gene clusters, with protein number reference.

***srw1* gene cluster**	***srw2* gene cluster**	**Protein**
35	28	Murein effector protein LrgB
36	29	Murein regulator LrgA
37	30	LysR family transcriptional regulator
38	31	Na+/H+ antiporter
39	32	Xanthine/uracil/vitamin C permease
40	33	Glyoxalase/bleomycin resistance protein/dioxygenase
41	34	Glutathione S-transferase domain protein
45	38	3-oxoacyl-(acyl-carrier-protein) reductase

A 3D structure of Serrawettin W1 biosynthesis protein from *Serratia* strain AS13, with 1,313 residues, was successfully predicted and 25 residues were identified as binding sites (**Table 15, Supplementary data 1**).

3D structures of all 5 modules of Serrawettin W2 biosynthesis protein from *Serratia* strain PWN146, with 5,929 residues, were successfully predicted and 165 residues were identified as binding sites (**Table 16, Supplementary data 2**). These high number of binding sites suggest that Serrawettin W2 has a very complex biosynthesis process.

No studies concerning 3D structures of Serrawettins biosynthesis proteins and binding sites were previously published.

Our results are congruent with previously proposed biosynthetic pathways (**LI, H. et al., 2005**; **C. SU, et al. 2016**).

These results contribute to better understand Serrawettins biosynthesis process, as they show that several additional, regulatory and transport proteins are involved and that many biosynthesis protein residues may actively participate in Serrawettins production.

Seventh, molecular dynamic simulations were performed to analyse protein-ligand interactions.

3D structures of Serrawettin W2 and TOL1 protein were successfully predicted.

Due to limited computational capability, molecular dynamic simulations duration was too brief to fully explore protein-ligand interactions between TOL1 protein and Serrawettins (**Supplementary data 5 and 7**, **Tables 31, 34, 36 and 38**).

Nevertheless, we determined that residues 75 to 77 in Serrawettin W1 and residues 99 to 101 in Serrawettin W2 are putative areas of protein-ligand interactions (**Figures 32 and 37**).

Conclusion

We confirmed that *Serratia* strains Arv-22-2.5c, Arv-22-2.6, Arv-29-3.9, NBRC 102599^{T} and AS13 have *srw1* gene and that *Serratia* strains A88copa7 and A88copa13 have *srw2* gene.
Through ANTISMASH software, we also identified another 15 *Serratia* strains with *srw1* and another 11 *Serratia* strains with *srw2* gene.
Selected *Serratia* strains concentrated supernatants showed, as expected, overall better nematicidal activity than supernatants. Therefore, concentrated supernatants of *Serratia* strains A88C3, A88C4, A88C6, Arv-29-3.11b, Arv-29-3.9, A88copa7 and Leaf50 were able to kill all *C. elegans* after 24 h. Selected *Serratia* strains supernatants had a better nematicidal activity against *B. xylophilus* than against *C. elegans*. Only supernatant from *Pseudomonas* strain M47Tronco1 was able to kill all *C. elegans* after 24 h. All concentrated supernatants were able to kill more than 57% of *B. xylophilus* after 72 h. Concentrated supernatants from *Serratia* strains M24T3, A25T1, A88C4, A88C6, Arv-20-4.2, Arv-22-2.5c and Arv-22-2.6 were able to kill all *B. xylophilus* after 72 h. Concentrated supernatants of *Serratia* strains with Serrawettins were able to kill more than 77% of *B. xylophilus* after 72 h. Concentrated supernatant of *Serratia* strains with both serralysin/serine protease bands and a Serrawettin gene demonstrated a higher nematicidal activity against *B. xylophilus*.
Serratia strains M24T3, A25T1, A88C3, A88copa7 and Leaf50 were able to attract more *C. elegans* than *E. coli* OP50. Supernatant from *Serratia* strains A25T1, A88C3, A88C4 and A88C6 were able to attract more *C. elegans* than *E. coli* OP50.
From the twenty-five amino lipids extracted and purified from supernatants of *Serratia* strains with *srw1* and *srw2* genes, amino lipids 8D, 9C, 16C, 17C, 18F, 18G, 19D and 19E were able to kill more than 46% of *B. xylophilus* in 48 h.
HPLC analysis revealed a common peak to these amino lipids, between 3.478 and 3.863 min of retention time that does not correspond to Serrawettin W1 or to W2. Further studies must be conducted to identify these amino lipids and to better understand their nematicidal activity.
Gene cluster analysis revealed 13 proteins, common to all 17 *srw1* gene clusters, and 24 proteins, common to all 11 *srw2* gene clusters, classified as additional, regulatory and transport proteins.
Eight proteins common to all *srw1* and *srw2* gene clusters were identified as Murein effector protein LrgB, Murein regulator LrgA, LysR family transcriptional regulator, Na+/H+ antiporter, Xanthine/uracil/vitamin C permease, Glyoxalase/bleomycin resistance protein/dioxygenase, Glutathione S-transferase domain protein and 3-oxoacyl-(acyl-carrier-protein) reductase.
Phyre2 3DLigandSite predicted 25 residues as binding sites of Serrawettin W1 biosynthesis protein and 165 residues as binding sites of Serrawettin W2 biosynthesis protein.
Molecular dynamic simulations selected residues 75-77 of Serrawettin W1 and residues 99-101 of Serrawettin W2 as probable candidates to protein-ligand interaction.
We conclude that some *Serratia* strains, their supernatants and specific amino lipids contribute to nematicidal activity against *B. xylophilus.* Serratomolides biosynthesis process and protein-ligand interactions between TOL1 and Serratomolides deserve to be entirely studied.

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Annexes

```
-- ==================================================================================
-- Author: Ana Catarina Marques
-- Creation date: 12/01/2019
-- Description: ANOVA2 and Tukey statistical tests for Mortality tests with R script.
-- Name: Mortality Tests - Statistics
-- ==================================================================================
        >data <- read_csv("tables/statistic/mortality.csv")

        >mortality.24 <- aov(data$t24 ~ data$test)
        >M24 = TukeyHSD(mortality.24)$`data$test`
        >matrix24 = matrix(NA, ncol = 2, nrow = NA)
        >matrix24 = M24[, 4]
        >write.csv(matrix24, file = "Mortality24.csv")

        >mortality.48 <- aov(data$t48 ~ data$test)
        >M48 = TukeyHSD(mortality.48)$`data$test`
        >matrix48 = matrix(NA, ncol = 2, nrow = NA)
        >matrix48 = M48[, 4]
        >write.csv(matrix48, file = "Mortality48.csv")

        >mortality.72 <- aov(data$t72 ~ data$test)
        >M72 = TukeyHSD(mortality.72)$`data$test`
        >matrix72 = matrix(NA, ncol = 2, nrow = NA)
        >matrix72 = M72[, 4]
        >write.csv(matrix72, file = "Mortality72.csv")
        END
```

Annex 1 R script for Statistical analysis of Mortality tests.

```
-- ==========================================================================================
-- Author: Ana Catarina Marques
-- Creating date: 09/01/2019
-- Description: Chi-Square statistical tests for Attraction and Avoidance tests with R script.
-- Name: Attraction and Avoidance Tests - Statistics
-- ==========================================================================================

        >library(dplyr)
        >data <- read_csv("tables/statistic/attraction.csv")
        >l = list(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25)

        >chi_square = matrix(NA, ncol = 4, nrow = 11)
        >for(i in l){
                hour2 = data[i,] %>%
                select(t2,c2)
                chi2 = chisq.test(hour2)
                p_value2 = chi2[['p.value']]
                chi_square[i,1] = chi2[['statistic']]
                chi_square[i,2] = p_value2
        }

        >for (i in l){
                hour24 = data[i,] %>%
                select(t24,c24)
                chi24 = chisq.test(hour24)
                p_value24 = c24[['p.value']]
                valores_chi[i,3] = chi24[['statistic']]
                valores_chi[i,4] = p_value24
        }
        END
```

Annex 2 R script for Statistical analysis of Attraction and Avoidance tests.

```
-- ==========================================================================================
-- Author: GROMACS comands modificated by Ana Catarina Marques
-- Creating date: 20/05/2019
-- Description: Protein in water simulation with GROMACS software
-- Name: Serrawettin W1 biosythesis protein in water
-- ==========================================================================================

        >pdb2gmx -f AS13.pdb -o AS13.gro -water spc216
        >15
        >editconf -f AS13.gro -o AS13_box.gro -c -d 1.0 -bt cubic
        >solvate -cp AS13_box.gro -cs scp216.gro -o AS13_solv.gro -p topol.top
        >grompp -f ions.mdp -c AS13_solv.gro -p topol.top -o ions.tpr
        >genion -s ions.tpr -o AS13_solv_ions.gro -p topol.top -pname CL -nname CL -neural
        >grompp -f em.mdp -c AS13_solv_ions.gro -p topol.top -o em.tpr
        >mdrun -v -deffnm em
        >grompp -f nvt.mdp -c em.gro -r em.cpt -p topol.top -o nvt.tpr
        >mdrun -v -deffnm nvt
        >grompp -f npt.mdp -c nvt.gro -r nvt.cpt -p topol.top -o npt.tpr
        >mdrun -v -deffnm npt
        >grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -o md.tpr
        >mdrun -v -deffnm md

        END
```

Annex 3 Simulation commands of Serrawettin W1 biosythesis protein in water.

```
-- ==========================================================================================
-- Author: GROMACS commands modified by Ana Catarina Marques
-- Creating date: 20/05/2019
-- Description: Protein ligand simulations with GROMACS software
-- Name: TOLI PROTEIN WITH LIGANDS IN WATER
-- ==========================================================================================

        >pdb2gmx -f TOLI.pdb -o TOLI.gro
        >1
        >1
        >perl sort_mol2_bonds.pl SRW.mol2 SRW_fix.mol2
        >python3 cgenff-charm2gmx_py3.py SRW SRW_fix.mol2 SRW.str charmm36-mar2019.ff
        >editconf -f SRW_ini.pdb -o SRW.gro
        >editconf -f complex.gro -o box.gro -bt dodecahedron -d 1.0
        >solvate -cp box.gro -cs scp216.gro -o solv.gro -p topol.top
        >grompp -f ions.mdp -c solv.gro -p topol.top -o ions.tpr
        >genion -s ions.tpr -o solv_ions.gro -p topol.top -pname CL -nname CL -neural
        >grompp -f em.mdp -c solv_ions.gro -p topol.top -o em.tpr
        >mdrun -v -deffnm em
        >make_ndx -f SRW.gro -o index_SRW.ndx
        >0 & ! a H*
        >q
        >genrestr -f SRW.gro -n index_SRW.ndx -o posre_SRW.itp -fc 1000 1000 1000
        >make_ndx -f em.gro -o index.ndx
        >1|13
        >q
        >grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -n index.ndx -o nvt.tpr
        >mdrun -v -deffnm nvt
        >grompp -f npt.mdp -c nvt.gro -t nvt.cpt -r nvt.gro -p topol.top -n index.ndx -o npt.tpr
        >mdrun -v -deffnm npt
        >grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -n index.ndx -o md.tpr
        >mdrun -v -deffnm md

        END
```

Annex 4 Simulation commands of TOL1 protein with ligands in water.

```
-- ==========================================================================================
-- Author: GROMACS commands modified by Ana Catarina Marques
-- Creating date: 23/05/2019
-- Description: Molecular dynamic simulations analysis
-- Name: TOLI PROTEIN WITH LIGANDS IN WATER
-- ==========================================================================================
        >trjconv -s md.tpr -f md.xtc -o md_center.xtc -center -pbc mol -ur compact
        >trjconv -s md.tpr -f md_center.xtc -o start.pdb -dump 0
        >trjconv -s md.tpr -f md_center.xtc -o md_fit.xtc -fit rot+trans

        RMSD analysis:
        >g_rms -s md.tpr -f md_fit.xtc -n index.ndx -o rmsd.xvg

        RMSF analysis:
        >g_rmsf -s md.tpr -f md_fit.xtc -n index.ndx -o rmsf.xvg

        Distance analysis:
        >g_dist -s md.tpr -f md_fit.xtc -n index.ndx -o distance.xvg
```

Annex 5 Molecular dynamic simulations analysis

	S. marcescens ATCC 13880	*S. marcescens* CDC_813-60 DP21	*S. marcescens* strain UMH8	*S. marcescens* IOMTU 115	*S. nematodiphila* DZ0503SBS1 DSM 21420	*S. marcescens* VGH107	*S. marcescens* EGD-HP20	*S. marcescens* WW4	S. strain FS14
1									
2									
3									
4									
5									
6									
7									
8									
9									
10		KFL04448.1	ASM18649.1	BAO21119.1	KFF87821.1	EMF04461.1	ERH70676.1	AGE20163.1	
11	KFD15002.1	KFL04416.1	ASM18650.1	BAO21120.1	KFF87820.1	EMF04460.1	ERH70677.1	AGE20164.1	
12	KFD15001.1	KFL02178.1	ASM18651.1	BAO21121.1	KFF87819.1	EMF04459.1	ERH70678.1	AGE20165.1	AIA46718.1
13	KFD15000.1	KFL01211.1	ASM18652.1	BAO21122.1	KFF87818.1	EMF04458.1	ERH70679.1	AGE20166.1	AIA46717.1
14									
15	KFD14999.1	KFL02951.1	ASM18653.1	BAO21123.1	KFF87817.1	EMF04457.1	ERH70680.1	AGE20167.1	AIA46716.1
16					KFF87816.1				
17	KFD14998.1	KFL04871.1	ASM18654.1	BAO21124.1	KFF87815.1	EMF04456.1	ERH70681.1	AGE20168.1	AIA46715.1
18	KFD14997.1	KFL03528.1	ASM18655.1	BAO21125.1	KFF87814.1	EMF04455.1	ERH70682.1	AGE20169.1	AIA46714.1
19	KFD14996.1	KFL03461.1	ASM18656.1	BAO21126.1	KFF87813.1	EMF04454.1	ERH70683.1	AGE20170.1	AIA46713.1
20	KFD14995.1	KFL05217.1	ASM18657.1	BAO21127.1	KFF87812.1	EMF04453.1	ERH70684.1	AGE20171.1	AIA46712.1
21	KFD14994.1	KFL03855.1	ASM18658.1	BAO21128.1	KFF87811.1	EMF04452.1	ERH70685.1	AGE20172.1	AIA46711.1
22	KFD14993.1	KFL03341.1	ASM18659.1	BAO21129.1	KFF87810.1	EMF04451.1	ERH70686.1	AGE20173.1	AIA46710.1
23	KFD14992.1	KFL04748.1	ASM18660.1	BAO21130.1	KFF87809.1	EMF04450.1	ERH70687.1	AGE20174.1	AIA46709.1
24						EMF04449.1			
25									
26	KFD14991.1	KFL04562.1	ASM19892.1	BAO21131.1			ERH70688.1	AGE20175.1	AIA46708.1
27	KFD14990.1	KFL01955.1	ASM18661.1	BAO21132.1	KFF87808.1	EMF04448.1	ERH70689.1	AGE20176.1	AIA46707.1
28	KFD14989.1								
29							ERH70690.1		AIA46706.1
30	KFD14988.1	KFL03718.1	ASM18662.1	BAO21133.1	KFF87807.1	EMF04447.1	ERH70691.1	AGE20177.1	AIA46705.1
31	KFD14987.1	KFL02732.1	ASM18663.1	BAO21135.1	KFF87806.1	EMF04446.1	ERH70692.1	AGE20178.1	AIA46704.1
32	KFD14986.1	KFL05425.1	ASM18664.1	BAO21136.1	KFF87805.1	EMF04445.1	ERH70693.1	AGE20179.1	AIA46703.1
33	KFD14985.1	KFL04184.1	ASM19893.1	BAO21137.1	KFF87804.1	EMF04444.1	ERH70694.1	AGE20180.1	AIA46702.1
34	KFD14984.1	KFL05097.1	ASM18665.1	BAO21138.1	KFF87803.1	EMF04443.1	ERH70695.1	AGE20181.1	AIA46701.1
35	KFD14983.1	KFL03693.1	ASM18666.1	BAO21139.1	KFF87802.1	EMF04442.1	ERH70696.1	AGE20182.1	AIA46700.1
36	KFD14982.1	KFL01258.1	ASM18667.1	BAO21140.1	KFF87801.1	EMF04441.1	ERH70697.1	AGE20183.1	AIA46699.1
37	KFD14981.1	KFL02555.1	ASM18668.1	BAO21141.1	KFF87800.1	EMF04440.1	ERH70698.1	AGE20184.1	AIA46698.1
38	KFD14980.1	KFL05361.1	ASM18669.1	BAO21142.1	KFF87799.1	EMF04439.1	ERH70699.1	AGE20185.1	AIA46697.1
39	KFD14979.1	KFL04460.1	ASM18670.1	BAO21143.1	KFF87798.1	EMF04438.1	ERH70700.1	AGE20186.1	AIA46696.1
40	KFD14978.1	KFL02686.1	ASM18671.1	BAO21144.1	KFF87797.1	EMF04437.1	ERH70701.1	AGE20187.1	AIA46695.1
41	KFD14977.1	KFL05295.1	ASM18672.1	BAO21145.1	KFF87796.1	EMF04436.1	ERH70702.1	AGE20188.1	AIA46694.1
42	KFD14976.1	KFL01470.1	ASM18673.1	BAO21146.1	KFF87795.1	EMF04435.1	ERH70703.1	AGE20189.1	AIA46693.1
43	KFD14975.1	KFL01261.1	ASM18674.1	BAO21147.1	KFF87794.1	EMF04434.1	ERH70704.1	AGE20190.1	AIA46692.1
44					KFF87793.1	EMF04433.1	ERH70705.1	AGE20191.1	AIA46691.1
45	KFD14974.1	KFL04091.1	ASM18675.1	BAO21148.1	KFF87792.1	EMF04432.1	ERH70706.1	AGE20192.1	AIA46690.1
46									
47	KFD14973.1	KFL05541.1	ASM18676.1	BAO21149.1	KFF87791.1	EMF04431.1	ERH70707.1	AGE20193.1	AIA46689.1
48									
49	KFD14972.1	KFL01340.1	ASM18677.1	BAO21150.1	KFF87790.1	EMF04430.1	ERH70708.1	AGE20194.1	AIA46688.1
50									
51	KFD14971.1	KFL02338.1	ASM18678.1	BAO21151.1	KFF87789.1	EMF04429.1	ERH70488.1	AGE20195.1	AIA46687.1
52	KFD14970.1	KFL02532.1	ASM18679.1	BAO21152.1	KFF87788.1	EMF04428.1	ERH70709.1	AGE20196.1	AIA46686.1
53									
54									
55	KFD14969.1	KFL03204.1	ASM18680.1	BAO21153.1	KFF87787.1	EMF04427.1	ERH70710.1	AGE20197.1	AIA46685.1
56									
57	KFD14968.1	KFL03297.1	ASM18681.1	BAO21154.1	KFF87786.1	EMF04426.1	ERH70711.1	AGE20198.1	AIA46684.1
58	KFD14967.1	KFL01191.1	ASM18682.1		KFF87785.1	EMF04425.1	ERH70712.1	AGE20199.1	AIA46683.1
59	KFD14966.1	KFL03048.1	ASM18683.1		KFF87784.1	EMF04424.1	ERH70713.1	AGE20200.1	AIA46682.1
60		KFL04717.1	ASM18684.1	BAO21155.1	KFF87783.1	EMF04423.1	ERH70714.1		AIA46681.1
61								AGE20201.1	
62									
63	KFD14964.1	KFL04093.1	ASM18685.1	BAO21156.1	KFF87782.1	EMF04422.1	ERH70715.1	AGE20202.1	AIA46680.1
64	KFD14963.1	KFL02836.1	ASM18686.1	BAO21157.1					

Annex 6 Accession numbers of identified proteins of Serrawettin W1 clusters of *Serratia* strains ATCC 13880, CDC_813-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EGD-HP20, WW4 and FS14 by AntiSMASH and NCBI BlastP softwares. Pink - Additional proteins, Green - Regulatory proteins, Blue - Transport proteins, Yellow - Core protein, White - other proteins.

	S. marcescens BIDMC 81	*S.* strain TEL NODE_13	*S. grimesii* isolate BXF1	*S. grimesii* strain A2	*S.* strain AS13	*S. plymuthica* AS9	*S.* strain AS12		*S. plymuthica* NBRC 102599^T
1			SMZ58692.1						
2	EZQ62943.1	KLE36465.1	SMZ58693.1	KFB89940.1	AEG30265.1	AEF47606.1	WP_013814709.1		
3	EZQ62942.1	KLE36466.1	SMZ58694.1	KFB89939.1	AEG30266.1	AEF47607.1	WP_004948651.1		
4	EZQ62941.1	KLE36467.1	SMZ58695.1	KFB89938.1	AEG30267.1	AEF47608.1	WP_013814710.1		
5	EZQ62940.1	KLE36468.1	SMZ58696.1	KFB89937.1	AEG30268.1	AEF47609.1	WP_004948655.1		
6	EZQ62939.1	KLE36469.1	SMZ58697.1						
7									
8					AEG30269.1	AEF47610.1	WP_013814711.1		
9	EZQ62938.1	KLE36470.1	SMZ58698.1	KFB89936.1	AEG30270.1	AEF47611.1	WP_013814712.1		
10	EZQ62937.1	KLE36471.1	SMZ58699.1	KFB89935.1	AEG30271.1	AEF47612.1	WP_013814713.1		
11	EZQ62936.1	KLE36472.1	SMZ58700.1	KFB89934.1	AEG30272.1	AEF47613.1	WP_013814714.1		
12	EZQ62935.1	KLE36473.1	SMZ58701.1	KFB89933.1	AEG30273.1	AEF47614.1	WP_004948665.1		
13	EZQ62934.1	KLE36474.1	SMZ58702.1		AEG30274.1	AEF47615.1	WP_013814715.1		
14				KFB89932.1					
15	EZQ62933.1	KLE36475.1	SMZ58703.1	KFB89931.1	AEG30275.1	AEF47616.1	WP_013814716.1		
16								65	SQI30623.1
17								66	SQI30627.1
18								67	SQI30640.1
19								68	SQI30643.1
20								69	SQI30646.1
21	EZQ62932.1	KLE36476.1	SMZ58704.1	KFB89930.1	AEG30276.1	AEF47617.1	WP_013814717.1	70	SQI30648.1
22	EZQ62931.1	KLE36477.1	SMZ58705.1	KFB89929.1	AEG30277.1	AEF47618.1	WP_013814718.1	71	SQI30650.1
23	EZQ62930.1	KLE36478.1	SMZ58706.1	KFB89928.1	AEG30278.1	AEF47619.1	WP_013814719.1	72	SQI30652.1
24		KLE36479.1						73	SQI30658.1
25					AEG30279.1	AEF47620.1	WP_013814720.1	74	SQI30662.1
26	EZQ62929.1		SMZ58707.1	KFB89927.1	AEG30280.1	AEF47621.1	WP_004948691.1	75	SQI30664.1
27	EZQ62928.1	KLE36480.1	SMZ58708.1	KFB89926.1	AEG30281.1	AEF47622.1	WP_000462905.1	76	SQI45910.1
28								77	SQI45911.1
29								78	WP_063202315.1
30	EZQ62927.1	KLE36481.1						79	SQI45912.1
31	EZQ62926.1	KLE36482.1						80	SQI45913.1
32	EZQ62925.1	KLE36483.1	SMZ58709.1	KFB89925.1	AEG30282.1	AEF47623.1	WP_004948702.1	32	WP_063202311.1
33	EZQ62924.1	KLE36558.1	SMZ58710.1	KFB89924.1	AEG30283.1	AEF47624.1	WP_013814721.1	33	WP_063202309.1
34	EZQ62923.1	KLE36484.1	SMZ58711.1	KFB89923.1	AEG30284.1	AEF47625.1	WP_013814722.1	34	WP_063202307.1
35	EZQ62922.1	KLE36485.1	SMZ58712.1	KFB89922.1	AEG30285.1	AEF47626.1	WP_013814723.1	35	WP_064799207.1
36	EZQ62921.1	KLE36486.1	SMZ58713.1	KFB89921.1	AEG30286.1	AEF47627.1	WP_013814724.1	36	WP_063202305.1
37	EZQ62920.1	KLE36487.1	SMZ58714.1	KFB89920.1	AEG30287.1	AEF47628.1	WP_013814725.1	37	WP_006328328.1
38	EZQ62919.1	KLE36488.1	SMZ58715.1	KFB89919.1	AEG30288.1	AEF47629.1	WP_013814726.1	38	WP_063202303.1
39	EZQ62918.1	KLE36489.1	SMZ58716.1	KFB89918.1	AEG30289.1	AEF47630.1	WP_013814727.1	39	WP_020439894.1
40	EZQ62917.1	KLE36490.1	SMZ58717.1	KFB89917.1	AEG30290.1	AEF47631.1	WP_013814728.1	40	WP_063202301.1
41	EZQ62916.1	KLE36491.1	SMZ58718.1	KFB89916.1	AEG30291.1	AEF47632.1	WP_013814729.1	41	WP_004948715.1
42	EZQ62915.1	KLE36492.1							
43	EZQ62914.1	KLE36493.1	SMZ58719.1	KFB89915.1	AEG30292.1	AEF47633.1	WP_013814730.1	43	WP_062868861.1
44			SMZ58720.1	KFB89914.1	AEG30293.1	AEF47634.1	WP_013814731.1	44	WP_063202299.1
45	EZQ62913.1	KLE36494.1	SMZ58721.1	KFB89913.1	AEG30294.1	AEF47635.1	WP_013814732.1	45	WP_063202297.1
46			SMZ58722.1	KFB89912.1					
47	EZQ62912.1	KLE36495.1							
48			SMZ58723.1	KFB89911.1					
49	EZQ62911.1	KLE36496.1							
50			SMZ58724.1	KFB89910.1					
51	EZQ62910.1	KLE36497.1	SMZ58725.1	KFB89909.1	AEG30295.1	AEF47636.1	WP_006317814.1	51	WP_006317814.1
52	EZQ62909.1	KLE36498.1	SMZ58726.1	KFB89908.1	AEG30296.1	AEF47637.1	WP_013814733.1	52	WP_063202295.1
53	EZQ62908.1	KLE36559.1							
54	EZQ62907.1	KLE36499.1							
55			SMZ58727.1		AEG30297.1	AEF47638.1	WP_013814734.1	55	WP_062868864.1
56				KFB89936.1				56	
57	EZQ62906.1	KLE36500.1	SMZ58728.1	KFB89907.1	AEG30298.1	AEF47639.1	WP_013814735.1	57	WP_063202322.1
58	EZQ62905.1	KLE36501.1	SMZ58729.1	KFB89906.1	AEG30299.1	AEF47640.1	WP_004948738.1	58	WP_126528840.1
59	EZQ62904.1	KLE36502.1	SMZ58730.1	KFB89905.1	AEG30300.1	AEF47641.1	WP_004948741.1	59	WP_062868866.1
60	EZQ62903.1	KLE36503.1	SMZ58731.1	KFB89904.1	AEG30301.1	AEF47642.1	WP_013814736.1	60	WP_006328339.1
61									
62					AEG30302.1	AEF47643.1	WP_006317804.1	62	WP_006317804.1
63	EZQ62902.1	KLE36504.1			AEG30303.1	AEF47644.1	WP_013814737.1	63	WP_062868868.1
64									

Annex 7 Accession numbers of identified proteins of Serrawettin W1 clusters of *Serratia* strains BIDMC81, TEL NODE_13, NBRC 102599, BXF1, A2, AS13, AS9 and AS12 by AntiSMASH and NCBI BlastP softwares. Pink - Additional proteins, Green - Regulatory proteins, Blue - Transport proteins, Yellow - Core protein, White - other proteins.

34 - **Core biosynthesis Serrawettin W1, responsible for Serrawettin W1 lipopeptide biosynthesis.**
35 - Murein effector protein LrgB, with hydrolase activity.
36 - Murein regulator LrgA, with hydrolase activity.
37 - LysR family transcriptional regulator with a DNA binding and a transcription factor activity capable of a specific interaction with DNA.
38 - Na+/H+ antiporter, enables the transport of sodium ion across the membrane.
39 - Xanthine/uracil/vitamin C permease, with a transmembrane transport activity.
40 - Glyoxalase/bleomycin resistance protein/dioxygenase, with dioxygenase activity.
41 - Glutathione S-transferase domain protein, with dioxygenase activity.
45 - 3-oxoacyl-(acyl-carrier-protein) reductase, responsible for 3-oxoacyl-[acyl-carrier-protein], 3-oxo-glutaryl-[acp] methyl ester and 3-oxo-pimeloyl-[acp] methyl ester reductase activity and NAD binding.
51 - Single-stranded DNA-binding protein, responsible for DNA replication, recombination and repair.
52 - Excinuclease ABC subunit A, a damage recognition complex that recognises and processes DNA lesions.
57 - Maltose O-acetyltransferase catalyses the reaction acetyl-CoA + maltose = CoA + acetyl-maltose.
60 - Aromatic amino acid aminotransferase with L-phenylalanine: 2-oxoglutarate aminotransferase activity and selectively interacts with vitamin B6.

Annex 8 Proteins common to all seventeen clusters with Serrawettin W1 biosynthesis protein.

65 - Regulator of ribonuclease activity B.
66 - tRNA-(MS[2]IO[6]A)-hydroxylase (MiaE).
67 - Alcohol dehydrogenase
68 - Uncharacterized protein conserved in bacteria.
69 - Predicted acetyltransferase.
70 - Valine-tRNA ligase.
71 - DNA polymerase III subunit chi.
72 - Cytosol aminopeptidase.
73 - Lipopolysaccharide export system permease protein lptF.
74 - Lipopolysaccharide export system permease protein lptG.
75 - Putative prophage CPS-53 integrase.
76 - Uncharacterised protein.
77 - Uncharacterised protein.
78 - 99 bp at 3' side: Protein involved in cell division.
79 - Protein involved in cell division.
80 - Uncharacterised protein respectively.

Annex 9 Proteins exclusive to cluster of *S. plymuthica* NBRC 102599^{T}.

21 - inner membrane protein.
22 - sodium/pantothenate symporter.
23 - ribosomal protein L11 methyltransferase.
27 - DNA-binding transcriptional regulator Fis.
32 - Lipid A biosynthesis lauroyl acyltransferase.
33 - CTP synthase.
43 - Multiple stress resistance protein BhsA precursor.

Annex 10 Proteins present in every Serrawettin W1 clusters except in *S. plymuthica* NBRC 102599^{T} cluster.

	S. marcescens PWN146	*S.* sp. SSNIH1	*S. marcescens* SM39	*S. marcescens* SmUNAM836	*S. marcescens* BIDMC 44	*S. ureilytica* Lr5/4 LG59
1	SAY45228.1		BAO35805.1	ALE98092.1	ETX44741.1	KKO56054.1
2	SAY45229.1		BAO35806.1	ALE98093.1	ETX44742.1	KKO56543.1
3	SAY45230.1		BAO35807.1	ALE98094.1	ETX44743.1	KKO58503.1
4	SAY45231.1	AUY16856.1	BAO35808.1	ALE98095.1	ETX44744.1	KKO58608.1
5						
6						
7	SAY45232.1	AUY16857.1	BAO35809.1	ALE98096.1	ETX44745.1	KKO58450.1
8	SAY45233.1	AUY16858.1	BAO35810.1	ALE98097.1	ETX44746.1	KKO55998.1
9	SAY45234.1	AUY16859.1	BAO35811.1	ALE98098.1	ETX44747.1	KKO58722.1
10	SAY45235.1	AUY16860.1	BAO35812.1	ALE98099.1	ETX44748.1	KKO55898.1
11	SAY45236.1	AUY16861.1	BAO35813.1	ALE98100.1	ETX44749.1	KKO60356.1
12	SAY45237.1	AUY16862.1	BAO35814.1	ALE98101.1	ETX44750.1	KKO57730.1
13	SAY45238.1	AUY16863.1	BAO35815.1	ALE98102.1	ETX44751.1	KKO58288.1
14	SAY45239.1	AUY16864.1	BAO35816.1	ALE98103.1	ETX44752.1	KKO58582.1
15	SAY45240.1	AUY16865.1	BAO35817.1	ALE98104.1	ETX44753.1	KKO58632.1
16		AUY16866.1	BAO35818.1		ETX44754.1	KKO55853.1
17		AUY17174.1	BAO35819.1			
18	SAY45241.1			ALE98105.1	ETX44755.1	KKO57611.1
19	SAY45242.1	AUY16867.1	BAO35820.1	ALE98106.1	ETX44756.1	KKO58924.1
20		AUY16868.1				
21						
22	SAY45243.1	AUY16869.1	BAO35821.1	ALE98107.1	ETX44757.1	KKO58181.1
23	SAY45244.1	AUY16870.1	BAO35822.1	ALE98108.1	ETX44758.1	KKO57272.1
24	SAY45245.1	AUY16871.1	BAO35823.1	ALE98109.1	ETX44759.1	KKO57011.1
25	SAY45246.1	AUY17175.1	BAO35824.1	ALE98110.1	ETX44760.1	KKO58031.1
26		AUY16872.1				
27	SAY45247.1	AUY16873.1	BAO35825.1	ALE98111.1	ETX44761.1	KKO55915.1
28	SAY45248.1	AUY16874.1	BAO35826.1	ALE98112.1	ETX44762.1	KKO57005.1
29	SAY45249.1	AUY16875.1	BAO35827.1	ALE98113.1	ETX44763.1	KKO55736.1
30	SAY45250.1	AUY16876.1	BAO35828.1	ALE98114.1	ETX44764.1	KKO55858.1
31	SAY45251.1	AUY16877.1	BAO35829.1	ALE98115.1	ETX44765.1	KKO55913.1
32	SAY45252.1	AUY16878.1	BAO35830.1	ALE98116.1	ETX44766.1	KKO58939.1
33	SAY45253.1	AUY16879.1	BAO35831.1	ALE98117.1	ETX44767.1	KKO57238.1
34	SAY45254.1	AUY16880.1	BAO35832.1	ALE98118.1	ETX44768.1	KKO57218.1
35	SAY45255.1	AUY16881.1	BAO35833.1	ALE98119.1	ETX44769.1	KKO57955.1
36	SAY45256.1	AUY16882.1	BAO35834.1	ALE98120.1	ETX44770.1	KKO56111.1
37						
38	SAY45257.1	AUY16883.1	BAO35835.1	ALE98121.1	ETX44771.1	KKO58381.1
39						
40						
41						KKO56541.1
42	SAY45258.1					
43						KKO56600.1
44	SAY45259.1					
45	SAY45260.1					
46						
47	SAY45261.1	AUY16884.1	BAO35836.1	ALE98122.1	ETX44772.1	KKO56864.1
48	SAY45262.1	AUY16885.1	BAO35837.1	ALE98123.1	ETX44773.1	KKO57935.1
49						KKO56767.1
50						KKO59604.1
51	SAY45263.1	AUY16886.1	BAO35838.1	ALE98124.1	ETX44774.1	
52	SAY45264.1	AUY16887.1	BAO35839.1	ALE98125.1	ETX44775.1	KKO57029.1
53	SAY45265.1	AUY16888.1	BAO35840.1	ALE98126.1	ETX44776.1	KKO56861.1
54	SAY45266.1	AUY16889.1	BAO35841.1	ALE98127.1	ETX44777.1	KKO55965.1
55	SAY45267.1		BAO35842.1	ALE98128.1	ETX44778.1	KKO57271.1
56		AUY16890.1				
57	SAY45268.1	AUY16891.1	BAO35843.1	ALE98129.1	ETX44779.1	
58		AUY16892.1	BAO35844.1	ALE98130.1	ETX44780.1	
59		AUY16893.1	BAO35845.1	ALE98131.1	ETX44781.1	

Annex 11 Accession numbers of identified proteins of Serrawettin W2 clusters of *Serratia* strains PWN146, SSNIH1, SM39, SmUNAM836, BIDMC44 and Lr5/4 LG59 by AntiSMASH and NCBI BlastP softwares. Pink - Additional proteins, Green - Regulatory proteins, Blue - Transport proteins, Yellow -Core protein, White - other proteins.

	S. marcescens RSC-14	*S. marcescens* subsp. *marcescens* AH0650_Sm1 AG2	*S. marcescens* subsp. *marcescens* Db11	*S.* sp. SCBI		*S.* sp. YD25
1	ALD45128.1	KMU50681.1	CDG14223.1	AIM23781.1	1	AOF01114.1
2	ALD45127.1	KMU50682.1	CDG14224.1	AIM23782.1	2	AOF01115.1
3	ALD45126.1	KMU50683.1	CDG14225.1	AIM23783.1	3	AOF01116.1
4	ALD45125.1	KMU50684.1	CDG14226.1		4	AOF01117.1
5				AIM23784.1		
6				AIM23785.1		
7	ALD45124.1	KMU50685.1	CDG14227.1	AIM23786.1	7	AOF01118.1
8	ALD45123.1	KMU50686.1	CDG14228.1	AIM23787.1	8	AOF01119.1
9	ALD45122.1	KMU50687.1	CDG14229.1	AIM23788.1	9	AOF01120.1
10	ALD45121.1	KMU50688.1	CDG14230.1	AIM23789.1	10	AOF01121.1
11	ALD45120.1	KMU50689.1	CDG14231.1	AIM23790.1	11	AOF01122.1
12	ALD45119.1	KMU50690.1	CDG14232.1	AIM23791.1	12	AOF01123.1
13	ALD45118.1	KMU50691.1	CDG14233.1	AIM23792.1	13	AOF01124.1
14	ALD45117.1	KMU50692.1	CDG14234.1	AIM23793.1	14	AOF01125.1
15	ALD45116.1	KMU50693.1	CDG14235.1	AIM23794.1	15	AOF01126.1
16	ALD45115.1		CDG14236.1	AIM23795.1	16	AOF01127.1
17	ALD45114.1		CDG14237.1		17	AOF01128.1
18		KMU50694.1		AIM23796.1	18	
19	ALD45113.1	KMU50695.1	CDG14238.1	AIM23797.1	19	AOF01129.1
20						
21		KMU50696.1	CDG14239.1			
22	ALD45112.1	KMU50697.1	CDG14240.1	AIM23798.1	22	AOF01130.1
23	ALD45111.1	KMU50698.1	CDG14241.1		23	AOF01131.1
24	ALD45110.1	KMU50699.1	CDG14242.1	AIM23799.1	24	AOF01132.1
25	ALD47357.1	KMU50700.1	CDG14243.1	AIM23800.1	25	AOF02337.1
26					26	
27	ALD45109.1	KMU50701.1	CDG14244.1	AIM23801.1	27	AOF02338.1
28	ALD45108.1	KMU50702.1	CDG14245.1	AIM23802.1	28	AOF01133.1
29	ALD45107.1	KMU50703.1	CDG14246.1	AIM23803.1	29	AOF01134.1
30	ALD45106.1	KMU50704.1	CDG14247.1	AIM23804.1	30	AOF01135.1
31	ALD45105.1	KMU50705.1	CDG14248.1	AIM23805.1	31	AOF01136.1
32	ALD45104.1	KMU50706.1	CDG14249.1	AIM23806.1	32	AOF01137.1
33	ALD45103.1	KMU50707.1	CDG14250.1	AIM23807.1	33	AOF01138.1
34	ALD45102.1	KMU50708.1	CDG14251.1	AIM23808.1	34	AOF01139.1
35	ALD45101.1	KMU50709.1	CDG14252.1	AIM23809.1	35	AOF01140.1
36	ALD45100.1	KMU50710.1	CDG14253.1	AIM23810.1	36	AOF01141.1
37					37	AOF01142.1
38	ALD45099.1	KMU50711.1	CDG14254.1	AIM23811.1	38	AOF01143.1
39					39	AOF01144.1
40					40	AOF01145.1
41	ALD45098.1	KMU50712.1	CDG14255.1	AIM23812.1	60	AOF01146.1
42				AIM23813.1	61	AOF01147.1
43	ALD45097.1	KMU50713.1	CDG14256.1		62	AOF01148.1
44					63	AOF01149.1
45					64	AOF01150.1
46					65	AOF01151.1
47	ALD45096.1	KMU50714.1	CDG14257.1	AIM23814.1	66	AOF01152.1
48	ALD45095.1	KMU50715.1	CDG14258.1	AIM23815.1	67	AOF01153.1
49	ALD47356.1			AIM23816.1	68	AOF01154.1
50	ALD45094.1			AIM23817.1		
51		KMU50716.1	CDG14259.1			
52	ALD47355.1	KMU50717.1	CDG14260.1	AIM23818.1		
53	ALD45093.1	KMU50718.1	CDG14261.1	AIM23819.1		
54	ALD45092.1	KMU50719.1	CDG14262.1	AIM23820.1		
55	ALD45091.1	KMU50720.1	CDG14263.1	AIM23821.1		
56						
57		KMU50721.1	CDG14264.1			
58						
59						

Annex 12 Accession numbers of identified proteins of Serrawettin W2 clusters of *Serratia* strains RSC-14, AH0650_Sm1 AG2, Db11, SCBI and YD25 by AntiSMASH and NCBI BlastP softwares. Pink - Additional proteins, Green - Regulatory proteins, Blue - Transport proteins, Yellow - Core protein, White- other proteins.

7 - RNase E specificity factor CsrD, with L-phenylalanine: 2-oxoglutarate aminotransferase activity and selectively interacts with vitamin B6.
8 - Putative acrylyl-CoA reductase.
9 - Sulfoxide reductase catalytic subunit YedY precursor.
10 - Sulfoxide reductase heme-binding subunit YedZ, both part of the MsrPQ system capable of repairing oxidized periplasmic proteins.
11 - 3-dehydroquinate dehydratase, involved on aromatic amino acids biosynthesis pathway.
12 - Biotin carboxyl carrier protein and
13 - Biotin carboxylase subunit, are components of the acetyl-CoA carboxylase complex.
14 - Putative membrane protein.
15 - Sodium/pantothenate symporter, with a transport activity enables the pantothenate movement across the membrane.
19 - DNA-binding protein Fis putative membrane protein, with a DNA binding and a transcription factor activity capable of a specific interaction with DNA.
22 - Putative exported protein, providing cell adhesion.
24 - Lipid A biosynthesis lauroyl (or palmitoleoyl) acyltransferase family protein, with catalytic activity.
25 - Glutamine amidotransferase class-I family protein, selectively interacts with a carbohydrate derivative.
27 - Non-ribosomal peptide synthetase, responsible for Serrawettin W2 lipopeptide biosynthesis.
28 - Murein effector protein LrgB and
29 - Murein regulator LrgA, both with a hydrolase activity.
30 - LysR-family transcriptional regulator, capable of carbonate dehydratase activity, DNA binding and a transcription factor activity.
31 - Sodium, potassium, lithium and rubidium/H+ antiporter and
32 - xanthine/uracil/vitamin C permease, both with transmembrane transporter activity.
33 - Glyoxalase/bleomycin resistance protein, with dioxygenase activity.
34 - Glutathione S-transferase domain-containing protein, with transferase activity.
35 - Lipocalin-like domain protein.
36 - Multiple stress resistance protein BhsA precursor.
38 - 3-oxoacyl-[acyl-carrier-protein] reductase responsible for 3-oxoacyl-[acyl-carrier-protein], 3-oxo-glutaryl-[acp] methyl ester and 3-oxo-pimeloyl-[acp] methyl ester reductase activity and NAD binding.

Annex 13 Proteins common to all eleven clusters with Serrawettin W2 biosynthesis protein.

60 - Carboxymuconolactone decarboxylase.
61 - MFS transporter
62 - hypothetical protein.
63 - Tautomerase.
64 - hypothetical protein.
65 - hypothetical protein.
66 - hypothetical protein.
67 - LysR family transcriptional regulator.
68 - ssDNA-binding protein.

Annex 14 Proteins exclusive to cluster of *Serratia* strain YD25.

Supplementary data

Supplementary data 1: Serrawettin W1 biosynthesis protein from *Serratia* strain AS13.
https://drive.google.com/file/d/1GySF37EgrkbM4O1v2efpo-t5cO107TvB/view?usp=sharing

Supplementary data 2: Serrawettin W2 biosynthesis protein from *Serratia* strain YD25.
https://drive.google.com/open?id=1InIsvb_0m6kBdAEG8s5MyCcfsAuPD5La

Supplementary data 3: Molecular dynamic simulation with Serrawettin W1 biosynthesis protein from *Serratia* strain AS13 in water.
https://drive.google.com/open?id=1RC7gg6VqzE55HcsgBhN-pXFYgOSYSgo9

Supplementary data 4: System of molecular dynamic simulation with Serrawettin W1 biosynthesis protein from *Serratia* strain AS13 in water.
https://drive.google.com/open?id=1CTgtqE6Rp9HF5ZHOg1NSEyO6SUA2zeqH

Supplementary data 5: Molecular dynamic simulation of TOL1 protein with Serrawettin W1.
https://drive.google.com/open?id=1X-MtChZro1p6NqAsKZkeNPt_P-htGY1q

Supplementary data 6: System of molecular dynamic simulation of TOL1 protein with Serrawettin W1.
https://drive.google.com/open?id=1N9Fu-_sFAc7rwraobYIBArXYb-mReySy

Supplementary data 7: Molecular dynamic simulation of TOL1 protein with Serrawettin W2.
https://drive.google.com/open?id=16-R_rkWPqP1iz3X8vYhcbd_ppqrC60Lk

Supplementary data 8: System of molecular dynamic simulation of TOL1 protein with Serrawettin W2.
https://drive.google.com/open?id=1SuOyLbAD5JHjdU6GAyeNBtqxq9m3E6-r