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Extracellular Proteins from A Novel *Serratia* Isolate Capable Of Killing *Phytophthora erythroseptica*

By

Brittany A. Mangum

A thesis

submitted in partial fulfillment

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To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Brittany Mangum find it satisfactory and recommend that it be accepted.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

AI	Aggressiveness Index
BSA	Bovine Serum Albumin
CFU	Colony Forming Unit
DMSO	Dimethyl Sulfoxide
EC	Effective Concentration
EHM	Extrahaustorial Membrane
ESI	Electrospray Ionization
ESP	Extracellular Serine Protease
GC	Gas Chromatography
GFP	Green Fluorescent Protein
HEMO	Hemolysin
HPLC	High Performance Liquid Chromatography
IPTG	Isopropyl ß-D-1-thiogalactopyranoside
ITS	Internal Transcribed Spacers
LAX	LB + Ampicillin + X-gal
LAXI	LB + Ampicillin + X-gal + IPTG
LB	Luria-Bertani
LC	Liquid Chromatography
LIC	Ligation Independent Cloning
MAMP	Microbe Associated Molecular Patterns
MCS	Multiple Cloning Sites
MIC	Minimum Inhibition Concentration

MS	Mass Spectroscopy
NLS	Nuclear Localization Signal
NRE	Non-Rhizobia Endophytic
OmpA	Outer membrane Protein A
PAGE	Polyacrylamide Gel Electrophoresis
PAMP	Pathogen Associated Molecular Patterns
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PEG	Polyethylene Glycol
PMSF	Phenylmethylsulfonyl Fluoride
PRR	Pattern Recognition Receptors
PSM	Peptide Spectrum Match
RSS	Rapid Silver Stain
TEM	Transmission Electron Microscopy
TIC	Total Inhibition Concentration
TOF	Time-of-flight
TSB	Tryptic Soy Broth
X-gal	5-Bromo-4-Chloro-3-Indolyl β-D-Galactopyranoside
ZE	Zoospore Exudate

Extracellular Proteins from A Novel *Serratia* Isolate Capable of Killing *Phytophthora erythroseptica*

Thesis Abstract-Idaho State University (2023)

A novel strain of the organism *Serratia plymuthica* has shown inhibitory effects against *Phytophthora erythroseptica*. *P. erythroseptica* is a chytrid fungus known to cause wilt and pink rot in tubers. We have identified four virulence factors (outer membrane protein A (OmpA), extracellular serine protease (ESP), hemolysin 1, and hemolysin 2 from the spent media of the isolate which may account for the inhibition. The spent medium was sent to Boise State University for proteomic sequencing. Utilizing bioinformatic methods to identify the proteins detected and their underlying gene sequence of a closely related genome, we designed PCR primers to amplify the top candidate genes. Utilizing various cloning systems (pBAD TOPO TA, pUC18, and aLICator), we were able to identify the correct vector-host combination that enabled us to clone three of the four gene targets. Additionally, we have transformed recombinant plasmid DNA into an expression host and preliminary expression experiments have been conducted.

Keywords: cloning, inhibition, OmpA, *Phytopthora*, *Serratia*

Chapter I: Literature Review

Phytophthora

True Fungus vs Oomycete?

The genus *Phytophthora* contains eukaryotic microorganisms within the kingdom *Straminiphila*, *Oomycota* phylum, and *Oomycetes* class. Over 150 *Phytophthora* species have been categorized, 60% of species being plant pathogens. For example, *Phytophthora infestans* infects potatoes and tomatoes causing late blight, *Phytophthora erythroseptica* cause pink rot, and *Phytophthora ramorum* causes sudden oak death (Wang & Jiao, 2019; Yang et al., 2017). Oomycetes were once considered to be true fungi due similar phenotypes, morphological features such as the presence of mycelia and spores, mode of nutrition, and environmental niches. However, there are several major distinctions which differentiate oomycetes from true fungi (Klinter et al., 2019). Traditionally oomycetes were informally grouped together as "lower fungi" including slime molds, chytrids, zygomycetes, and arbuscular mycorrhizae. True fungi or "higher fungi" includes ascomycetes (sac fungi) and basidiomycetes (club fungi) (Rossman & Palm-Hernández, 2006).

True fungi cell walls contain chitin, a polymer of β-1,4 linked N-acetylglucosamine responsible for maintaining the mechanical strength of the cell wall. Cell walls of oomycetes are composed of cellulose β-1,3 linked and β-1,6 linked glucan. Oomycetes can be further divided into two taxonomic sister groups Peronosporomyctes, mainly plant pathogens such as *Phytophthora*, and Saprolegniomyctes, saprophytes. Saprolegniomyctes may contain varying amounts of β-1,4 linked N-acetylglucosamine in their cell wall, but Peronosporomyctes do not (Klinter et al., 2019).

Using microscopy hyphae and mitochondria from oomycetes and true fungi can be visualized. Hyphae are fine branching filaments enabling both organisms to absorb nutrients and reproduce. True fungi, except *Zygomycota*, the mycelium is divided by cross-walls or septations that contain cells with haploid (1n) nuclei. Oomycetes hyphae are known as coenocytic due to the lack of cross-walls. Oomycetes cells contain diploid (2n) nuclei. *Zygomycota*, a true fungus, lacks septation however, hyphae contain haploid (1n) nuclei. In the 1970s, transmission electron microscopy (TEM) revealed mitochondrial cristae present in both oomycetes and true fungi. Cristae occur when the inner membrane of the mitochondrion folds inward. Most of the electron transport chain complexes and ATP synthase dimers are found in mitochondrial cristae. Therefore, the more cristae, the more energy that can be produced (Glancy et al., 2020). Oomycete's mitochondria contain tubular or fingerlike cristae, while true fungal mitochondria have flattened or sheetlike cristae (Rossman & Palm-Hernández, 2006).

Phylogenetic analysis has revealed oomycetes are more closely related to heterokont algae than true fungi. Heterokont algae include brown algae (*Phaeophyta*), yellow-green algae (*Xanthophyta*), golden algae (*Chrysophyta*), and diatoms (*Bacillariophyta*). Similar to oomycetes, heterokont algae have tubular mitochondrial cristae and two types of flagella. Oomycetes' zoospores possess two types of flagella. The first type of flagellum is located posteriorly and moves in a whiplike fashion while the second type of flagella is positioned anteriorly. A true fungus, *Chytridiomycota*, produces zoospores with only the posterior flagellum, differentiating it from oomycetes (Rossman & Palm-Hernández, 2006).

Genus

Prior to DNA based identification methods, *Phytophthora* species were characterized and divided into six groups based on three sporangium types, 2 antheridium types, host range,

sporangium morphology, presence or absence of chlamydospores, hyphal swellings, optimal growth temperature, colony morphology, and oogonium morphology. The classification system was devised by Waterhouse, a British mycologist, in the 1920s and later revised. Classification of species within the genus *Phytophthora* using the above criteria required an expert mycologist to differentiate new species. To aid in further differentiation mycologists were able to analyze physiological characteristics such as resistance to malachite green. The advancement in molecular technology has enable researchers to construct molecular genetic linkage maps, track clonal lineages within populations, and construct phylogenic maps. When DNA regions and genes from dozens of species are characterized, a phylogeny can be constructed, and species can be grouped into clades (Kroon et al., 2012).

Phylogenies were constructed based on DNA sequences of the 5.8S ribosomal RNA gene and flanking internal transcribed spacers (ITS1 and ITS2). The ITS's were ideal targets for polymerase chain reaction (PCR) amplification due to the highly conserved regions. However, within the scientific community there was doubt that the ITS regions could differentiate closely related species. Phylogenic resolution has been greatly enhanced by targeting "housekeeping" genes such as mitochondrial genes, nuclear genes, and proteins with known metabolic functions. Similar as the ITS genes, housekeeping genes have highly conserved regions ideal for universal primers, however, nucleotide variation is enhanced within given regions (Kroon et al., 2012).

Within the *Phytophthora* genus, ten clades have been categorized (Figure 1). Organisms within the ten clades are differentiated based on host, host tissue infected, sex (heterothallic or homothallic), type of zoosporangia (papillate, semipapillate, or nonpapillate), and either amphigynous or paragynous attachment to antheridia (Kroon et al., 2012). *Phytophthora* species can affect plant foliage (leaves), roots, or both. For instance, *P. infestans* is known to infect plant

foliage, *P. erythroseptica* is a soil-borne pathogen and infects plant roots, and *P. nicotianea* infects both roots and foliage (Kroon et al., 2012). *Phytophthora* species can reproduce sexually by either heterothallic or homothallic conditions. Heterothallic species are of a single mating type and depend on a compatible mating partner to reproduce sexually. Homothallic species produce both the male and female mating types for sexual reproduction. *Phytophthora* species can also be differentiated based on zoospore release. Zoospores can be released from the sporangia via exit pores that are well developed (papillate) or underdeveloped and appear nipple like (semi-papillate; Figure 2). During sexual reproduction the antheridium can attach to the oogonium either by paragynous or amphigynous attachment. Paragynous attachment is when the antheridium attaches to the side of the oogonium, while amphigynous pertains to the antheridium surrounding the oogonium (Figure 3; Glossary, 2023).

THE REST OF THE BODY TEXT IS CUT FOR CONCISENESS.

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