FEDERAL UNIVERSITY OF SÃO CARLOS

CENTER OF SCIENCES AND TECHNOLOGY

GRADUATION PROGRAM IN BIOTECHNOLOGY

ISOLATION OF THE ENDOPHYTIC AND RHIZOSPHERIC MICROBIOME ASSOCIATED WITH *POLYGALA* SPP.: EVALUATION OF THE BIOTECHNOLOGICAL POTENTIAL AND ANTIMICROBIAL ACTIVITY

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FELIPE DE PAULA NOGUEIRA CRUZ

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Biotechnology) in the Federal University of São Carlos, 2018.

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Epigraph

“There is no knowledge that is not power”

Ralph Waldo Emerson

Dedication

To my parents, Antonio and Gilda for all love, encouragement, contribution and support for achieving this goal.

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Firstly, thanks God for entrusting me with the mission here on Earth so that I could somehow contribute to the development of humanity in the search for knowledge.

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Abstract

Polygala spp. are plants commonly used for the treatment of injuries and sprains. Their roots contain methyl salicylate, a compound known for its analgesic properties. However, important compounds such as alkaloids, flavonoids, terpenes, lignins and coumarins were obtained from this genus. In this context, the *Polygala* spp. tissues could represent a promising source of natural products, since that several pharmacological effects have been reported for their crude extracts. Therefore, the microbiome associated to these plants and the antimicrobial potential of the natural product extracts produced by these microorganisms was investigated. In this study it has been demonstrated the isolation of a potent substance with a broad-spectrum activity produced by an actinobacteria Rizo Pp Ac-11 isolated from rhizospheric space of white-flower *Polygala* sp.. The natural product extract was carried out to a C_{18} fractionation in a H_2O/acetonitrile gradient and all fractions (Rizo Pp Ac-11A, Rizo Pp Ac-11B, Rizo Pp Ac-11C, Rizo Pp Ac-11D, Rizo Pp Ac-11E, Rizo Pp Ac-11F, Rizo Pp Ac-11G, Rizo Pp Ac-11H and Rizo Pp Ac-11I) were tested for antimicrobial activity. Excepting Rizo Pp Ac-11A and Rizo Pp Ac-11B, all fractions showed inhibition. However, the fraction Rizo Pp Ac-11C showed a broad-spectrum activity against all tested microorganisms, especially multi resistant pathogens. Considering that, this study demonstrated for the first time the isolation of microorganisms associated with *Polygala* spp. and the isolation of substances with broad-spectrum activity.

Keywords: Actinobacteria; Antimicrobial compounds; Atlantic Forest; Natural products, multi resistant pathogens; *Polygala* spp.; *Streptomyces*.

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List of abbreviations

16S rRNA: 16 subunity of Ribosome Ribonucleic acid.

ACN: Acetonitrile.

AIG: Anthrax Immunoglobulin.

ATCC: American Type Culture Collection.

BHI: Brain Heart Infusion medium.

CDC: Centers for Disease Control and Prevention.

COX: Cyclo-oxygenase

DMP: Department of Morphology and Pathology.

DMSO-d_6: Deuterated dimethyl sulfoxide.

DNA: Deoxyribonucleic acid.

EGCG: Epigallocatechin-3-gallate.

EtOAc: Ethyl acetate.

FB: Fermentation broth

FDA: Food and Drug Administration.

GDP: Gross Domestic Product

HNP: Defensin Human Neutrophil Peptide.

HPLC: High-performance liquid chromatography.

HTS: High Throughput Screening.

IAA: Indole acetic acid.

ICU: Intensive care unit.

IDSA: Infectious Diseases Society of America.

ISP_2: International Streptomyces Project medium 2.

LaMiB: Laboratory of Microbiology and Biomolecules.

LC-MS: Liquid Chromatography/ Mass Spectrometry

MeOH: Methanol.

MIC: Minimum Inhibitory Concentration.

MACB: Mycolic Acid-containing Bacteria

NMR: Nuclear Magnetic Resonance.

NPE: Natural Product Extract.

NRPS: Nonribosomal peptide synthetase.

OD: Optical Density.

PBS: Phosphate Buffered Saline.

PDA: Potato Dextrose Agar.

PDB: Potato Dextrose Broth.

PesCDA: Chitin deacetylase.

PKS: Polyketide synthase.

PPA: Prepenetration apparatus.

PPU: Polyester polyurethane.

TSA: Triptic Soy Agar.

UFSCar: Universidade Federal de São Carlos.

UV: Ultraviolet.

VOC: Volatile organic compound.

WHO: World Health Organization.

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Introduction

1. Introduction

The Biotechnological processes that use microorganisms for drug development have increased in recent years and represent a rich source of bioactive natural products that can be used as pharmaceuticals and agrochemicals and for biotechnological applications [Azevedo et al., 2000; Strobel et al., 2004; Gunatilaka, 2006; Aly et al., 2011; Piza et al., 2015].

Several studies have shown that plant tissues represent an important source of natural substances for pharmaceutical and biotechnological interest. Most of these compounds are produced by microorganisms that live in intimate interaction with the host plant without causing damage, therefore, they are known as endophytic [Azevedo et al., 2000; Ratti, 2009; Joseph & Pryia, 2011; Serrano et al., 2012]. These can be found in different structures and plant tissues, such as leaves, stems, roots, fruits, seeds and flowers [Strobel et al., 2004; Piza et al., 2015].

In this context, the genus *Polygala* is known for the abundant presence of methyl salicylate and is widely used for the treatment of lesions and sprains [Lorenzi & Matos, 2002; Nogueira et al., 2005]. Other relevant studies show that compounds such as alkaloids, flavonoids, terpenes, lignins and coumarins were also found in these plants, as well several pharmacological effects have been reported for crude extracts of *P. cyparissias* and *P. sabulosa*, such as anti-inflammatory, anxiolytic, trypanocidal, antinociceptive and antimicrobial activity in *P. myrtifolia* [Victório et al., 2011]. In the last years, biological actions in the nervous system have been attributed to these plant extracts.

LaMiB focuses on the research of bioactive substances produced by endophytes. Several studies have demonstrated the isolation of potent substances with high biotechnological potential produced by these microorganisms isolated from plants from Cerrado region of São Carlos, Brazil. However, other biomes are important sources of endophytes for the search for new bioactive substances.

Ratti (2009) conducted the first study in a preliminary prospecting and purification of microorganisms of biotechnological interest, isolated from *Solanum lycocarpum* Saint Hill (Lobeira). In this study, it was reported the bioactive potential of the crude extract of *Streptomyces tubercidicus*. And also reports the isolation of two different fractions with

antibiotic activity in *E. coli* and *S. aureus*, suggesting the presence of new bioactive compounds with antimicrobial activity. It is known that to produces tubercidin, a potent substance that can inhibit various metabolic processes, including pathogens such as *T. cruzi*, viruses, fungi and exhibits cytotoxic activity. However, there are few studies on the isolation of *S. tubercidicus* and only four have been published in the production of bioactive substances [Smulson & Suhadolnik, 1967; Kónya et al., 2008].

The study of Favoretto (2010) consisted in the isolation of endophytes from the aerial parts of *Butia capitata*, *Solanum lycocarpum*, *Miconia albicans* and *Aegiphila lhotzkiana*. The crude extracts produced by the isolated strains were able to inhibit the growth of *E. faecalis* and *S. aureus*.

A strain of *Paenibacillus polymyxa* was endophytically isolated from plants of the genus *Prunus* spp. [Serrano, 2009]. This Gram-positive bacillus can be found in several *habitats*, and due to its characteristic metabolism and production of substances, it is widely applied in commercial agriculture as biofertilizer, biological control and environmental remediation. In this study, it was reported that *P. polymyxa* was able to produce small molecules with high bioactivity against *S. aureus* and *E. coli*. In another study, Serrano et al. (2012) carried out studies to optimize the production conditions of these bioactive substances.

In the literature, *Nocardiopsis dassonvillei* is described as a microorganism isolated from the soil, while *Amycolatopsis orientalis* is a producer of vancomycin. They were isolated as endophytes from *Miconia albicans* for the first time by Piza et al. (2015). In this study, *A. orientalis* showed significant inhibition against *S. aureus*, *E. faecalis*, *C. albicans*, *E. coli* and *S. sonnei*, whereas *N. dassonvillei* showed inhibition only against *E. faecalis*.

In the study of Romano (2015), fractions with cytotoxic and antiparasitic activity were isolated from the endophytic *Paenibacillus terrae* isolated from *Tabebuia* spp.. This endophyte showed a high anti-tumor effect against cancer cell lines such as OVCar-8, HCT-116 and SF -295.

Thus, our group has great potential and intellectual structure for the development of this thesis. And the relevance of this study may contribute to the search for new bioactive compounds for the control of pathogenic microorganisms.

Studies conducted by other research groups corroborate the growing interest in microorganisms associated with plants as a source of bioactive compounds, because they have a great ability to produce substances with different types of actions [Strobel et al., 2004; Gunatilaka, 2006; Piza et al., 2015].

Li et al. (2008) isolated endophytic actinomycetes from medicinal plants in China. Their results showed that 31.7% of endophytic *Streptomyces* isolated presented cytotoxicity against tumor cells, *E. coli*, *S. aureus*, *S. epidermidis*, and *C. albicans*.

In a study conducted by Souza et al. (2008), a strain of *Streptomyces* was isolated from the Cerrado soils. This isolate was identified by the analysis of the 16S rRNA sequence and identified as a new *Streptomyces lunalinharesii* species. These results show that the Cerrado biome harbors a variety of unknown microorganisms with great potential for biotechnological applications.

Bascom-Slack et al., 2009 reported the isolation of 14 species of actinomycetes from plants collected in a forest in Peru. All isolates showed inhibitory activity against pathogenic bacteria and fungi.

Moreover, Xing et al. (2011) demonstrated that the endophytic fungus *Epicoccum nigrum* in species of *Dendrobium* spp. (*Orchidaceae*) presented a more intense bioactivity compared to ampicillin, whereas *Fusarium* spp., was effective against several pathogenic bacteria and fungi.

As there are no studies in the literature on the isolation of microorganisms associated with *Polygala* species from Atlantic Forest region, this thesis presents a unique study of the microbiome associated with these plants and substances produced by them, in addition to presenting the great potential of the *Polygalaceae* family, thus justifying the relevance of the research of these species.

In order to promote advances in the study of compounds obtained from microorganisms isolated from different structures of *Polygala* spp., this work was based on a multidisciplinary approach, involving research areas of the Federal University of São Carlos, Brazil, and the Sherman Laboratory from Life Sciences Institute - University of Michigan, USA.

The aim of this doctoral thesis was to acquire a better understanding of the molecular structure of the isolated substances and their synthetic pathways, in order to develop new medicines and other substances of biotechnological interest. The objectives of this work were:

1. Isolation of the endophytic and rhizospheric microbiome associated with the *Polygala* genus;
2. Analysis of the isolation frequency profile;
3. Evaluation of the enzymatic potential;
4. Evaluation of the production of secondary metabolites with antimicrobial activity;
5. Purification of the bioactive substances present in the natural products extracts;
6. Optimization of a protocol for nucleic acids isolation; and
7. Determination of the molecular mass of the bioactive compounds.

Literature review

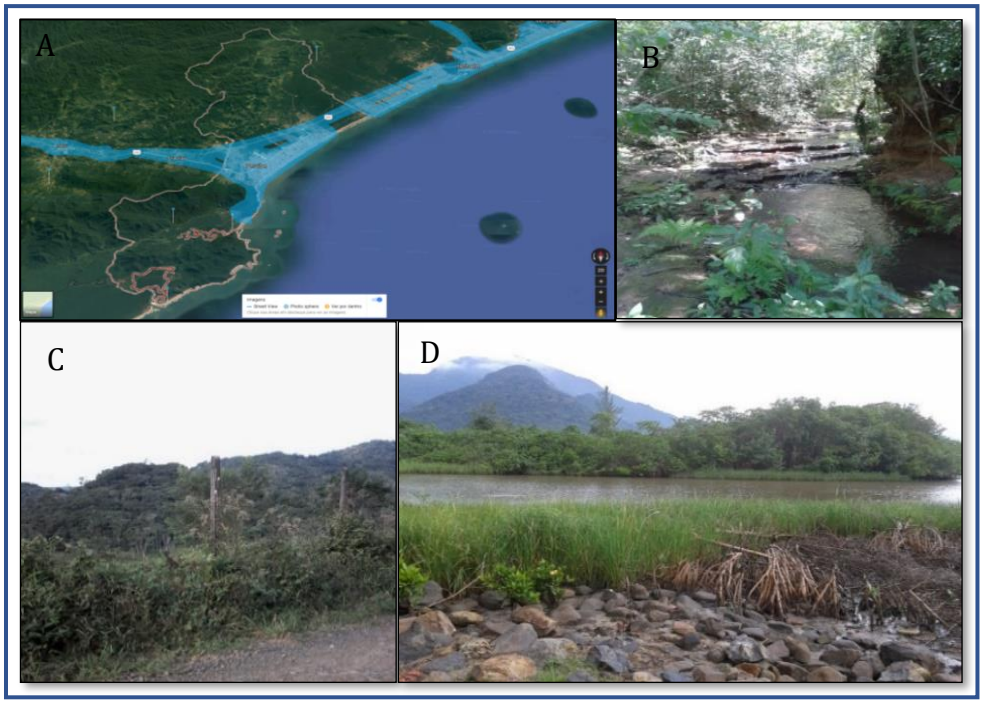
2. Literature review

2.1. The Atlantic forest biome

The Brazilian territory presents an immense biodiversity, which represents about 20% of the total number of species of the planet. In this context, the country has great potential in the search for and development of new biotechnological products as well as the expansion of intellectual property.

The Atlantic Forest (Figure 1) is the biome located on the east coast of South America, which comprises a large complex of forest types, generally broad-leaved, rainy, tropical and subtropical, where the variety of formations and floristic compositions are modeled by climatic influences, relief and different types of soil. For this reason, the Atlantic Forest is considered one of the 25 global biodiversity conservation hotspots [Myers et al., 2000; Santos et al., 2014].

Figure 1: A: Location of the city of Peruíbe. **B, C** and **D:** Atlantic Forest biome located in Peruíbe.



Source: A: Google maps. **B, C and D:** Author.

Thus, the most promising plants and endophytes for biotechnological applications are those that grow in areas of high biodiversity or having different survival strategies or have a history of popular use. In terms of biodiversity, Brazil is a privileged country, as it has major biomes such as the Amazon, Atlantic Forest and Brazilian Tropical Savannah that together they account for nearly 80% of territory [Silva et al., 2011; Lacava & Sousa, 2016].

2.2. *Polygala* species and medicinal properties

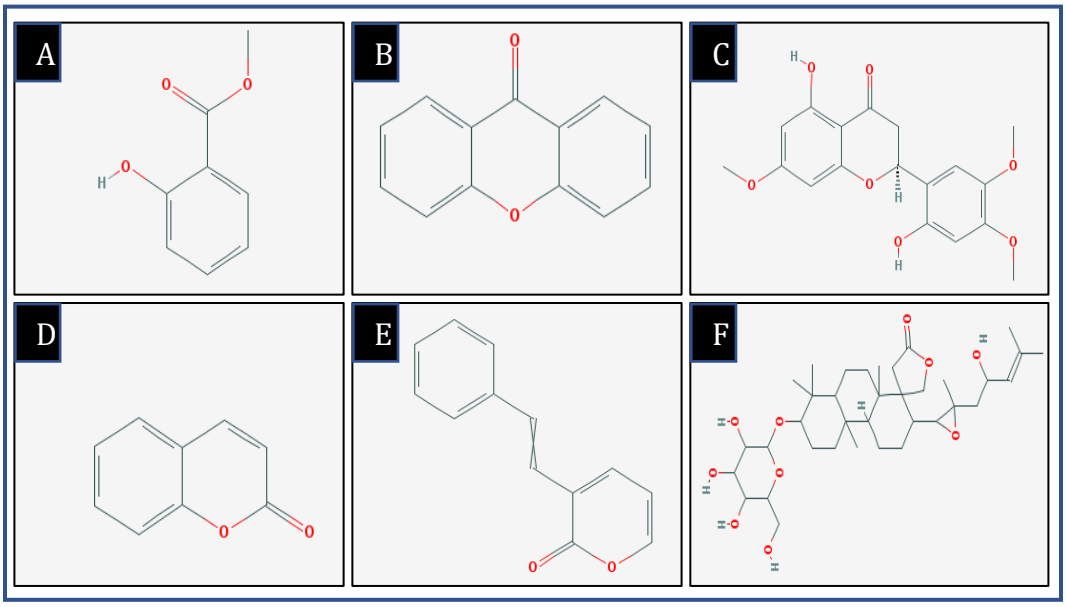
The *Polygala* genus (Figure 2) is the largest within the family *Polygalaceae*. They are herbs and sub-bushes characterized by their simple racemes, zygomorphic flowers, rhodium capsule fruit, seeds with endosperm and continuous or invaginated embryo. Its distribution is pantropical, and a huge number of species is concentrated in America and Africa [Marques 2003].

Figure 2: A: purple flowers *Polygala* sp.. **B:** Specimen of white flowers *Polygala* sp..

Source: Author.

A striking feature in many species of this genus such as *P. cyparissias* and *P. paniculata* is the accumulation of methyl salicylate in its roots, being the main compound responsible for the organoleptic characteristics in these species [Victório et al., 2011]. In addition, other *Polygala* species have long been reported in control or treatment of various diseases, and many studies have shown the presence of a variety of phytochemical compounds in these species such as alkaloids [Jin & Park, 1993], xanthones [Pinheiro et al., 1998; Dall'Acqua et al., 2002/2004], saponins [Nagai et al., 2001; Jia et al., 2004], glycosylated flavonoids [Rao & Raman, 2004], coumarins [Hamburger et al., 1985; Pizzolatti et al., 2002] and styrylpyrones [Pizzolatti et al., 2002], among others (Figure 3).

Figure 3: Basic structures of molecules reported in Polygala plants **A:** Methyl salicylate. **B:** Xanthone. **C:** Flavonoid. **D:** Coumarin. **E:** Styrylpyrone. **F:** Saponin.



Source: PubChem Substance Database.

Methyl salicylate is an ester derived from salicylic acid, originally isolated from the plant *Gaultheria procumbens* in 1843 by Cahours & Proctor and is known to be used as medicine due to anti-inflammatory, analgesic, antipyretic, and antithrombic properties [Li et al., 2002; Victório et al., 2011]. It has a similar mechanism of action of aspirin; based on primarily in the inhibition of the cyclo-oxygenase (COX) and possibly on suppression of

cyclooxygenase-2 (COX-2) transcription and salicylate (2-hydroxybenzoate); and when absorbed by the skin causes a slight tingling and feeling of freshness probably due to the presence of phenolic hydroxyl groups [Li et al., 2002; Victório et al., 2011]. In addition, it is reported that the activation of NF-κB act as hydroxyl radical scavenger, reacting with hydroxyl radicals, thereby intervening in apoptotic pathways [Li et al., 2002].

Among the natural compounds, the xanthones have great prominence due to their diverse pharmacological effects. This class of heterocyclic compounds contains dibenzo-γ-pyrone as their scaffold and classified according to contemporary substitutes in positions 1 to 8 of the central nucleus, which can be simple, glycosylated, pre-diluted, xanthonolignoid, bis-xanthone and mixed xanthones, furthermore, they can be subdivided according to their degree of oxygenation. In natural xanthones, there are groups substituted with methoxy, hydroxyl, alkyl, isopentenyl and glycosyl groups [Pinto et al., 2005; Cao et al., 2012].

Regarding prospecting of new molecules from the root extract of *Polygala japonica*, a medicinal plant from the south of China that has been used to treat pharyngolaryngitis, tonsillitis, stomatitis, pneumonia, calculus, wound, and diphtheria, Xue et al., 2007 isolated three new xanthones, which contains methoxy groups in their structure.

In a study conducted by Dao et al. (2012), xanthones were isolated from the *Polygala karensium* and these extracts were able to decrease the cytopathic effect and inhibit the enzyme neuramidase of the H1N1 virus.

Li et al. (2014) obtained xanthone-lignoids and glomexanthones from the ethanolic extract of *Polygala glomerata*. These showed moderate neuroprotective effects on L-glutamic acid-induced cellular damage in human neuroblastoma SK-N-SH cells.

Flavonoids consist of a very extensive class of natural products derived from chalcones. They are widely distributed by the Plant kingdom, presented from the roots to the flowers and fruits [Jiang et al., 2012].

Among the potential pharmacological effects of this class of natural products we can cite the antiviral, antitumor, anti-inflammatory and antioxidant activity [Jiang et al., 2012]. Structurally, flavonoids have a backbone containing 15 carbon atoms in their basic nucleus with two aromatic rings connected by a three-carbon bridge. This group includes the

flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones [Jiang et al., 2012].

Coumarins represents an important class of molecules which are naturally found in several plants and are widely used as anticoagulants in antithrombotic therapy. Derived from the metabolism of phenylalanine and synthesized through the shikimic acid route, these phenolic compounds presents several pharmacological properties ranging from the inhibition of lipid peroxidation, superoxide generation, to linoleic acid hydroperoxide-induced cytotoxicity, as well as acting as immunosuppressive and anti-inflammatory agents [Jiang et al., 2012; Li et al., 2017; Ziegler et al., 2017].

Styrylpyrones are a relatively unusual group of natural products, their molecular structures having a γ-, δ-lactone ring attached to a styryl or dihydrostyryl fragment. Possibly, the biosynthesis of this class of molecules is via the shikimic acid route. According to Herderich et al. (1997), the cinnamoyl-CoA acid under the action of the enzyme styrylpyrone synthase can incorporate two malonyl-CoA units and subsequently the polyketide derivative undergoes lactonization to result in the corresponding styrylpyrone. Among this class of molecules, hispidine and bisnoriangonine are the best, where the former was isolated from *Inonotus. hispidus* by Zopf in 1889 but was only identified in 1961 by Edwards and later by Bu'Lock in 1962 as 6 - (3 ', 4'-dihydroxystyryl) - 4-hydroxy-2-pyrone [Pizzolatti et al., 2004; Lee & Yun, 2011].

Saponins are one of the main bioactive components present in plants of the genus *Polygalaceae*. Most saponins have a variety of action spectro such as, antifungal, hemolytic, immuno-modulatory, and the ability to act in metabolism processes. In recent years, they have been widely used in several clinical trials and studies as treatment of people with dementia and amnesia [Liu et al., 2007].

Chemically, there are derived from the precursor squalene oxide with 30 carbon atoms. They can be grouped into steroid and triterpenoid glycosides, where. the difference between them is that the steroidal saponins have 3 methyl groups at least in relation to the triterpenes that are maintained with 30 carbon atoms. Structurally, the saponins found in the *Polygalaceae* family have in common the oleanane skeleton and are therefore probably synthesized via β-amirin as a common precursor [Jin et al., 2014].

In a recent study conducted by Jin et al. (2017) a high antimicrobial activity saponins that were isolated from the endophytic fungi *Fusarium oxysporum* PN8 and *Aspergillus niger* PN17 obtained from Chinese medicinal herb *Panax notoginseng*. In subsequent studies, Gu et al. (2018) led to the identification of two new dammarane-type of triterpenoid saponins. In addition, these compounds showed cytotoxicity activity on HL-60 (Human myeloid leukemia), SMMC-7712 (hepatocellular carcinoma), A-549 (lung cancer), MCF-7 (breast cancer) and SW480 (colon cancer) cell lines at a concentration of 40 μM.

Regarding to biological activities, *Polygala tenuifolia* stands out; substances such as polygalactic acid and tenuifoline isolated from *P. tenuifolia* were able to confer a neuroprotective effect in rats with scopolamine-induced cognitive dysfunction and prolong sleep time, respectively [Guo et al., 2016; Cao et al., 2016]. An euxantone isolated from the roots by Mak et al. (2000) could act directly on neuronal differentiation. However, other effects in nervous system has been reported in other species such as *Polygala paniculata* by Bettio et al. (2011) that showed interactions of compounds present in extract with the monoaminergic system and, consequently, an anti-depressive effect in rats.

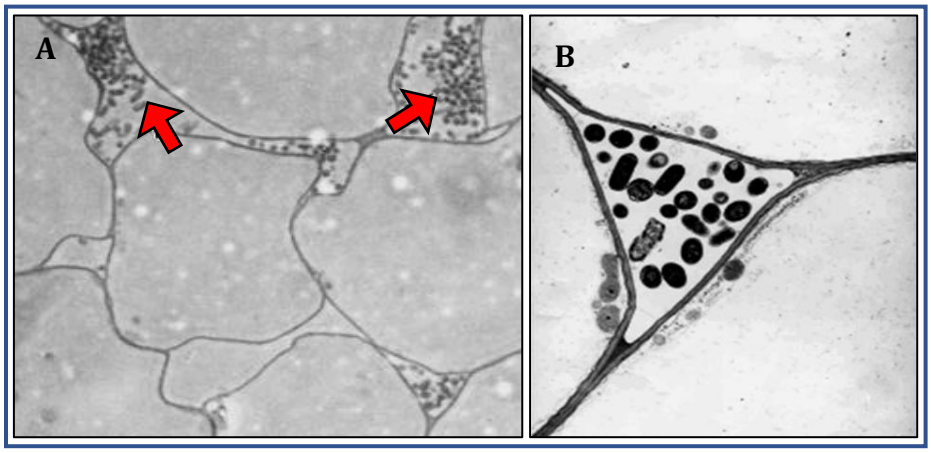
Other pharmacological effects have been described for the extracts of *Polygala telephioides* that is used as a detoxification agent for narcotics [Egashira et al., 2005]. In addition, in the studies of Li & Nohara (2000) and Li & Nohara (2000) was reported the identification of esters of oligosaccharides and C-glycosides of benzophenone in extracts of *P. telephioides*. In addition, other biological activities have been associated to *Polygala* extracts such as expectorant, sedative, tonic [Jiang & Tu, 2002; Lin et al., 2005], anti-stress [Kawashima et al., 2004], anti-depressive [Cheng et al., 2006], and *Polygala caudata* for the treatment of cough and hepatitis [Lin et al., 2005].

In Brazil it has been reported that trypanocidal activities, antinociceptive, anesthetic, anxiolytics and anticonvulsants properties in *Polygala sabulosa* extracts, [Duarte et al., 2007]. In addition to the study by Johann et al. (2011) it was reported the presence of substances with antifungal activity in *Polygala cyparissias*.

2.3. Endophytic and rhizospheric microorganisms

Endophytic microorganisms establish a mutualistic association with the host plant, they can be found in the aerial parts of the plant, but also in the roots (Figure 5), which are one of the main entry points. Whereas the plant provides an environment, protection and feeding, the microorganism, in turn, produces substances that can enhance growth and protect against biotic and abiotic stresses. This kind of interaction was first mentioned in the early nineteenth century, and only then, in 1866, Bary, outlined a possible distinction between endophytes and phytopathogens [Azevedo et al., 2000; Strobel, 2004; Serrano, 2012; Piza, 2015].

Figure 4: A: *Bacillus mojavensis* endophytically associated with maize. The red arrows indicate bacterial cells located in the intercellular spaces of the root cortex. **B:** Transmission electron micrograph of the same histological section showing bacteria between the intercellular spaces.

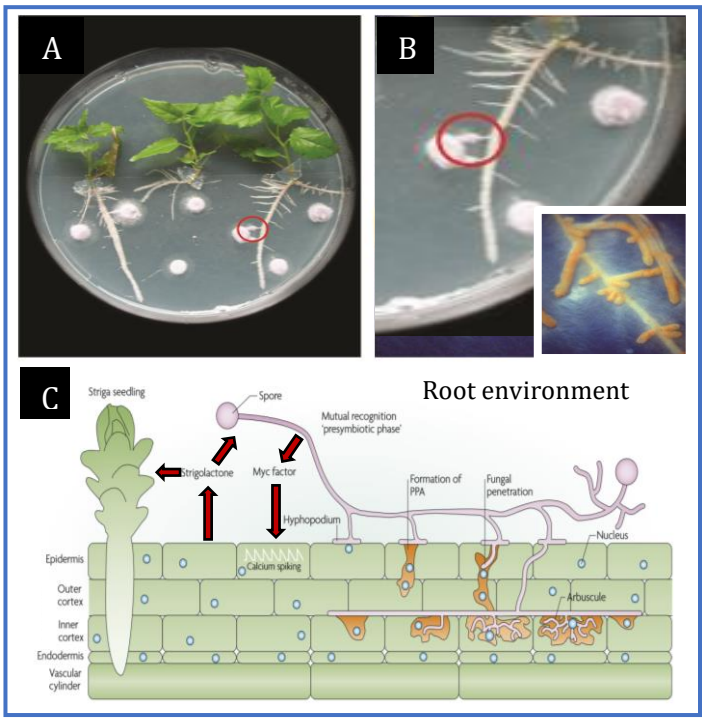


Source: Adapted from Bacon. & Hinton, 2002 and Bacon. & Hinton, 2011.

Another important type of endophytic association is mycorrhizae (Figure 6). This denomination was proposed by Frank in 1885 to describe this type of mutual association between obligatory symbiotic fungi and plants. These fungi that belongs to the Glomeromycota phylum promotes the absorption of water and nutrients, such as

phosphate and nitrogen, to the host plant [Parniske, 2008; Martin et al., 2016]. They are classified according to the morphological characteristics and species of fungi and plants involved, which the most common classes are: arbuscular mycorrhiza, ectomycorrhiza, orchid mycorrhiza and ericoid mycorrhiza [Parniske, 2008; Martin el at., 2016]. Among these, arbuscular mycorrhizae (Figure 7) are the most important. They are characterized by the presence of intracellular arbuscule in the cortical region of the root and the presence of inter and intracellular hyphae [Martin el at., 2016].

Figure 5: (A): Ectomycorrhizal association between *Populus tremula* and *Laccaria bicolor in vitro*. **(B):** Magnified image showing the colonization structure in roots of *P. tremula.* **(C):** Root formation and colonization by arbuscular mycorrhizae, which strigolactone molecules produced by the roots, induce germination of spores and branches of hyphae. In turn, fungi produce mycorrhizal factors that induces calcium oscillations in root epidermal cells and activate genes related to plant symbiosis. In response to chemical and mechanical stimulation, plant cells produce a prepenetration apparatus (PPA), where, later, a fungal hypha extending from the hyphopodium enters the PPA.

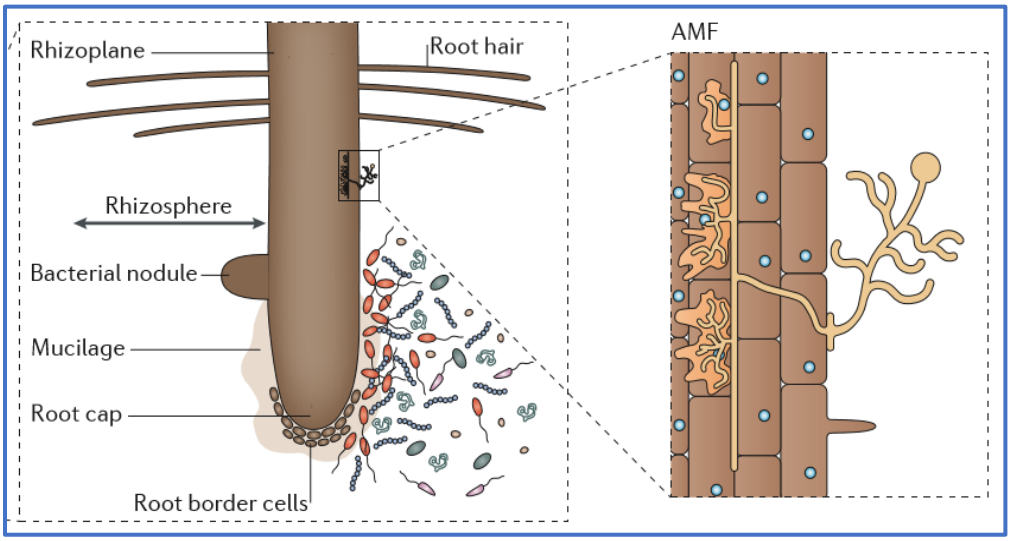


Source: Adapted from Parniske, 2008; Martin et al., 2016

In contrast, the rhizosphere (Figure 8), consists of the narrow soil zone that surrounds the roots of plants, is the habitat of numerous microorganisms and invertebrates, being considered one of the most dynamic interfaces on Earth [Philippot et al., 2013].

The micro community consists in bacteria, fungi (arbuscular mycorrhizae), oomycetes, viruses and archeas living in the rhizosphere. They are attracted and fed by nutrients, exudates, border cells and mucilage that are released by the root of the plant. In this context, the rhizosphere microbiota can exert profound effects on plant growth, nutrition and health. This microbiome can directly and indirectly affect the composition and biomass of the plant community in natural ecosystems. [Philippot et al., 2013]. These interactions may be due to altered nutrition or water absorption by the plant, or even by alteration of the plant defense system [Philippot et al., 2013].

Figure 6: Basic scheme of the rhizospheric space showing saprophytic and symbiotic bacteria and fungi, including arbuscular mycorrhizal fungi.



Source: Adapted from Philippot et al., 2013.

Typically, the process of colonization of internal plant tissues occurs in a specific tissue or through the vascular system or apoplast where the entrance orifices may be: wounds, lateral or germinating root areas, stomata and lenticels. However, the main

colonization pathways are lesions, root hairs and epidermal cells [Mesquita et al., 2013; Lacava & Sousa, 2016].

In addition, there is also evidence that the endophytic can penetrate the host plant enzymatically degrading the cell walls of plant cells by producing cellulases and pectinases or [Azevedo et al., 2000; Deshmukh et al., 2015]. This infection can also occur vertically through host seeds. In this context, the endophyte can colonize a plant throughout its life [Deshmukh et al., 2015; Lacava & Sousa, 2016], where this association can be maintained in part due to the production of cellulases that allow the saccharification of the plant cell walls [Zhang et al., 2017].

The production of biologically active metabolites, particularly plant growth regulators, is considered one of the most important mechanisms of action. These can act in the solubilization and mineralization of phosphate in the soil, being able to increase the use of other less soluble sources. They can also produce phytonutrients, such as indole acetic acid (IAA), which acts on root and root growth, thus increasing the nutrient and water surface of absorption [Godinho & Bhosle, 2013; Lacava & Sousa, 2016].

The great advantage of the application of these microorganisms is the reduction of the use of phosphate fertilizers. These exhibit characteristics of better use of natural phosphates and, other nutrients [Lacava & Sousa, 2016]. In addition, the microbiome may induce a systemic resistance response in the plant that is typically effective against multiple pathogens and insect pests [Philippot et al., 2013]. Several studies showed how those interactions can occur in rhizospheric space and influence communities above ground.

Liu et al., 2017 studied the action of siderophores produced by *Paenibacillus illinoisensis* and *Bacillus* sp. in peanut crops in calcareous soils. After the inoculation of the siderophores YZ29 and DZ13, the iron and chlorophyll content of the leaves, root activity, total nitrogen, phosphorus and potassium reserve, as well as grain quality and biomass were significantly improved in relation to controls.

Xiang et al., 2017 evaluated the action of rhizobacteria in the control of *Heterodera glycines* in soybean crops. In this study, a bacterium of the genus *Bacillus* could generate more than 50% mortality of this parasite. In the same year, Ossowick et al., 2017 studied the isolation of volatile compounds produced by *Pseudomonas donghuensis*. Strong

antifungal properties and significantly inhibition the phytopathogens *R. solani*, *F. culmorum*, *V. dahliae* and *P. ultimum* were shown.

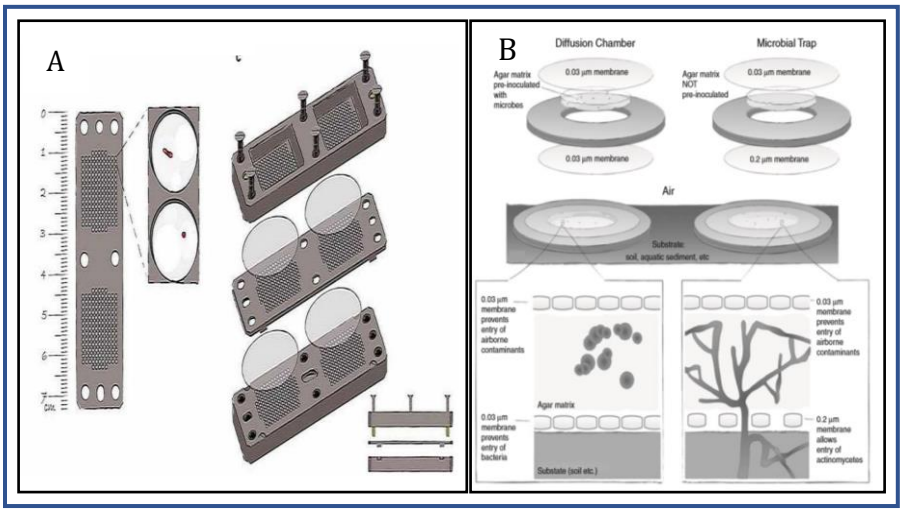
Therefore, the biotechnological applications of these different groups of microorganisms that live in association with plants consist in an unexplored source of bioproducts.

2.3.1. Bacteria

Usually, bacteria are associated with diseases, but the majority are harmless and even beneficial. As an example, bacteria of the genus *Rhizobium* spp, *Bradyrhizobium* spp and *Frankia* spp. which play a key role in the fixation of molecular atmospheric nitrogen. Other genera such as *Corynebacterium* spp, *Bacillus* spp and *Microbacterium* spp have great biotechnology importance due to their ability to excrete metabolic products as antimicrobial and enzymes [Hameed et al., 2004; Gage, 2004; Simon et al., 2014].

A high importance major study conducted by Ling et al., 2015 using a device named as iChip (Figure 9) could explore the isolation of non-cultivable soil bacteria.

Figure 7: A: Description of the iChip device. **B:** Schematic capture of the non-cultivable soil microbiota by iChip.



Source: Adapted from Ling et al., 2015; Lewis et al., 2010.

In this work the bacterium *Eleftheria terrae* was also identified, which produces a new antibiotic, teixobactin, a potent substance that showed strong inhibition against multi resistant pathogen strains such as methicillin resistant *S. aureus* (MRSA).

2.3.2. Actinomycetes

In terms of metabolites production, the *Streptomyces* genus stands out about the other microorganisms because of their variety of bioactive substances and secondary metabolites of economic interest, since more than 80% of the antibiotics produced industrially are processed by this group of microorganisms [Bérdy, 2005; Qin, 2011; Piza, 2015; Matsumoto & Takahashi, 2017].

In this work the potential of inoculation of the isolates against pathogens and the search of clusters of biosynthetic genes were investigated. And many other species of actinomycetes such as *Streptomyces argenteolus*, *Streptomyces galilaeus*, *Streptomyces peucetius*, *Microbispora amethystogenes*, *Micromonospora yulongensis*, and *Nocardioides albus* have also been described in the literature as endophytic [Lacava & Sousa, 2016].

Caraballo-Rodríguez et al., 2017 evaluated the production of natural compounds produced in cultures of actinomycetes and fungi isolated from the Brazilian medicinal plant *Lychnophora ericoides*. According to authors, 29 bioactive compounds were identified as polycyclic macrocycles, pyrrole indole alkaloids, angucyclines and leupeptin, where two compounds correspond to a new fungal metabolite and a new angucycline derived from actinobacteria.

In recent years another aspect that is gaining plenty of space in the research for new substances are volatile organic compounds (VOCs). It is produced in different combinations and quantities by microorganisms as metabolites and it can generate characteristic odors for certain species of bacteria. When these molecules are released, it can provide several activities such as plant growth and microbicide [Insam. & Seewald, 2010; Hofmann, 2013; Cordovéz et al., 2015].

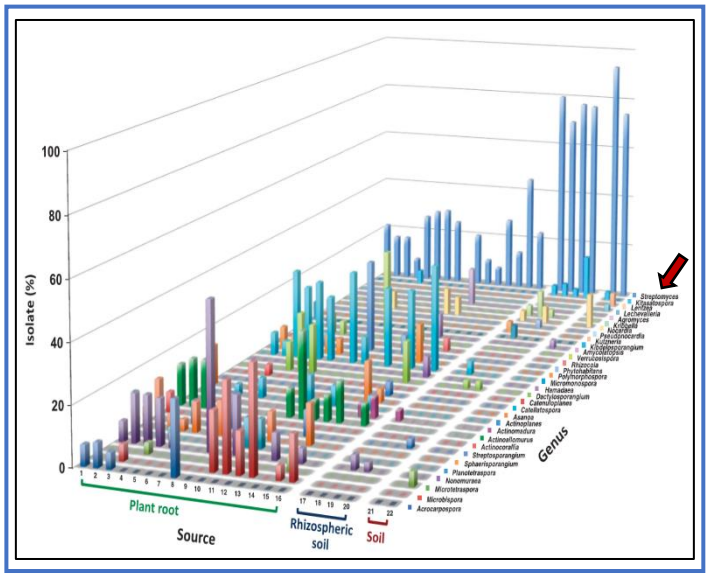
Cordovez et al., 2015 tested the antifungal activity and growth promotion by VOCs produced by several *Streptomyces spp*. isolated from rhizosphere of sugar beet plants

grown in a soil suppressive to *R. solani*. In this study, all strains of *Streptomyces* were able to retard the growth of *R. solani* and promote the growth of *A. thaliana*.

Schöller et al., 2002 characterized several VOCs from actinomycetes, which were sulfur compounds, isoprenoid compounds, isoprene, acetone, 1-butanol, 2-methyl-1-propanol, 3-methyl-3-buten-1-ol, 3-methyl-1-butanol, 2-methyl-1-butanol, cyclopentanone, dimethyl disulfide, dimethyl trisulfide, 2-phenylethanol, and geosmin.

The study of Matsumoto & Takahashi, 2017 included the isolation of actinomycetes endophytically associated with the roots and rhizosphere of several medicinal plants in Japan (Figure 10).

Figure 8: Diversity and isolation frequency of actinomycetes in different structures of medicinal plants from Japan. The numbers 1–3 correspond to Mondo grass (*Ophiopogon japonicus* ker-Gawler), 4–7; Kinginso (*Goody procera*), 8–9; Common Sorrel (*Rumex acetosa*), 10; Chusan Palm (*Trachycarpus fortunei*), 11; Mugwort (*Artemisia indica* var. maximowiczii), 12; Dokudami (*Houttuynia* sp), 13; Prickly Sow-thistle (*Sonchus asper*), 14; Fuki (*Petasites japonicus*), 15; Hellebore (*Helleborus orientalis*), 16 is *Pteridophyta* sp. In terms of diversity, streptomycetes (red arrow) were -predominant in surface soil (21 and 22) at the depths of 1 and 5 cm respectively, and rhizosphere (17-20) of Mondo grass and Kinginso.



Source: Adapted from Matsumoto & Takahashi, 2017.

The multidrug resistant *A. baumannii* is described as a dangerous agent by the Society of Infectious Diseases of America (SIDA). Following the introduction of antibiotics in clinical use, *A. baumannii* has become a major threat in intensive care units (ICUs) due to the development of resistance to broad-spectrum antibiotics [Huggins et al., 2016; Sommer et al., 2017; Chen et al., 2018]. *Streptomyces gandocaensis* isolated from the marine sediment of the island of Punta Mona, Costa Rica was able to produce new substances called cahuitamycins. These compounds showed potent inhibition of biofilms produced by *Acinetobacter baumannii* [Park et al., 2016].

In this way, actinomycetes can be considered as alternative suppliers of characteristic phytochemical compounds. In addition, they represent a huge unexplored reservoir of unique chemical structures. Thus, there are great opportunities to find new secondary metabolites with important biological activities.

In this context, other sources for obtaining these microorganisms have attracted the attention in recent years. The study conducted by Magarvey et al. (2004) identified a new taxon (*Micromonosporaceae*) in actinomycetes from marine sediment collected in Papua New Guinea.

2.3.3. Fungi

Fungi are considered a very rich and diverse source of natural products, including antitumor, cholesterol reducers, immunosuppressants and antibiotics [Strobel, 2004; Pan et al., 2017].

Tong et al., 2011 isolated endophytic fungi from the medicinal plant *Orthosiphon stamineus*, where 92% of them exhibited significant inhibitory activity against different species of bacterial pathogens and filamentous fungi. In same year Cao & Clardy identified 2 new naphthoquinones, delitzchianones produced by *Delitzchia winteri* and one pestalopyrone produced by *Phomatospora bellaminuta*. These compounds secreted by endophytic fungi isolated in Costa Rica have high antimalarial activity.

In a study conducted by Newmister et al., 2016, the fungus *Penicillium oxalicum* was isolated from marine sediment. This work described the implementation of a nitrone

synthase as a biocatalyst as well the characterization and enzymatic activity of OxaD, a potent alkaloid with insecticidal, antibacterial, antitumor and antiparasitic activity.

Several research groups in Brazil have already been able to chemically purify and characterize compounds with great biotechnological potential in biomes, such as the Amazon and Cerrado. Borges et al., 2011 structurally characterized six new azafilones produced by the endophytic fungus *Chaetomium globosum* isolated from leaves of *Tithonia diversifolia*. These are polyketides produced naturally by ascomycetes and some genera of the family *Xylariaceae*. They have wide biological activity that includes inhibition of the enzyme monoamine oxidase and antimicrobial activity.

The antimicrobial and anticancer activities were evaluated by Carvalho et al., 2012, in several fungi isolated from *Stryphnodendron adstringens*. The extract of *Nigrospora oryzae* promoted a selective antifungal activity and was able to inhibit the growth of *C. albicans* and *C. sphaerospermum*. Considering that the extracts of *Diaporthe phaseolorum* and *Xylaria* spp. were presented potent anticancer activities.

The endophytic fungal communities associated with *Myrciaria floribunda*, *Alchornea castaneifolia* and *Eugenia aff. bimarginata* were examined for their ability to produce antimicrobial activity. Thirty isolates were shown for antimicrobial activity against various pathogens with zones of inhibition varying from 7 to 35 mm in diameter [Vaz et al., 2012].

The hypoglycemic activity and other medicinal effects of the extract of *Baccharis spp.* are known a long time ago [Xavier et al., 1967; dos Reis Lívero et al., 2016]. Based on this concept, Vieira et al., 2014 isolated the fungal endophytes from *Baccharis trimera*. The main objective of this study was to test the antimicrobial activity of fungi obtained from leaves. The extracts obtained from *Epicoccum spp*., *Pestalotiopsis spp*., *Cochliobolus lunatus*, and *Nigrospora spp*. were presented the best minimum inhibitory concentration values against bacterial pathogens.

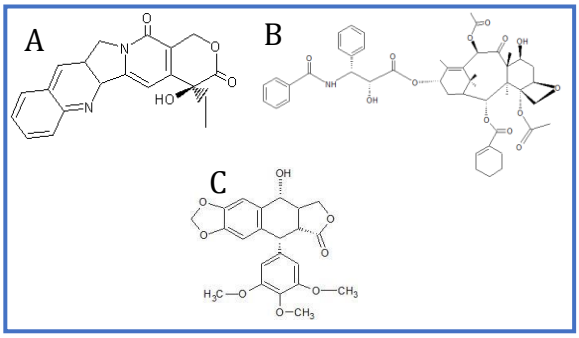
2.4. Medicines produced by microorganisms

The discovery of medicines in treatment of infectious diseases represents one of the greatest accomplishments of humankind. The introduction of antibiotics made it possible to treat diseases that were incurable before, allied to the discovery that

phytochemical compounds can also be produced by the plant-associated microbiome, has allowed the effective treatment of these, promoting the increase in longevity. These products of natural origin consist in metabolites of great importance in the interactions between the endophyte and the host plant, and may act in several processes, such as signaling, defense and symbiosis regulation [Strobel et al., 2004; Matsumoto & Takahashi]. However, the major classes of antibiotics were discovered between 1940 and 1962, and several decades passed without significant innovations until the discovery and development of oxazolidinones in 2010 (Figure 15). Currently there are several substances with antibiotic properties, however, few are interesting in clinical applications [Malajovich, 2012; Nicolaou & Rigol, 2017].

High impact studies have reported different compounds with powerful antimicrobial and antineoplastic activity (Figure 11), such as Camptothecin. Described by Wall et al., 1966 for the first time, this alkaloid is used as antitumor and antileukemic. The producer of this substance is the endophytic fungus *Entrophospora infrequens* isolated from the plants *Camptotheca acuminata* and *Nothapodytes foetida* [Musavi et al., 2015]. We can also mention another important compound that is widely used in the treatment of human papillomavirus (HPV) tumor lesions named podophyllotoxin. It was isolated for first time from *Podophyllum* plants and produced by endophytic fungi such as *Phialocephala fortinii* [Imbert, 1998; Eyberger et al., 2011].

Figure 9: Chemical structures of the main metabolites produced by endophytes (**A**) Camptothecin. (**B**) Paclitaxel. (**C**) Podophyllotoxin. Obtained using ACD/ChemSketch 2018.1.1.



Source: Author.

Paclitaxel (Taxol®), an important drug with antimitotic activity used against several malignancies was first isolated from a *Taxus brevifolia* plant, a native tree from Pacific [Wani et al., 1971]. However, *T. brevifolia* is currently almost extinct, taking between 100 to 200 years to reach maturity. In addition, to yield 1 kg of paclitaxel requires about 10,000 kg of bark *T. brevifolia*, which is equivalent to about 3,000 trees. The alternative was found by Stierle *et al*., 1993, they discovered that paclitaxel is also produced by *Taxomyces andreanae,* an endophytic fungus.

Brartemicin is produced by *Nonomuraea* sp., isolated from the Brazilian medicinal plant, *Artemisia vulgaris*, is a new antibiotic and can inhibits metastasis and tumor invasion processes [Igarashi *et al*., 2009].

Beauvericin is a powerful agent. It has insecticidal properties and can promote apoptosis in mammalian cells. Also, was described as a mycotoxin of the *Eniatina* family produced by several species of *Fusarium* spp. [Taevernier et al., 2016]. Recently, Campos et al., 2015 isolated several fungi from *Caesalpinia echinata*. In this study, it was demonstrated that Beauvericin has a potent cytotoxicity activity against *T. cruzi*. The other strains such as *Xylaria spp, Epicoccum sorghi, Fusarium spp, Nectria pseudotrichia, Talaromyces spp,* and *Aspergillusspp* were effective on growth inhibition of *B. cereus*; *C. albicans*; *C. tropicalis*; *E. coli*; *K. oxytoca*; *P. aeruginosa*; *S. aureus*; *S. typhimurium*.

According to these studies, the bioprospection of microorganisms in Brazilian medicinal plants can contribute to the research of new bioactive substances, since these biomes consists of an unexploited source of bioactive compounds.

2.5. Biotechnological Applications

2.5.1. Enzyme technology

Enzymes are defined as macromolecules catalysts, being able to increase the speed of the various biochemical reactions that occur in cells, where their biotechnological applications extend from the food industry to the pharmaceutical industry. In this context, microorganisms that are skilled in producing these biomolecules are widely used to obtain it, whereas filamentous fungi are the are used as a source of industrial enzymes due to their excellent extracellular protein production capacity [Corrêa et al., 2014; Lacava &

Sousa, 2016]. Endophytic fungi such as *Acremonium terricola*, *Aspergillus japonica*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Fusarium lateritium*, *Monodictys castaneae*, *Nigrospora sphaerica*, *Penicillium aurantiogriseum*, *Penicillium glandicola*, *Pestalotiopsis guepinii*, *Phoma tropica*, *Phomopsis archeri*, *Tetraploa aristata* and *Xylaria* sp. are examples of endophytes with potential for production of pectinases, cellulases, xylanases and proteases [Nair & Padmavathy, 2014].

Cord-Landwehr et al., 2016 identified and characterized the enzyme chitin deacetylase (PesCDA) isolated from the endophytic fungus *Pestalotiopsis* sp. The authors show that endophytes adopt survival strategies to avoid detection by the immune system of the plant. Since the cell walls of these microorganisms commonly contain chitin, it is possible that they modify chitin using chitin deacetylase enzymes.

In the study by Zhang et al., 2017, the potential of cellulase activity in endophytes isolated from different parts of *Angelica sinensis* was evaluated. In this work, three strains with high cellulase activity were identified.

Katoch et al., 2017 isolated an endophytic strain of *Aspergillus* from *Viola odorata* Linn with ability to inhibit the pancreatic lipase enzyme. The results showed that the extracts studied have great potential in the development of new treatments against obesity.

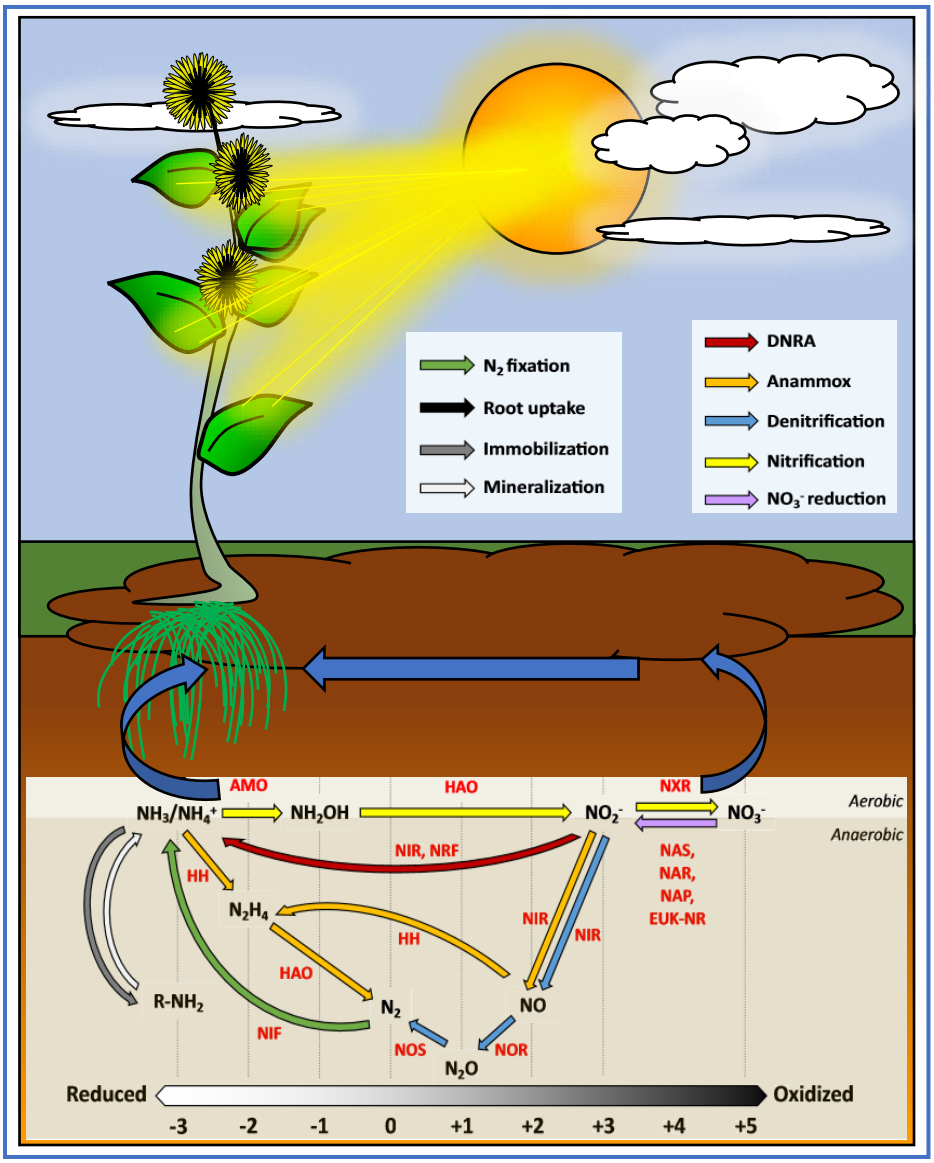
2.5.2. Agricultural systems: Promotion of plant growth & biological control

Modern agriculture is completely dependent on the use of agrochemicals, which has repercussions on the environment and human health, whereas, in recent years, issues related to sustainability and practices in the defense of the environment have gained great notoriety [Lacava & Sousa, 2016].

The emerging alternative for such practices is the biotechnological use of the microbiome associated with plants. Since Brazil has a predominantly agricultural GDP, these technologies associated to the great diversity can leverage in a significant way the processes of obtaining species more resistant to pests and diseases. According to Lacava & Sousa, 2016, microorganisms associated with plants can act directly and indirectly in the promotion of plant growth. Since these can solubilize substances like phosphate or even produce hormones, siderophores, antibiotics and enzymes.

Nitrogen (N_2) is an important nutrient for the development of plants. Like phosphorus, these are part of the composition of the DNA. Although these are present in large amounts in the atmosphere and soil respectively, N_2 is found in molecular form, which is not absorbed by plants. Under natural conditions, diazotrophic bacteria process atmospheric N_2 (Figure 12) for forms that can be assimilated by plants through the enzyme nitrogenase [Lacava & Sousa, 2016; Norman & Friesen, 2017].

Figure 10: Detailed diagram of the N_2 cycle in the soil.



Source: Adapted from Coskun et al., 2017.

Another important nutrient for plants, iron (Fe), as well as the other elements mentioned above, needs to be converted to Fe^{2+} to be absorbed by plants. In this context, several microorganisms have metabolic routes for Fe^{3+} chelation. The siderophores capture the Fe molecules by binding to membrane receptors, and consequently Fe is absorbed by the host plant. In addition, the chelation of Fe by siderophores can indirectly eliminate pathogens by stimulating the biosynthesis of other antimicrobial compounds [Lacava & Sousa, 2016].

In natural conditions, Phosphorus (P) is predominant in the inorganic form, insoluble and associated with metallic ions, or in its organic form, that are not absorbed by the plants. However, rhizosphere microorganisms such as *Pseudomonas* spp., *Bacillus* spp., *Rhizobium* spp., *Burkholderia* spp., *Achromobacter* spp., *Agrobacterium* spp., *Micrococcus* spp., *Flavobacterium* spp. and *Erwinia* spp. act in the solubilization of the phosphate, making it available to the plants [Lacava & Sousa, 2016].

In addition, the ability to synthesize phytohormones such as indole-acetic acid (IAA) has been widely explored. This nutrient is found up to 80% of the bacteria associated with plants. The IAA is responsible for stimulating rapid and long-term responses in plants, such as cell division and differentiation [Lacava & Sousa, 2016].

In other hand, the entomopathogenic characteristics of many microorganisms are also widely explored in agricultural systems in pest control, as well as the production of phytochemicals with antifungal activity in prophylaxis against phytopathogens [Lacava & Sousa, 2016]. Chitin is a polysaccharide that composes the exoskeleton of arthropods. *A. grandis* is an example of a pest that attacks cotton crops. This is controlled only with high cost agrochemicals, besides promoting ecological risks as the rupture of populations of predators and parasitoids. In this sense, the study by Quecine et al., 2011 involved the production and characterization of a chitinolytic extract produced by *Streptomyces* sp. endophytically isolated from citrus. In addition, the extract showed high levels of inhibition against several phytopathogenic fungi.

Xylella fastidiosa transmitted by sharpshooter insects (*Oncometopia facialis*) is an economically important pathogen that causes Citrus Variegated Chlorosis in citrus crops and other specific strains can cause diseases in other cultures such as grape, almond, peach, coffee and plum. Pria Júnior et al., 2008 investigated the efficacy of the

entomopathogenic fungus *Metarhizium anisopliae* to control the sharpshooter *O. facialis* under conditions of bioassay in citrus plants. The results showed that the sharpshooter was susceptible to the entomopathogenic action and the colonization of *O. facialis* occurred between 24 and 72 hours.

2.6. Other biotechnological applications

2.6.1. Biopigments

The pigments produced by microorganisms represent a great biotechnological potential in various activities such as food, cosmetics, textile, construction, pharmaceutical and diagnostic industries. In this context, these may be an alternative to animal and plant dyes [Frisvad et al., 2013].

Studies show that mixed microbial cultures can also induce the production of molecules that are not produced under usual culture conditions. Onaka et al., 2011 have shown that mixed cultures of *Tsukamurella pulmonis* and actinomycetes like *Streptomyces lividans* can induce the production of different pigments and secondary metabolites. According to the authors, substances such as mycolic acid present in the cell wall of *T. pulmonis* act in this process of activation of cryptic genes.

2.6.2. Bioremediation

The bioremediation process explores the metabolic properties of organisms to degrade contaminants. However, studies indicate that plant-endophyte collaboration can play a key role in the degradation of harmful contaminants in the rhizosphere [Stępniewska & Kuźniar, 2013; Nair & Padmavathy, 2014].

Nowadays, polyester polyurethane (PPU) composite is a large pollutant that is difficult to break down. In order of explore the ability of metabolites production that degrade the plastic, Russel et al., 2011 used endophytic fungi, where *Pestalotiopsis microspora* was able to grow exclusively in PPU as the only source of carbon under aerobic conditions and anaerobic. The study also suggests that the enzyme serine hydrolase is responsible for the degradation of PPU.

2.6.3. Biofuels

In recent years, biofuels in the form of plant-derived lipids and ethanol from the fermentation of sugars and starch have gained prominence and are providing an alternative to the use of fossil fuels [Strobel, 2014; Strobel, 2015; Liao et al., 2016].

Studies have found that various endophytic fungi, such as *Muscodor albus*, can produce volatile hydrocarbons (Micodiesel) while growing on agricultural residue substrates [Strobel, 2014; Strobel, 2015]. According to Wu et al., 2017, these fungi produce lignocellulolytic enzymes that convert lignocellulose to micodiesel. In addition, this study analyzed the potential of micodiesel production in four endophytes *Hypoxylon* sp. CI4A, *Hypoxylon* sp. EC38, *Hypoxylon* sp. CO27 and *Daldinia eschscholzii* EC12. The analysis of their genomes showed the presence of clusters of genes of active enzymes of carbohydrates, suggesting that these species can produce the micodiesel.

2.6.4. Effluent treatment

Among the components present in the sewage system, microorganisms deserve special attention due to their action in the treatment of effluent, acting in the removal of organic matter, especially of its biodegradable part, present in the sanitary, industrial liquid effluents and in the percolates of sanitary landfills, reducing the risks of environmental pollution and the contamination of rivers and lakes [Von Sperling, 2005; Borrel et al., 2012]. In this context, aerobic and anaerobic routes are used to treat sewage, where the aerobic pathway consists of accelerating the process of oxidation and natural decomposition of the organic matter that occurs in the receiving water bodies. The organic matter is converted into bacterial biomass and a part is mineralized to CO_2 and H_2O. The bacterial biomass can be separated from the treated waste by simple decantation [Von Sperling, 2005].

In contrast, the anaerobic route consists of a association of different species of microorganisms that, in the absence of molecular oxygen, promotes the transformation of complex organic compounds into simpler products such as methane and carbon dioxide. The different groups of microorganisms involved in anaerobic digestion are very

specialized and each group acts in specific reactions. Among methanogenic bacteria, *Methanosaeta* spp, *Methanosarcina* spp. stands out [Sun et al., 2014].

Methane, produced by hydrogenotrophic methanogenic archaea that act by reducing CO_2 to methane and using H*2* as an electron donor, releasing H_2O. In other hand the acetotroph microorganisms produce methane and CO_2 from the reduction of the acetate. The last one is a crucial in anaerobic reactors, since about 70% of the methane produced in these systems results from the degradation of acetate [Sun et al., 2014].

The advantages presented over aerobic processes are that they do not require aeration, they have reduced production of excess sludge and the generated methane gas can be reused as an energy resource. However, these reactors are less efficient in relation to aerobic processes, necessitating a post-treatment system to remove pollutants [Borrel et al., 2012; Sun et al., 2014].

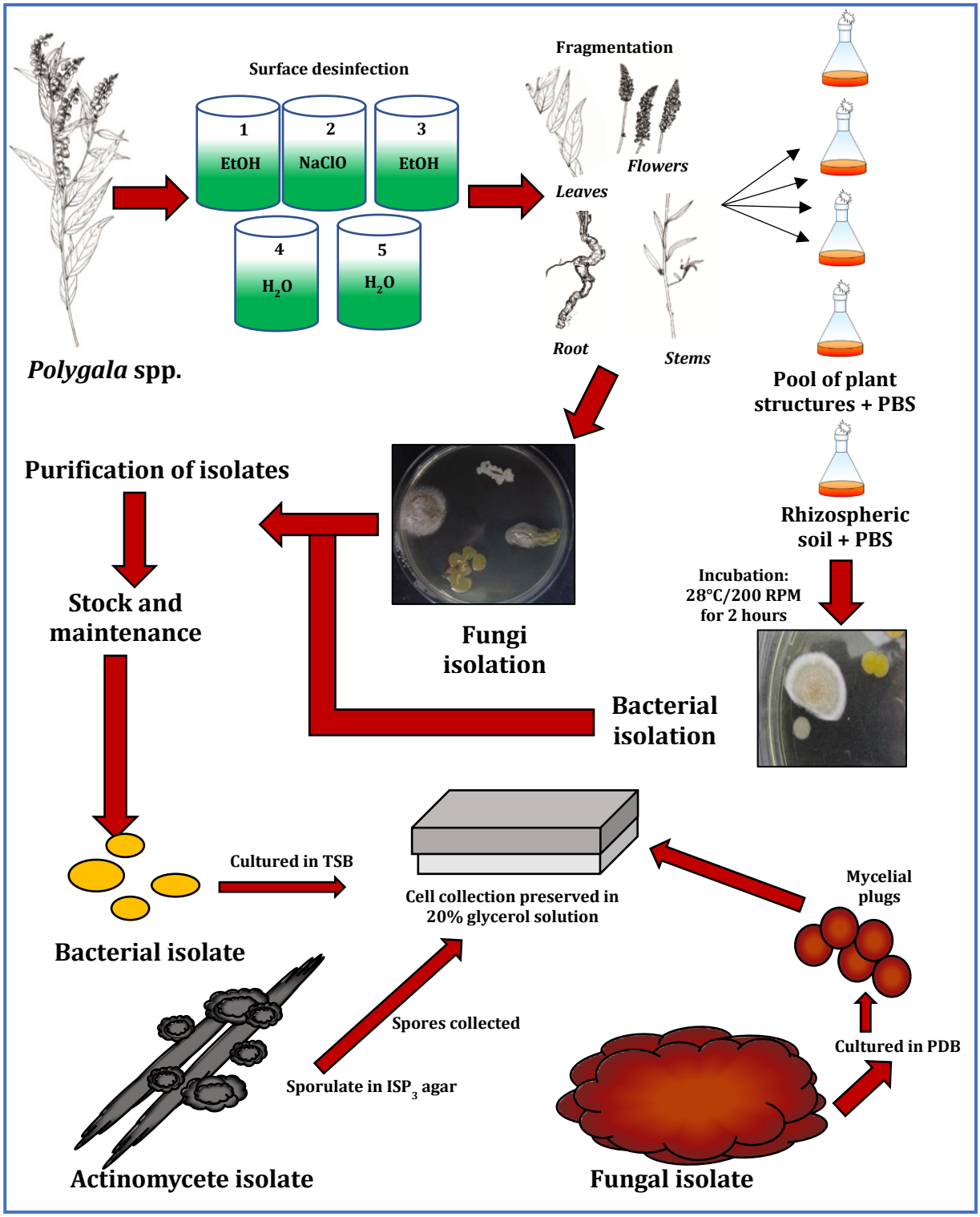
Material & methods

3. Material & methods

3.1. Sample collection and processing

The samples of *Polygala* spp and rhizospheric soil were collected in the city of Peruíbe – SP - Brazil (Latitude: -24º 19 '12 " S/ Longitude: 46º 59' 54" W) and transported in pots containing the original soil to the Laboratory of Microbiology and Biomolecules (LaMiB) at Federal University of São Carlos – SP – Brazil (SisGen – registration number: AF1A75A). In the first stage, superficial disinfection of the plant structures was carried out to eliminate the epiphytic population. This process consisted in serial washes in 70% ethanol for 2 min, NaClO for 3 min, 70% ethanol for 1 min and a double rinse with distilled H_2O. Subsequently, each plant structure (roots, stems, leaves and flowers) was incubated in phosphate-buffered solution (PBS) (NaCl: 8.0 g/L; KCl: 0.2 g/L; Na_2HPO_4: 1.44 g/L; KH_2PO_4: 0.24 g/L; pH: 7.4) under agitation at 28°C. Subsequently, decimal dilutions were performed and 100 µL aliquots were added in Petri dishes containing TSA supplemented with Benomyl (50 µg/mL), and again incubated at 28°C until bacterial growth. For the isolation of actinomycetes, ISP_2 medium (*International Streptomyces Project* – Malt extract: 10 g/L; Yeast Extract: 4 g/L; glucose: 4 g/L) was used. Finally, for fungi isolation, fragments of each plant structure were placed onto plates containing Potato Dextrose Agar (PDA) medium and incubated at 28 °C until fungal microbiota growth (Figure 13) [Araújo et al., 2014] For rhizospheric population, 10 g of rhizospheric soil from both species were placed in individual Erlenmeyer flasks containing 90 ml of sterile PBS. Samples were incubated in same conditions and 100 µL of decimal dilutions were inoculated in respective medium and incubated at same conditions until growth [Andreote et al., 2008].

Figure 11: Schematical representation of epiphytic microbiome removal and process for obtaining the population associated to *Polygala* spp.



Source: Author.

Part 1: Assessment of the biotechnological potential of isolates

3.2. Detection of enzymatical activity

Firstly, a pre-culture was grown in 3mL of tryptic soy broth (TSB) and incubated for 48 to 72 hours at 28°C. A 2 µL of the culture were transferred to M9 enzymatical solid medium [200 mL/L of stock solution (64 g/L $Na_2HPO_4.7H_2O$; 15 g/L KH_2PO_4; 2.5 g/L NaCl; 5 g/L NH_4Cl)]; 2.0 mL/L 1 M $MgSO_4$; 10 g/L; 0.1 mL/L $CaCl_2$ 1 M; 15 g/L agar, pH 7.2, supplemented with (0.5%, yeast extract, and 1% soluble starch for amylase activity); cellulase activity (0.5% yeast extract and 1% carboxymethylcellulose), pectin-pectate lyase (0.5% yeast extract and 1% pectin, pH 8.0) and pectin–polygalacturonase (0.5% yeast extract and 1% pectin, pH 5.0). The lipase/esterase media consisted in: peptone - 10 g/L; NaCl - 5 g/L; $CaCl_2.H_2O$ - 0.1 g/L; agar - 15 g/L; pH 7.4, supplemented with 1% (v/v) of Tween 20 and Tween 80 for lipolithic and esterastic activities respectively. For protease medium was used (5 g/L of tryptone; 2.5 g/L of yeast extract; 1.0 g/L of glucose; 2.5 g/L of NaCl; 15 g/L of agar; pH was adjusted to 7.0); after autoclaving, 100 mL skimmed milk was added to medium to one liter.

The isolates were incubated for 48 h at 28°C. The visualization of the cellulase activity consisted in using Congo red-dye as revealer, subsequently a wash of NaCl 5 M. Amylase and pectinases were used iodine tincture. For protease, lipase and esterase activity the enzymatic production could be visualized as a bright halo around the colonies (Oliveira et al., 2006; Specian et al.,2016).

Part 2: Investigation of antimicrobial activity

3.3. Evaluation of the antimicrobial activity of isolated fungi

The isolated fungi were grown in 100 mL of potato dextrose broth (PDB) for 10 days at 28 °C. After this growth period, the fermentation broths (FB) were collected by filtration using a polypropylene mesh. From an overnight culture at 37 °C in BHI (Brain-heart infusion agar), the target strains (*Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 11775 and *Candida albicans* ATCC 10231) obtained from cultures belonging to the

Microbial Cultures Collection of LaMiB – UFSCar – SP – Brazil were diluted at an OD_{600} of 0.3 to 0.5 and diluted 1:10 and then inoculated into Petri dishes containing BHI agar. A volume of 100 µL of the FBs was inoculated into 6 mm diameter wells made in agar plates containing the previously seeded microorganisms. The plates were incubated at 37°C for 12-24 hours in order to evaluate the activity of FBs.

3.4. Antimicrobial screening for bacteria and actinomycetes

The Overlay test [Piza et al., 2015] was carried out for a primary selection on solid media. For an initial selection, the isolates were pre-inoculated and grown at 28°C (the bacterial isolates in TSA medium for 24-48 hours and actinomycetes in ISP_2 for 7 days). After the reactivation, the isolates were inoculated in the center of plates containing the respective solid medium according to the kind of microorganism and incubated at 28°C for 3-7 days. The pathogens *S. aureus* ATCC 29213, *E. coli* ATCC 11775 and *C. albicans* ATCC 10231 were seeded in BHI broth and incubated at 37°C by overnight. The pathogen strains were diluted to an OD_{600} of 0.3 to 0.5 and diluted 1:10 and then inoculated into Petri dishes containing BHI agar. Then, the isolates were inactivated with chloroform (1 mL, 20 min for exposition, 30 min for evaporation of chloroform residues) and afterwards, 25 mL of semi-solid BHI previously inoculated with test microorganisms was poured onto the inactivated isolate. The plates were incubated at 37°C for 12-24 hours in order to verify the bioactivity. As controls, 200 µg of oxacillin (1 mg/mL) was used as positive and DMSO as negative.

3.5. Natural product extract library

Due to the great biotechnological importance, the group of actinomycetes isolated from the *Polygala* species was selected for assays in the Laboratory of Prof. Dr. David Sherman - Life Sciences Institute - University of Michigan (Shipment registration number at SisGen: R9C14B9).

To build the “Polygala isolates library”, the actinomycetes strains were grown in ISP_3 agar (Oatmeal: 20 g/L; trace salts solution: 1mL/L ($FeSO_4$. $7H_2O$: 1 g/L; $MnCl_2.4H_20$: 1 g/L; $ZnSO_4$. $7H_2O$: 1 g/L); pH: 7.2, Agar: 18 g/L) and incubated for 7 days at 28°C for sporulation. The spores were collected and preserved in 20% glycerol at -80°C.

To build a Natural Products Extracts (NPE) library, a volume of 10 µL were transferred to round bottom (12 mL capacity) tubes containing 3 mL of ISP_2 and incubated at 220 rpm/28°C for 3 days. Subsequently, the cultures were inoculated into 100 mL of ISP_2 in 250 mL capacity Fernbach flasks. The culture was maintained under the same conditions for 7 days. After this time, the culture was centrifuged at 4,500 rpm for 10 min. Subsequently, a solid phase extraction was carried out using polypropylene mesh packages containing 1.5 g of Amberlite® XAD16 resin (Sigma-Aldrich ™) were made and then added to the crude extract. They were overnight incubated on a rotary shaker under the same conditions. After this time, the resin packets were removed and packed into glass tubes containing 20 mL of MeOH: EtOAc (1: 1). Each extract was dried in Speed-vac and resuspended at a concentration of 50 mg/mL in DMSO and stocked at -80 °C for High Throughput Screening (HTS) tests.

In addition, another NPE library was built using the combined culture method [Onaka et al., 2011]. Each isolate and *Rhodococcus erythropolys* strain were individually cultured in 3 mL of V-22 broth (starch: 10 g/L, glucose: 5 g/L, yeast extract: 2 g/L, tryptone: 1 g/L; K_2 HPO_4: 1 g/L; $MgSO_4$ $.7H_2O$: 0.5 g/L; $CaCO_3$: 3 g/L) for 7 days/37 ° C. After this period, mixed cultures (3 mL of the isolate culture with 1 mL of the *R. erythropolis* culture) were transferred to a 100 mL of A-3M broth (glucose: 5 g/L; glycerol: 20 mL/L; starch extract: 3 g/L; Pharmamedia: 15 g/L; Diaion HP-20: 10 g/L) under the same conditions and the crude extract produced according to the methodology described above.

3.6. Antimicrobial activity of NPEs

The actinomycetes strains which presented the highest rates for bioactivity in overlay assay were selected and pre-cultivated in 50 mL ISP_2 for 72 hours/ 28°C. After this period, 5 mL of the culture (OD_{600} = 1.6 to 1.8) was transferred to Fernbach flasks containing 95 mL of ISP_2, and cultured for 3, 5, 7 and 9 days. In each period their respective

extract was produced following the procedure described in 3.5. The target strains (Table 1) belonging to Laboratory of Prof. Dr. David Sherman - Life Sciences Institute - University of Michigan were cultured in appropriate media at 37°C for 12 hours and diluted to an OD_{600}: 0.3 to 0.5 and diluted again to 1:10 in 25 mL of molten media and then inoculated into Petri dishes. The extracts were diluted in DMSO in a concentration of 50 mg/mL. And finally, concentrations of NPEs (200 µg, 50 µg and 10 µg) were inoculated in sterile discs placed onto the plate. As controls, 200 µg of ciprofloxacin (1 mg/mL) as positive and a negative control a sterile DMSO were used. All measurements were performed in duplicates.

Table 1: Pathogenic strains used in tests for antimicrobial activity.

Strain	Code	Medium
Acinetobacter baumannii	ATCC 17978	Müeller-Hinton II
Bacillus anthracis	34F2	BHI
Escherichia coli	TolC	Luria-Bertani/ Müeller-Hinton
UP Escherichia coli	CFT-073	Luria-Bertani/ Müeller-Hinton
Klebsiella pneumoniae	ATCC 29665	BHI
Lysteria monocytogenes	ATCC 19115	Luria-Bertani / BHI
Salmonella enterica	ATCC 14028	Luria-Bertani/ Müeller-Hinton
Shigella flexneri	BS 103	BHI/ Luria-Bertani
MR *Staphylococcus aureus*	ATCC 43300	Luria-Bertani /Müeller-Hinton

3.7. Isolation and purification of bioactive metabolites

The high bioactive extracts were concentrated in vacuo and purified by Reverse Phase (RP) chromatography (column: 30 x 2.6 cm, silica: YMC Gel ODS-A, 12 nm pore, particle size:150 µm) in water and acetonitrile gradient (100: 0 → 0: 100). Each fraction was dried in Speed-vac and resuspended at a concentration of 50 mg/mL in DMSO and tested for antimicrobial activity following the same methodology described in 3.6.

The most active fraction from each NPE was subjected to another step of purification was carried out using a pre-packed reverse phase column chromatography (Sep-Pak-C_{18}, 70 mL volume). As mobile phase an eluent system composed of water and methanol (100: 0 → 0: 100) was used. Each fraction and sub-fraction obtained were evaluated for antimicrobial activity following the same procedure described in 3.6.

3.7.1. Separation and purification of active fractions by HPLC

In a second step, the active fraction obtained from RP chromatography was subjected to RP-HPLC (Shimadzu CBM-20A) using the reverse phase analytical column (Waters XBridge BHE 250 x 10 mm C_{18}; 130Å, 5 µm). The mobile phase was composed of: Milli Q water supplemented with 0.1% formic acid (solution A) and methanol supplemented with 0.1% formic acid (solution B), with a flow rate of 3.0-4.0 mL/min in 30 minutes, where a volume of 10 µL was injected. The samples were eluted in the column in an isocratic manner (80% H_2O) for 100 minutes at wavelength of 200 to 500 nm, and the peaks were collected automatically. The obtained fractions were dried in speed-vac and resuspended in the concentration of 1mg/ml for antimicrobial evaluation tests following the same method described in 3.6.

3.8. Minimum inhibitory concentration of the compounds

This bioassay was based on the work of Li, et al., 2009; Bötcher et al., 2013 and Park et al., 2016. For this purpose, an overnight culture of each pathogen was diluted in culture medium until it reaches an OD_{600} between 0.3 and 0.6. These were again incubated for a period of 2 to 3 hours. After this time, cultures were diluted back to OD_{600} between 0.003 and 0.007. Then, a volume of 95 µL was distributed in each well and in triplicate. To make up to a volume of 100 µL, 5 µL of the compound diluted in DMSO were added in different concentrations. The plates were incubated at 37 °C and the optical density of each well was measured 12 hours after administration of compound BS-39 using a microtiter

plate reader. For positive control, the Ciprofloxacin at same concentration gradient, and the negative control a DMSO diluted at 5% were used.

3.9. Determination of molecular mass of bioactive compounds

For determination of the molecular mass, a LC/MS (Agilent 6520 Q-TOF Mass Spectrometer) analyzes was performed at Life Sciences Institute - University of Michigan by Prof. Dr. Ashootosh Tripathi, using the pure compound dissolved in 1:1 MeOH/H_2O in the concentration of 1 mg/mL.

3.10. DNA isolation

The DNA isolation protocol for the isolate Rizo Pp Ac-11 was optimized using a modification of the method proposed by Huber & Godfrey, 1987 for disruption of the cell wall of Rizo Pp Ac-11. Firstly, a 2-days culture was performed to avoid the secondary metabolites production, then the cells were harvested and washed twice and resuspended in 25% sucrose-Tris buffer (0.05 M, pH 8,0) and 1mg of lysozyme. The suspension was incubated at 37°C with agitation for 30 -60 min. To improve the lysis, was added a volume of EDTA 0,25 M, pH 8,0. Then, the purification of the total DNA was carried out using the protocol proposed by Aljanabi e Martinez, 1997, then, the DNA was eluted in 50-100 µL of ultrapure H_2O and incubated at 37°C for rehydration.

3.11. Statistical analysis

The results were analyzed using the software Graph Pad Prism 8.0.1 (San Diego, California, USA). For all data obtained, the Shapiro-Wilk test was applied. Subsequently, one-way ANOVA (One-way Analysis of Variance) followed by Dunnett's multiple comparisons test using a statistical significance at $p < 0.05$ (95%). Finally, the IC_{50} was measured by non-linear regressions of the values found for each concentration in at least three independent experiments.

Results & Discussion

4. Results and discussion

4.1. Isolation of the endophytic and rhizospheric population

For this study, a total of 30 individuals of the species of purple-flower *Polygala* sp. and 33 individuals of white-flower *Polygala* spp. were collected in April/2016 (Autumn) and September/2016 (Winter) in the city of Peruíbe – South coast of São Paulo State – Brazil (Figure 14). In the first isolation, the number of individuals of the purple-flower species presented greater compared to the white-flower *Polygala* sp. Regarding the microbial population, similar abundances were observed in rhizosphere, except in the white-flower rhizospheric space. The population was larger in the root groups, branches and leaves. On the other hand, in the second isolation, there was a great increase in the availability of individuals of both species. The white flower *Polygala* spp. rhizospheric population increased significantly while the endophytic population showed a slight decrease compared to the first isolation. For the microorganisms associated with the purple-flower *Polygala* spp., there was no significant alteration of the population in any of the analyzed structures (Figures 15 and 16).

Figure 12: Atlantic forest area where specimens of *Polygala* spp. were collected.

Figure 13: Isolation profile of the endophytic and rhizospheric population in *Polygala* spp. The collection was carried out in months of April and September of 2016 containing the number of isolates according to Family and Kingdom.

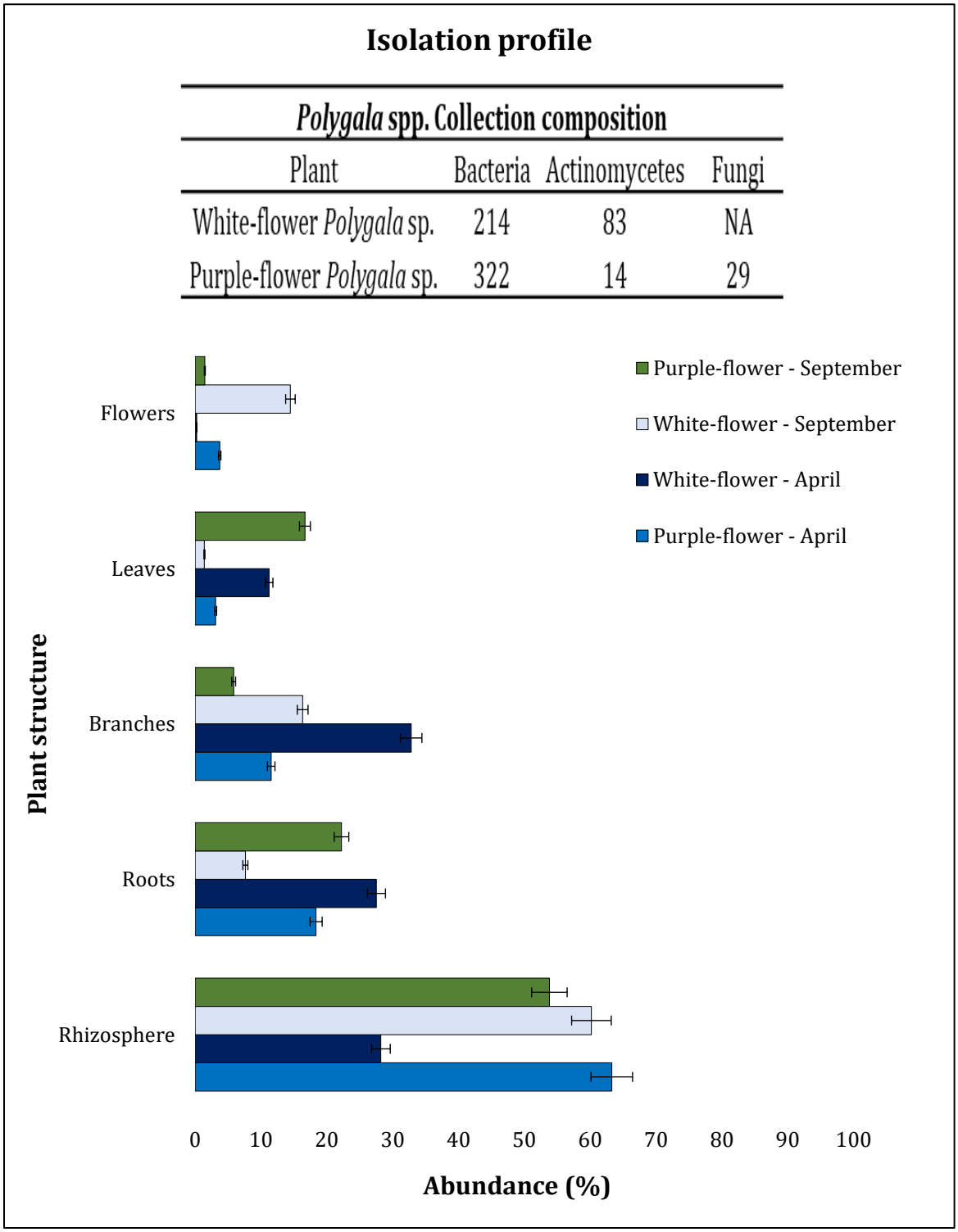
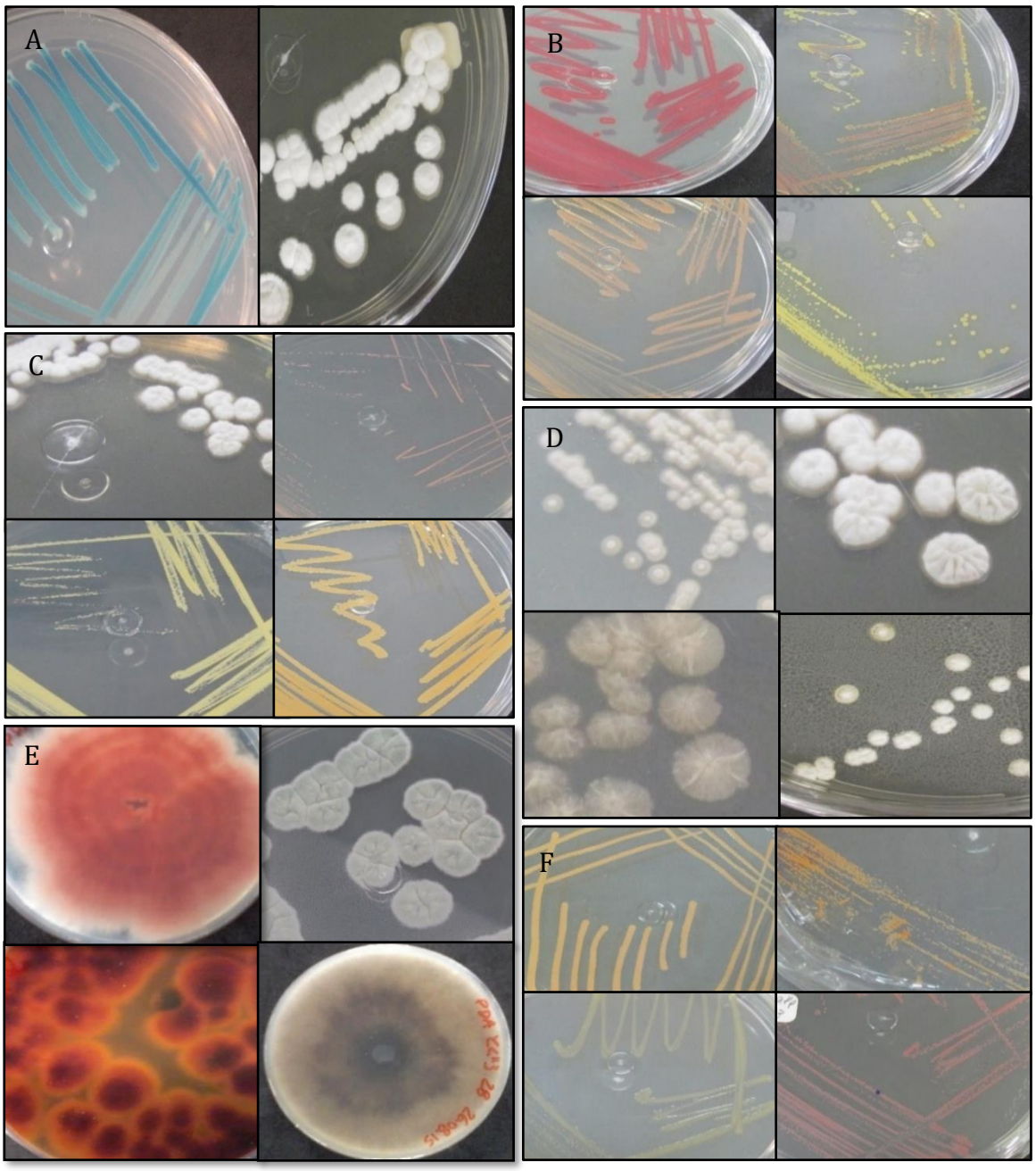


Figure 14: Diversity profile of rhizospheric and endophytic microorganisms isolated from *Polygala* spp.. **A:** Bacteria associated with the white-flower *Polygala* rhizospheric space. **B:** Purple-flower *Polygala* rhizospheric bacteria. **C:** Endophytic bacteria of the purple-flower *Polygala*. **D:** Endophytic actinomycetes isolated from both species. **E:** Endophytic fungi of the purple-flower *Polygala*. **F:** Endophytic bacteria isolated from white-flower specimen.



Part 1: Assessment of the biotechnological potential of isolates

4.2. Detection of enzymatic activity

Analyzing the enzymatic potential of the isolated population associated to purple-flower *Polygala*, it was observed that the most abundant enzymatic activities detected were pectinase at pH 8.0 (pectate lyase), protease and amylase, respectively. Other enzymatic activities, such as lipolytic and estereolytic, were significant in the rhizosphere group. Cellulolytic activity was observed in stem isolates, and pectinase activity at pH 5.0 (polygalacturonase) was detected predominantly in the roots. For the group of bacteria isolated from the white-flower of *Polygala*, the most abundant enzymatic activities detected were pectate lyase followed by protease and amylase, respectively. Again, lipolytic and estereolytic activities were significant in the rhizosphere group. Cellulolytic activity was observed in leaf and branch isolates, and the polygalacturonase enzyme was detected predominantly in the roots (Supplementary tables 1 and 2). The statistical data of the enzymatic activity are presented in Figure 17.

Regarding the production of multiple enzymes (Figure 18), an isolate from the rhizosphere of purple-flower *Polygala* sp. produced six different enzymes (Amylase, lipolytic, proteolytic, esterase, and pectinase in both pH values). Six isolates from rhizosphere, roots and branches produced five enzymatical activities. Thirty-three showed 3 enzymes, 55 isolates showed two different activities and 138 produced at least a unique enzyme (Figure 19).

Figure 15: Profile of the enzymatic production of bacterial isolates from both species of *Polygala* spp. grouped by enzyme and structure.

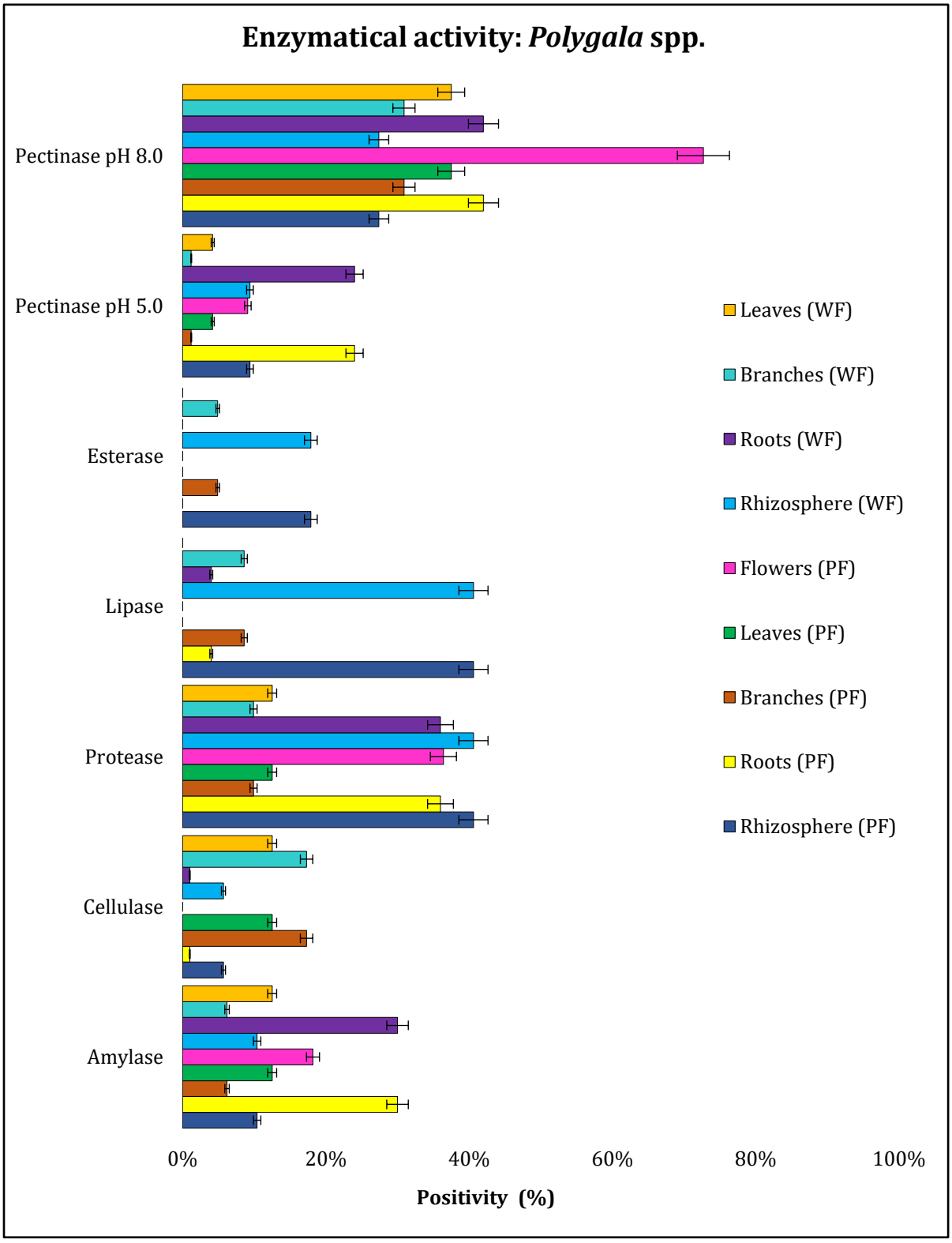
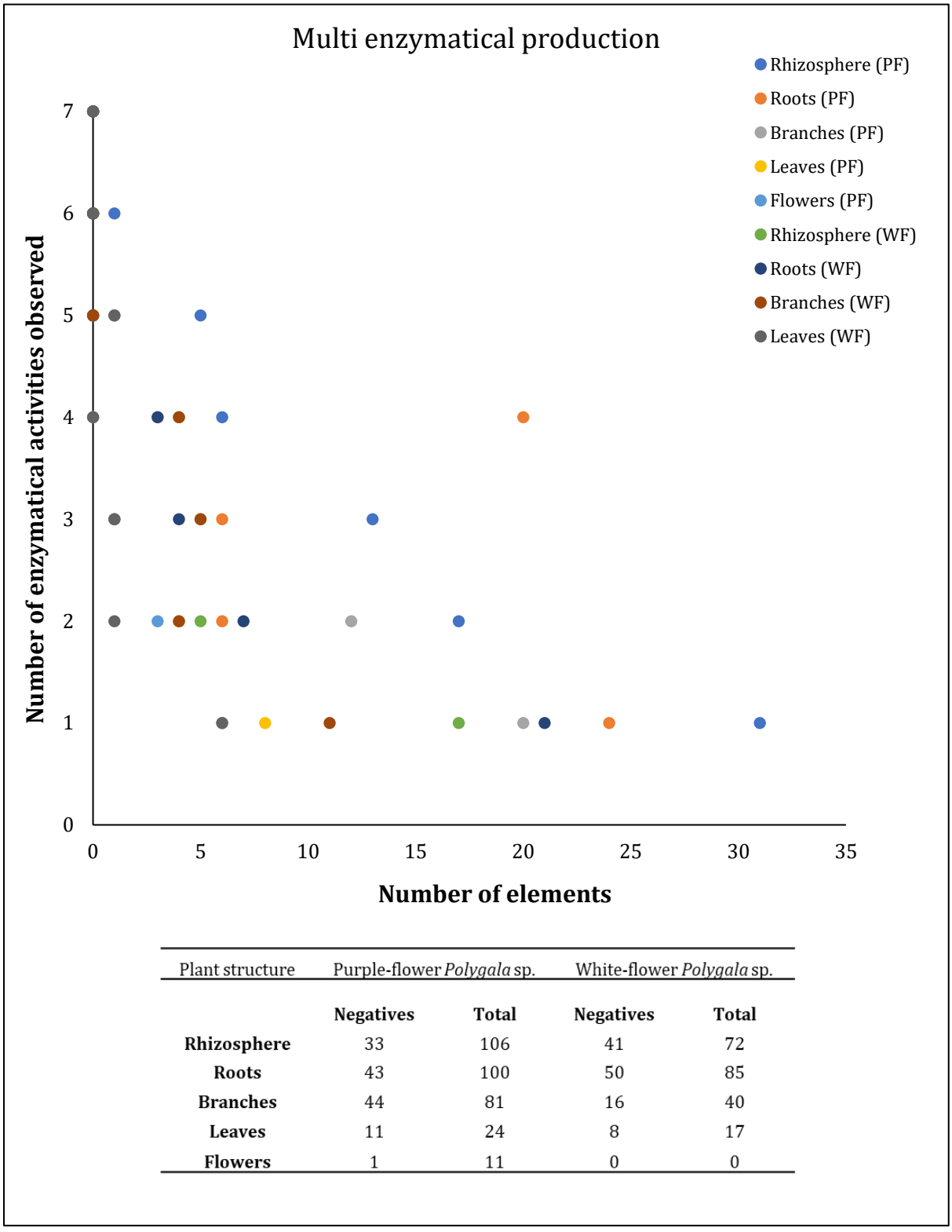
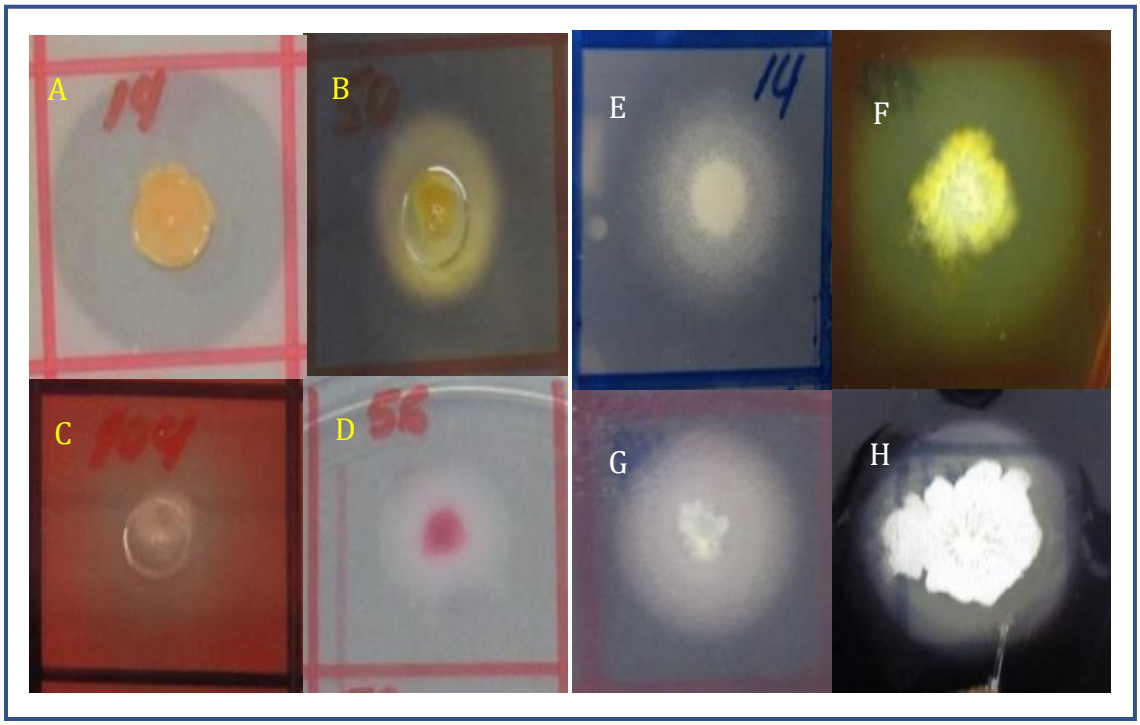


Figure 16: Multi enzymatic production by bacteria associated with *Polygala* spp. grouped by enzyme and structure including population used in each group.



Plant structure	Purple-flower *Polygala* sp.		White-flower *Polygala* sp.	
	Negatives	**Total**	**Negatives**	**Total**
Rhizosphere	33	106	41	72
Roots	43	100	50	85
Branches	44	81	16	40
Leaves	11	24	8	17
Flowers	1	11	0	0

Figure 17: Enzymatical production in different solid media for isolates of purple-flower *Polygala* sp.. **A:** Proteolytic. **B:** Pectin at pH 8.0. **C:** Cellulase. **D:** Esterase. Enzymatic production for white-flower *Polygala* sp. isolates. **E:** Lipase. **F:** Pectin at pH 5.0. **G:** Esterase. **H:** Amylase.



Biotechnological applications involving microbial enzymes have been widespread in recent years. They can degrade or convert phenolic compounds, nitriles and amines, which are present in toxic chemical compounds from industrial and domestic waste. In addition, there are innumerous advantages in their applications such as the minimized ecological impacts, reduced processing time, low energy input, cost effectiveness and nontoxic properties [Singh et al., 2016].

Microbial proteases are classified as acid, neutral and alkaline. They are used in several areas of biotechnology such as food, pharmaceuticals, cosmetics, leather and detergents, representing the major group of industrial hydrolytic enzymes. [Dorra et al., 2018].

Lipases and esterases constitute the most versatile enzyme family of enzymes composed of proteases, dehalogenases, epoxide hydrolases and peroxidases, which

contains an alpha-beta hydrolase fold. These family members share relatively homologous structural arrangements and preserved catalytic site, suggesting a possible common ancestor.

Lipases are capable of making the complete hydrolysis of insoluble fats, oils and esters of fatty acids. Therefore, they are considered to be excellent substances for classical chemical synthesis applications due to their versatility in catalyzing several reactions such as biotechnological processes in food, petroleum, chemical, biodiesel, effluent treatment, pulp, pharmaceutical and detergent industries [Singh et al., 2016; Patel et al., 2018; Lotti et al., 2018].

On the other hand, esterases are differ from lipases in terms of substrate specificity and lack of interfacial activation. Biotechnological applications for these enzymes include the production of pure compounds, pharmaceutical (stereo-selectivity of reactions), food and beverages as a flavor enhancer and in agricultural systems they can hydrolyze insecticidal compounds, paper manufacturing and cosmetology [Singh et al., 2016; Littlechild, 2017].

Baoune et al., 2018 isolated endophytic actinobacteria from naturally-grown plant roots in oil-contaminated sandy soil in Algeria. Petroleum hydrocarbons are well known for their high toxicity and recalcitrant properties. Its increasing use throughout the world has led to environmental contamination. In this sense, phytoremediation with microbe associated with plants is an interesting approach to the degradation of petroleum. The oil-tolerant isolates belonged to the *Streptomyces* genus, which played an important role in the degradation of aromatic hydrocarbons (C_6-C_{30}), aromatic and polycyclic n-alkanes, as well as presented a wide range of plant growth promoting characteristics, such as siderophores, phosphate solubilization, 1-aminocyclopropane-1-carboxylate deaminase, nitrogen fixation and indole-3-acetic acid production and biosurfactant production.

Bibi et al., 2017 isolated bacteria producing hydrolytic enzymes from seven different mangroves collected in the coastal area of Thuwal, Jeddah, Saudi Arabia, where 46 endophytic and rhizospheric bacteria were isolated, such as *Erwinia*, *Vibrio*, *Psychrobacter*, *Aidingimonas*, *Marinobacter*, *Cromohalobacter*, *Halomonas*, *Microbulbifer*, *Alteromonas* and *Bacillus*. Using different enzymatic media, they were able to produce

various enzymes of industrial importance such as cellulase, protease, lipase and amylase. In addition, several isolates were also active against fungal pathogens.

The Indian Tree of Heaven (*Ailanthus excelsa*) is a large tree commonly known in traditional medicine. It contains bitter principles known as quassinoids, and several pharmacological effects have been described including anti-tumor, anti-viral and anti-tuberculosis [Dell'Agli et al., 2008]. In addition, Roy et al., 2018 discovered that the tannic acid could be hydrolyzed in solid media by endophytic actinobacteria obtained from leaves of *Ailanthus excelsa* Roxb.

Amylases are extremely important in industrial processes and are mainly involved in food, paper, detergent, textile and pharmaceutical industries. In the study of Chaiyaso et al., 2018, a new species of red yeast *Sporidiobolus pararoseus* KX709872, was described as a producer of α-amylase and amyloglucosidase. Moreover, these enzymes showed ability to convert rice residues from canteen waste into biomass and lipids.

Cellulases are heterogeneous family of enzymes that catalyze the hydrolysis of cellulose, the main component of plant biomass. In this context, these enzymes have a broad spectrum in terms of biotechnological applications due to their synergistic action. Regarding the economic and sustainable models of bioproducts production, these enzymes has also been used for the development of the bioprocess for the recycling the used printed papers and biofuels production [Singh et al., 2016; Escuder-Rodríguez et al., 2018].

As mentioned before, sustainable models are gaining a prominent position in biotechnological applications. Faced with the inevitable depletion of petroleum supply, the interest in alternative energy sources has increased. In this context, biofuels such as bioethanol are produced from the conversion of biomass from vegetal sources and agro-industrial wastes [Sharma et al., 2016].

New technologies have been described in the last decades for biofuels production. Sasaki et al., 2018, developed a co-culture system for bioethanol production using two engineered *Saccharomyces cerevisiae* strains and the brown macroalgae *Ecklonia kurome* as biomass source. According to the authors, the advantage of this platform is the

possibility to adjust the composition of yeast cell populations depending on the carbohydrate components.

Pectinases are mainly used in maceration of plant tissues (reduction of juice viscosity, extraction of vegetable oils, coffee and tea fermentation, fiber crops degumming) and wastewater treatment [Kashyap et al., 2001; Patidar et al., 2018, Amin et al., 2018]. Moreover, according to Habrylo, et al., 2018, these enzymes occupy about fifth of the position in terms of biotechnological applications in the worldwide market of enzymes.

Damodharan et al., 2018 investigated the plant growth promotion and salinity stress alleviation in *Solanum lycopersicum* cv. Micro-Tom (tomato). In this study, the authors discovered a new *Streptomyces* SK68 strain, isolated from the rhizosphere of peanut plant. This actinobacteria exhibited the following enzymatical activities: xylanase, cellulase, amylase, and pectinase and degraded hypoxanthine, casein, and L-tyrosine. In addition, the strain SK68 inoculated on tomato plants under salt stress showed significant increase in plant biomass compared to control and salt-stressed (180 mmol/L).

In Oumer & Abate, 2018, the pectinolytic potential was investigated in isolates (95: actinomycetes (21.06%), bacteria (65.26%), and fungi (13.68%)) obtained from coffee pulp samples. The results showed that 31.58% of isolates indicated pectinase activity. In addition, the molecular identification showed that 70% of the isolates with high pectinase activity are members of *Bacillus* genus.

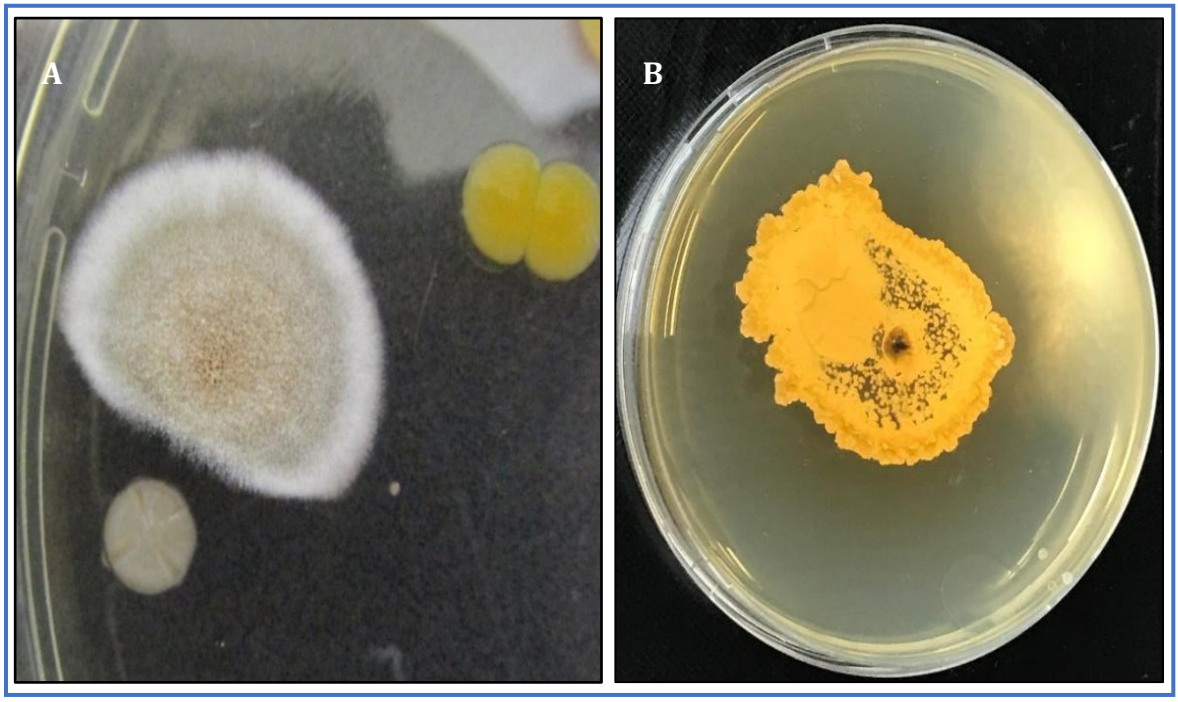
Part 2: Investigation of antimicrobial activity metabolites

Antibiotics consist of a class of organic or designed products that specifically works against bacteria and fungi, but not against viruses [Nicolaou & Rigol, 2017]. This prompted the interest of using microorganisms as a source for the discovery of secondary metabolites with activity against human and plant pathogens [Strobel et al., 2004; Matsumoto & Takahashi, 2017]. Consequently, the need to exploit niches that harbor microorganisms that produce bioactive metabolites is fundamental, since during the last few years the continued and uncontrolled use of antibiotics favored the spread of resistance, becoming a serious threat to the treatment of infectious diseases [Joseph &

Pryia, 2011; Caraballo-Rodriguez et al., 2017; Nicolaou & Rigol, 2017; Matsumoto & Takahashi, 2017].

Our investigations at LaMiB - UFSCar and at the Professor David Sherman's Laboratory in Life Sciences Institute - University of Michigan have shown that the endophytic and rhizospheric microbiome associated with *Polygala* species represents an unexplored source of compounds of biotechnological and pharmaceutical interest applied to substances with antagonistic potential against microorganisms of interest in Public Health (Figure 20).

Figure 18: A: Bioactivity produced by the endophyte II-Folha255 during the isolation of the endophytic microbiome of purple-flower *Polygala* sp.. **B:** Bioactivity of Rizo Pp Ac 11 from rhizospheric space of the white-flower *Polygala* sp..

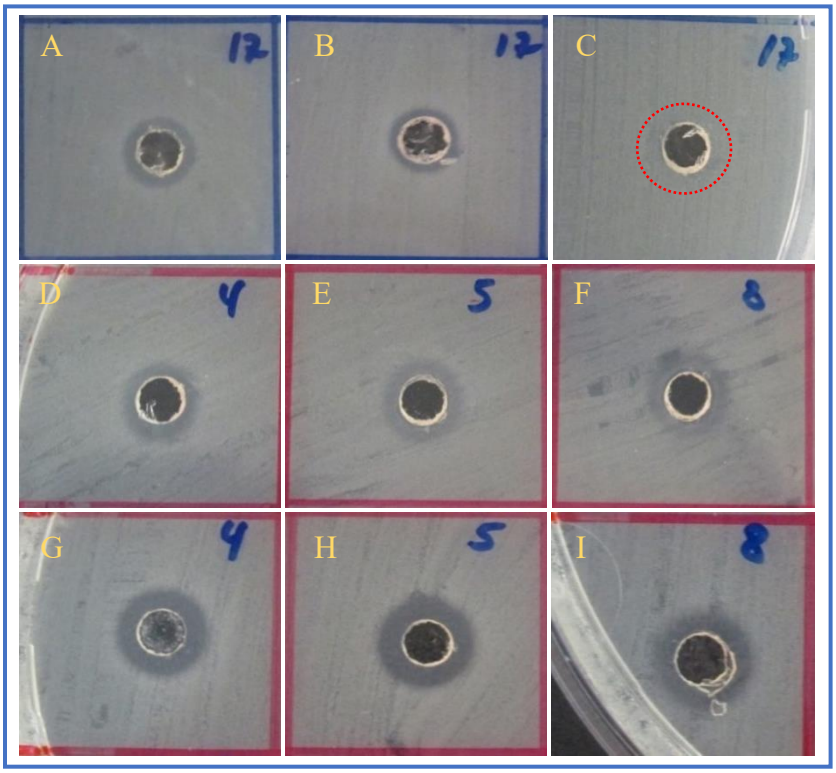


In this study, we highlight the NPE produced by the Rizo Pp Ac-11 isolate. This actinomycete isolated from the white-flower *Polygala* rhizosphere, in just three days of fermentation could produce a powerful antagonistic and broad-spectrum activity against all the tested pathogenic strains.

4.3. Evaluation of the antimicrobial activity of fungi NPEs

Fungi are known for the diverse ecological relationships and versatility in obtaining secondary metabolites, which several compounds exhibit antibiotic. In this study, the antimicrobial potential of extracts from 29 endophytic fungi isolates (Supplementary table 3) was evaluated by the well diffusion method [Ratti, 2009]. Among these, only 8 extracts showed inhibition activity (*S. aureus* ATCC 29213: 24.14%, *E. coli* ATCC 11775: 24.14% and *C. albicans* ATCC 10231: 6.9%), whereas the isolate GLRF-24 showed antimicrobial activity against all pathogens tested. However, the most bioactive extracts were Roxo 03, Roxo 00* and Roxo 01, which inhibited *S. aureus* and *E. coli* (Figure 21).

Figure 19: Bioactivity of NPEs produced by endophytic fungi isolated from *Polygala* spp.. **A**, **B** and **C**: GLRF-24 isolate against *S. aureus*, *E. coli* and *C. albicans* respectively. **D**, **E** and **F**: Bioactivity of Roxo 03, Roxo 00* and Roxo 01 NPEs against *E. coli*. **G**, **H** and **I**: Bioactivity of Roxo 03, Roxo 00* and Roxo 01 extracts against *S. aureus*.



Ochratoxin A is a mycotoxin produced by some species of fungi of the *Aspergillus* and *Penicillium* genera. Its importance is due to its toxic properties as carcinogenic, nephrotoxic, teratogenic, neurotoxic, among others.

Pereira et al. (2015) investigated four hundred fungi extracts, and the NPE produced by *Mycosphaerella* sp. endophytically isolated from *Eugenia bimarginata* presented high antifungal activity against *C. neoformans* and *C. gattii*, with MIC values of 31.2 µg/mL and 7.8 µg/mL, respectively.

The study by Pan et al. (2017) included the isolation of 53 endophytic fungi from the Chinese medicinal plant *Fritillaria unibracteata* var. *wabuensis*, where all extracts exhibited antioxidant activities.

In the study by Park et al. (2017), it was explored the antimicrobial potential of endophytic fungi isolated from *Panax ginseng* Meyer from several regions of Korea against the phytopathogens *Alternaria panax*, *Botrytis cinerea*, *Cylindrocarpon destructans*, *Pythium* sp. and *Rhizoctonia solani*. Among 1,300 isolates, the NPE produced by the endophyte *Trichoderma polysporum* was able to inhibit all tested pathogens.

Silva Ribeiro et al. (2018) conducted a study in which a fungi endophytically associated with *Pachystachys lutea* were identified. The isolates belonging to the genera *Colletotrichum*, *Phyllosticta*, *Xylaria*, *Nemania* and *Alternaria* were able to inhibit the growth of pathogenic fungi like *Diaporthe* sp. PL09.

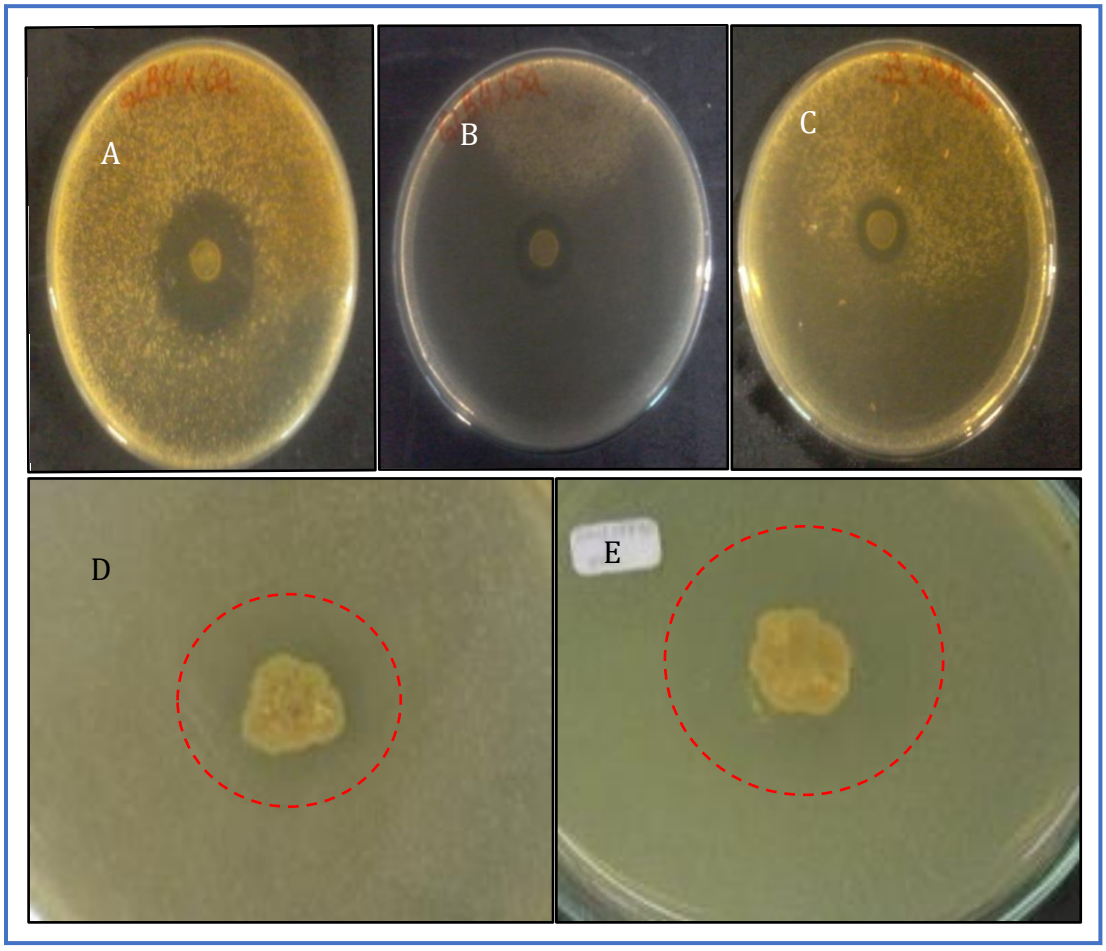
Su et al. (2018) isolated and studied the effects of bafilomycin C1. This compound produced by *Streptomyces albolongus* was able to strongly inhibit the opportunistic fungus *Candida albicans* by destroying the cell membrane. In addition, molecular analyzes have shown that bafilomycin C1 was able to promote the decrease of ergosterol, an important component of the cell membrane, and in contrast, the elevation of farnesol expression, which is a precursor of steroids in fungi.

4.4. Screening for antimicrobial activity in bacterial isolates

The initial screening of the antimicrobial activity by the Overlay assay for the bacteria and actinomycetes (Figure 22) showed that 9 isolates presented potent bioactivity against

the tested microorganisms. Therefore, these were selected for the production kinetics of extracts containing bioactive products (Supplementary table 4).

Figure 20: Bioactivity observed in the overlay assay by the endophytic GLB-4 bacterial isolate against **A:** *C. albicans*. **B:** *S. aureus*. **C:** *E. coli*. **D** and **E:** Bioactivity produced by the endophytic actinomycete Rizo Pp Ac 16 isolated from white-flower *Polygala* sp. roots of against *S. aureus*.

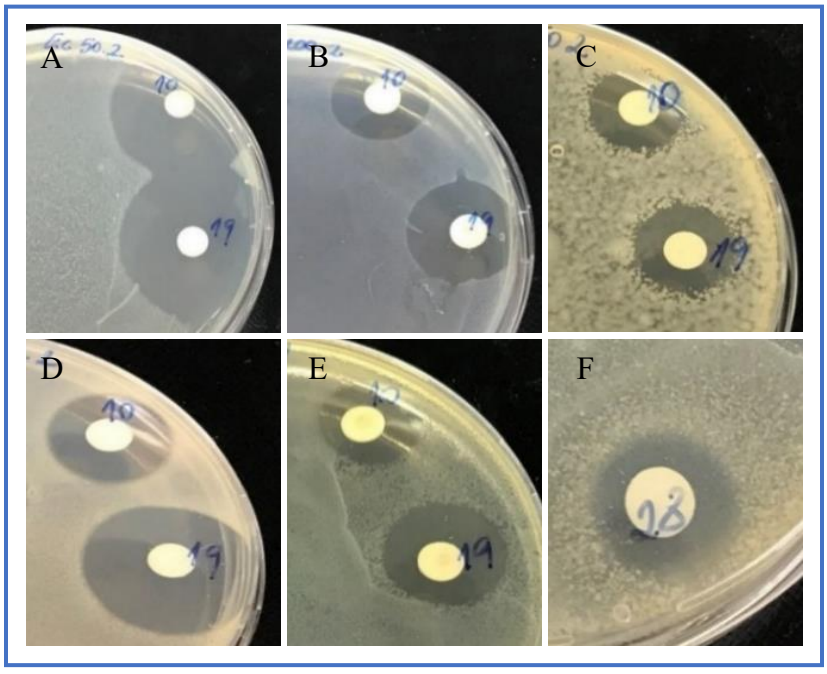


The selected isolates (Table 2) were cultured over the period of 3, 5, 7 and 9 days. For each culture time, the respective NPEs were produced and, tested by disk diffusion technique at concentrations of 200, 50 and 10 µg/disk (Figure 23).

Table 2: Isolates selected for NPE production.

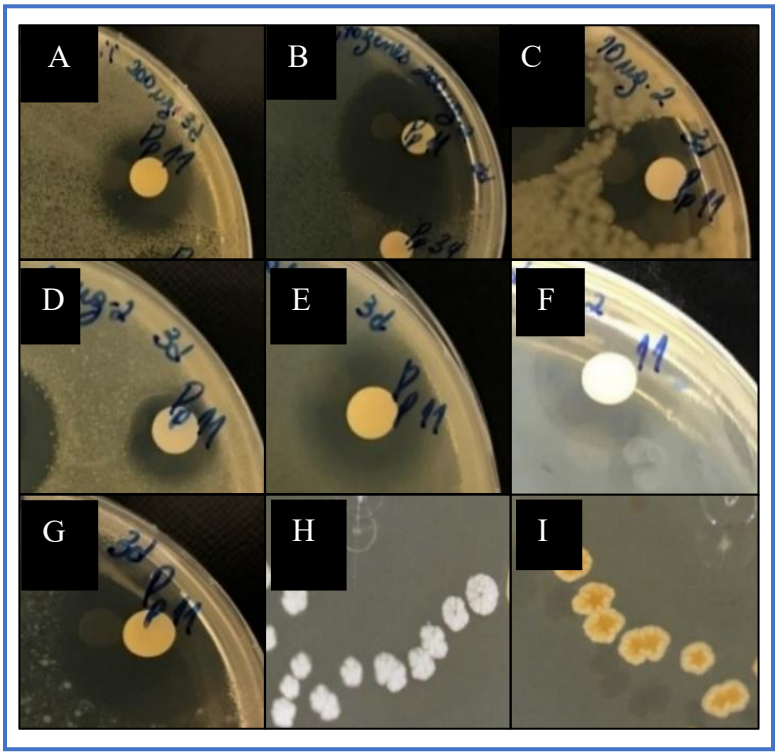
Strain	*S. aureus*	*E. coli*	*C. albicans*
Rizo Pv Ac 01 branco	Positive	Negative	Negative
Rizo Pv Ac 03?	Positive	Positive	Negative
Rizo Pp Ac 09	Positive	Positive	Positive
Rizo Pp Ac 10	Positive	Positive	Positive
Rizo Pp Ac 11	Positive	Positive	Positive
Rizo Pp Ac 19	Positive	Positive	Positive
Rizo Pp Ac 28-1	Positive	Positive	Negative
Rizo Pp Ac 43-1	Positive	Positive	Negative
Raíz PpP 16	Positive	Positive	Positive

Figure 21: Bioactivity of the extracts produced by the Rizo Pp Ac-10, Rizo Pp Ac-19 and Rizo Pp Ac-28 strains against indicators of Public Health **A:** *E. coli* in 50 µg. **B:** *L monocytogenes* in 200 µg. **C:** *B. anthracis* in 50 µg. **D:** MRSA in 10 µg. **E:** *S. flexneri* in 200 µg. **F:** Rizo Pp Ac-28 in 9 days of culture against MRSA in 10 µg.



Among the extracts tested, (based on NPE concentration on disk, range of activity and size of the inhibition area) the Rizo Pp Ac-11 NPE showed high and broad-spectrum activity against all pathogens, especially resistant pathogens in 3 days of cultivation (Figure 24), whereas the other NPEs showed the highest inhibition from the fifth day of culture (Rizo Pp Ac 10, Rizo Pp Ac 19 and Rizo Pp Ac 28-1).

Figure 22: Bioactivity produced by the Rizo Pp Ac-11 NPE in a disk diffusion test against pathogenic microorganisms. **A:** *A. baumannii* in 200 µg. **B:** *L monocytogenes* in 200 µg. **C:** *B. anthracis* in 10 µg. **D:** MRSA in 10 µg. **E:** *S. enterica* in 200 µg. **F:** *S. flexneri* in 200 µg. **G:** *E. coli* in 200 µg. **H** and **I:** Morphological characteristics of the surface and base of the isolate on ISP_2 agar.



Therefore, this isolate was selected to large-scale cultivation. However, studies to optimize and improve the bioactive metabolites production are critical. According to Bode et al. (2002), the OSMAC (One Strain - Many Compounds) concept consists in a strategy for small changes in cultivation that can completely shift the metabolic profile in several strains. In addition, the authors reported that high concentrations of phosphate or even

usual amino acids in media composition may act as inducers of production of selected metabolites. However, these components in high concentrations are generally described in the literature as secondary metabolism repressors [Romano et al., 2018].

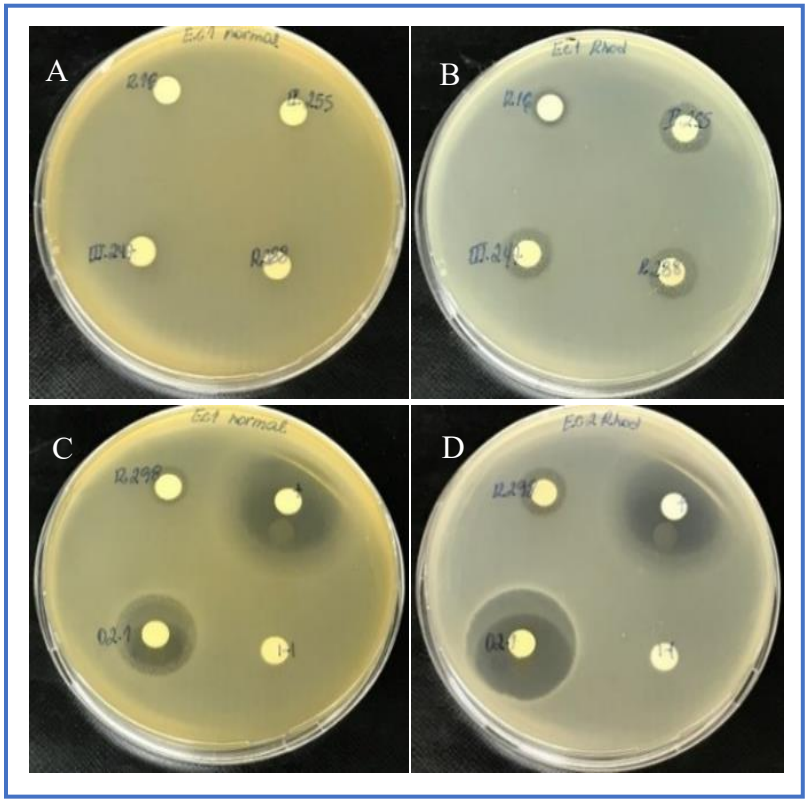
4.4.1. Activating cryptic metabolites production using combined cultures

Soil is an extremely diversified habitat where microorganisms compete to survive. In this context, actinomycetes are influenced by other microorganisms to activate routes of secondary metabolism, thus controlling the other microbes [Onaka, 2017]. On the other hand, Tamburini & Mastromei (2000), defined the cryptic genes as silent DNA sequences that are not expressed during the life cycle of a microorganism. This ability occurs in only a few members of a large population by mutation, recombination, insertion processes, or other genetic mechanisms. Based on this hypothesis, the endophytic isolated actinomycetes (Rizo Pp Ac-02-1, Raíz PpP 16, Raíz PpP 288, Raíz PpP 298, III Raíz 247 and II-Folha-255) were submitted to the combined culture with the objective of activating its secondary metabolism, where the activating strain used was *Rhodococcus erythropolis*, which contains mycolic acid in its composition. However, other strains may also be used for instance, *Dietzia* spp., *Nocardia* spp., *Williamsia* spp., *Gordonia* spp., *Mycobacterium* spp. and *Corynebacterium* spp. [Onaka et al., 2011; Onaka, 2017; Romano et al., 2018]. And according to several studies, this strategy has been used successfully to induce secondary metabolism, creating new and previously undetected compounds [Chagas & Pupo, 2018]. Moreover, the study of Asamizu et al. (2015) suggests that some factors, such as culture extracts or mycolic acids are not sufficient to activate these cryptic genes in *Streptomyces lividans* in monoculture conditions. According to their investigation, direct attachment of *S. lividans* cells on the mycolic acid-containing bacteria (MACB) it is crucial to a successful activation of secondary metabolism.

From the group of actinomycetes tested in co-cultures, the isolate Rizo Pp Ac-02-1 showed the higher rates of inhibition in pathogens (based on concentration of NPE inoculated in disk and size of inhibition area). However, to confirm the efficacy of the experiment, a new stage of cultivation and production of the NPEs was carried out in 3, 5,

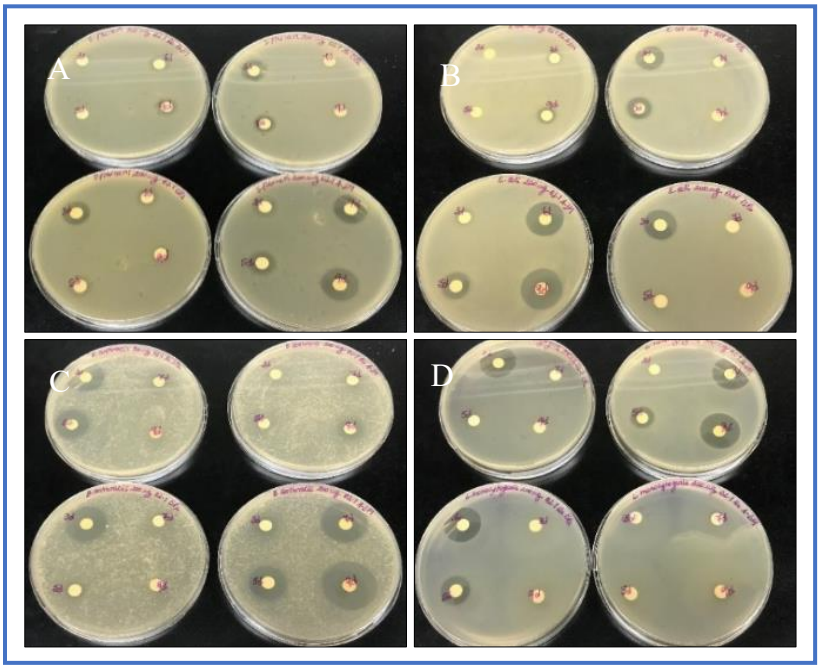
7 and 9 days of culture using ISP_2, ISP_2 combined with *R. erythropolis*, A-3M and A-3M combined with *R. erythropolis* (Figure 25).

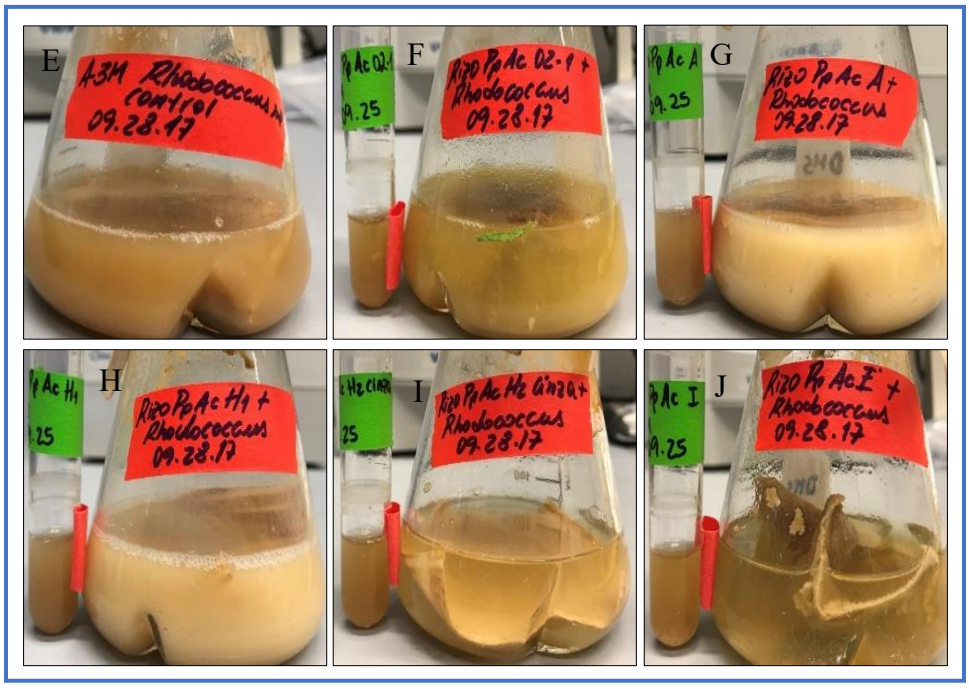
Figure 23: Example of activation of cryptic genes producing bioactive substances in the group of endophytic actinomycetes against *E. coli*. In **A** and **C**, the culture was performed only on A-3M medium for 7 days. However, in **B** and **D** the culture was carried out in A-3M medium combined with the activating strain *R. erythropolis*.



However, the Rizo Pp Ac-02-1 NPE obtained from the culture using A-3M medium showed the best inhibition results. Although the combined culture of Rizo Pp Ac-02-1 changed the staining of the A-3M medium in the presence of *R. erythropolis* (indicating the activation of cryptic genes [Onaka et al. 2017]) inhibition was not as effective in relation to the monocultures Rizo Pp Ac-02-1 (Figure 26).

Figure 24: Inhibition of pathogenic strains by the Rizo Pp Ac-02-1 NPEs in different cultures. **A:** *S. flexneri*. **B:** *E. coli*. **C:** *B. anthracis*. **D:** *L. monocytogenes.* Endophytic actinomycetes cultures in A-3M medium combined with *R. erythropolis*, where in **E** we can observe the pure culture of *R. erythropolis* and in **F**, **G**, **H**, **I** and **J** the cultures combined with the pure cultures in the tubes with the green label. According to Onaka et al., 2011, the color change of the combined culture suggests the activation of cryptic genes.





As previously mentioned, the *Streptomyces* genus stands out for its potential production of secondary metabolites; however, the expression of most groups of metabolic biosynthetic genes is enigmatic or silent, and this type of methodology indicates that approximately 90% of *Streptomyces* species present alterations in secondary metabolism in combined cultures when compared to pure cultures, a very effective method for the discovery of bioactive natural products. These mixed cultures can simulate competitive natural environments by stimulating microbial interactions [Bérdy, 2005; Qin, 2011, Onaka et al., 2011; Asamizu et al., 2015; Onaka, 2017; Chagas & Pupo, 2018].

Wakefield et al. (2017), reported bacterial and fungal metabolites by co-cultivation of the marine-derived fungal isolate *Aspergillus fumigatus* MR2012 and two bacterial strains obtained from hyper-arid desert isolates, *Streptomyces leeuwenhoekii* (C34 and C58). Co-cultivation of the fungal isolate MR2012 with the bacterial strain C34 led to the production of luteoride D, pseurotin G, terezine D and 11-O-methyl pseurotin A. In another combination, the fungus MR2012 was tested in co-cultivation with strain C58, and a double production of chaxapeptin was detected. Additionally, the bacterial metabolite pentalenic acid was detected and isolated. And from the monoculture of MR2012, a new diketopiperazine metabolite named brevianamide X was discovered.

The study of Park et al. (2017), a new polyketide glycoside known as gordonic acid, was discovered from a mixed culture of *Gordonia* sp. KMC005 and *Streptomyces tendae* KMC006. Gordonic acid antimicrobial activity was tested against *Bacillus subtilis* KCTC1021, *Staphylococcus aureus* KCTC1916, *Micrococcus luteus* KCCM11548, *Enterococcus hirae* KCCM11768 and *Escherichia coli* KCTC2593. The antagonism assay revealed activity only against *M. luteus* and *E. hirae* at a concentration of 10 µg/disk.

Chagas & Pupo (2018) studied the changes in metabolic profiles using the co-cultivation of endophytic fungus *Phomopsis* sp. FLe6 and *Streptomyces albospinus* RLe7 isolated from *Lychnophora ericoides*. The authors identified the cytotoxic compound cytochalasin H produced by the fungus *Phomopsis* sp. FLe6. Although cytochalasin H is described as toxic to various plants, it did not present toxicity to *S. albospinus* RLe7, suggesting that this microorganism does not require a defense mechanism to prevent the potentially harmful effects of this fungal compound.

In Kamdem et al. (2018), the endophytic fungus, *Bionectria* sp., obtained from seeds of the tropical plant *Raphiata edigera* was co-cultivated with *Bacillus subtilis* and *Streptomyces lividans*. In this study several compounds such as *o*-aminobenzoic acid derivatives, bionectriamines A and B, Penicolinate A (which exhibited potent cytotoxic activity against the human ovarian cancer cell line A2780 with an IC50 value of 4.1µM) were reported, while in axenic culture of *Bionectria* sp a new alkaloid 1,2-dihydrophenopyrrozin was isolated.

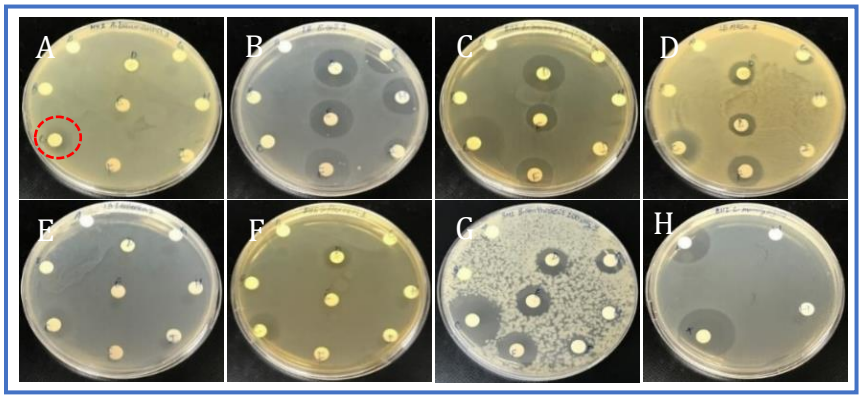
4.4.2. Evaluation of extract fractions activity of Rizo Pp Ac-11

For this purpose, the isolate was inoculated into flasks containing 100 mL of ISP_2 broth and incubated under the same culture conditions described previously. After this period, each culture was transferred to flasks containing 1.2 L of ISP_2, totaling a 20 L culture. Subsequently, the NPE produced from this large-scale culture was subjected to a C_{18} column fractionation in water and acetonitrile gradient (Table 3), and each fraction was subjected to bioassays at the concentration of 200 µg/disk (Figure 27).

Table 3: Composition of each fraction from Rizo Pp Ac-11 NPE.

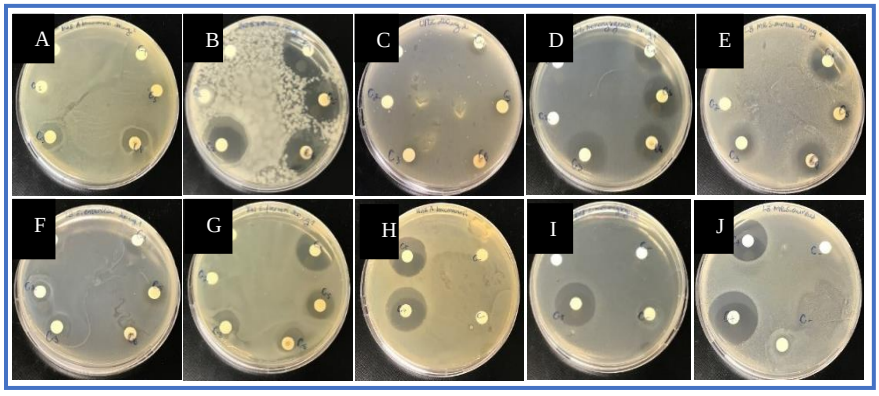
Fractions of Rizo Pp Ac-11 NPE obtained from C_{18} column	
Fraction	**Composition**
Rizo Pp Ac-11A	100% H_2O
Rizo Pp Ac-11B	90% H_2O 10% ACN
Rizo Pp Ac-11C	75% H_2O 25% ACN
Rizo Pp Ac-11D	60% H_2O 40% ACN
Rizo Pp Ac-11E	45% H_2O 55% ACN
Rizo Pp Ac-11F	30% H_2O 70% ACN
Rizo Pp Ac-11G	15% H_2O 85% ACN
Rizo Pp Ac-11H	100% CAN
Rizo Pp Ac-11I	MeOH:EtOAc (wash)

Figure 25: Disk diffusion assay of fractions of Rizo Pp Ac-11 NPE. **A:** *A. baumannii*. **B:** *E. coli*. **C:** *L. monocytogenes*. **D:** MRSA. **E:** *S. enterica*. **F:** *S. flexneri*. **G:** *B. anthracis*. **H:** Test controls.



After identifying the target fraction (fraction Rizo Pp Ac-11C), a new separation step using pre-packaged reverse phase column chromatography of the Sep-Pak-C_{18} was performed. Sbsequently, six sub-fractions (C_1, C_2, C_3, C_4, C_5 and C_6) were obtained. Again, these were subjected to the same bioassays described previously. Then, after a new bioassay, it was possible to identify the active part of the broad spectrum (C_4) contained in the extract produced by the Rizo Pp Ac 11 isolate, which was later concentrated in speed-vac and analyzed for HPLC purification (Figure 28).

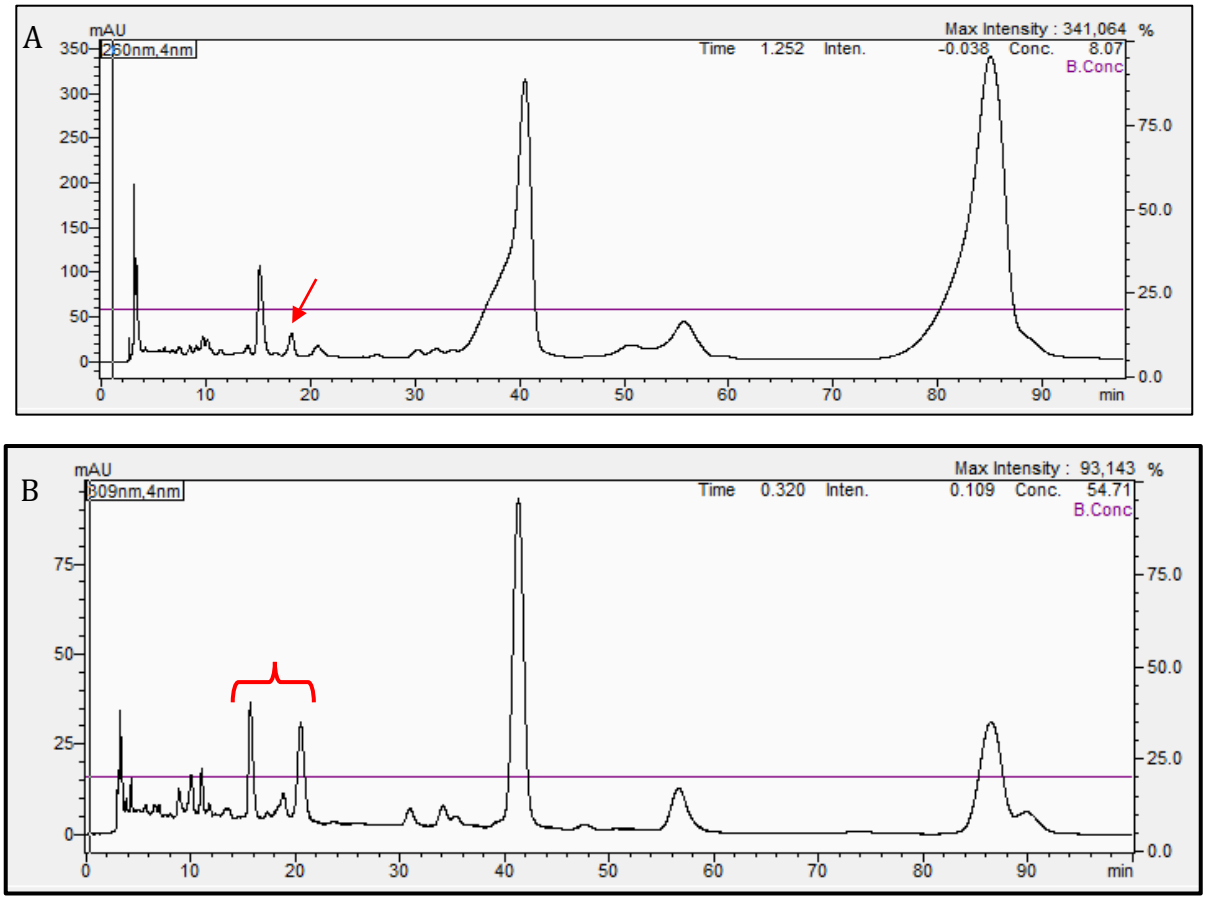
Figure 26: Disk diffusion test of partitions from fraction C obtained by Sep-Pak C_{18} chromatography. **A:** *A. baumannii*. **B:** *B. anthracis*. **C:** *E. coli* CFT073. **D:** *L. monocytogenes*. **E:** MRSA. **F:** *S. enterica*. **G:** *S. flexneri*. **H, I and J:** Controls for *A. baumannii*, *L. monocytogenes* and MR *S. aureus* respectively.



4.4.3. Evaluation of separation and purification by HPLC and bioactivity

The molecules contained in the C_4 sub-fraction were separated and purified by HPLC. The sample was prepared in two different partitions using 100% methanol (pMeOH) and methanol and water 1:1 (pMeOH/H_2O) to trap the peaks, where the separation profile is shown in Figure 29.

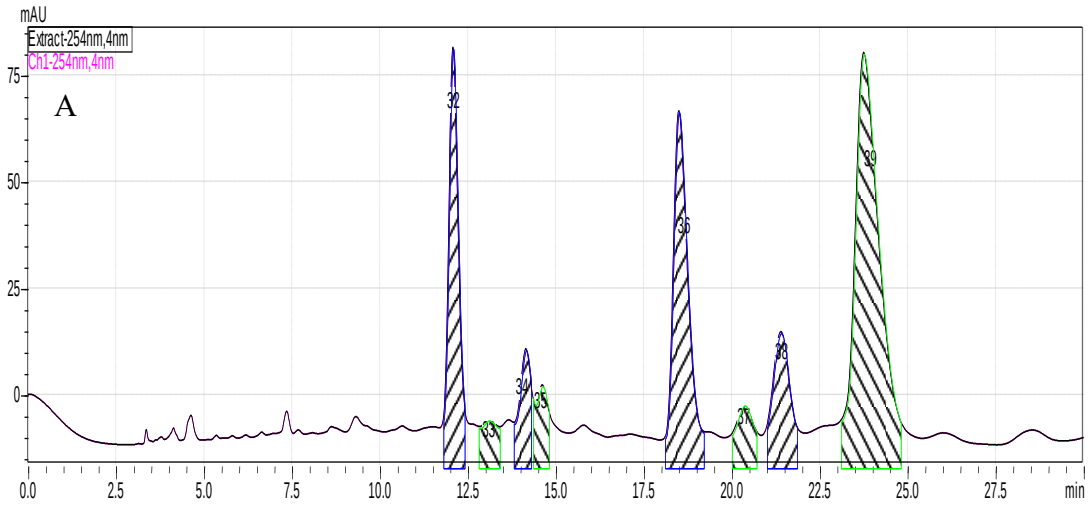
Figure 27: Separation profile of the pMeOH (**A**) and pMeOH/H_2O (**B**) partitions within 100 minutes of running, where the red arrow indicates the bioactive peak: BA-34 in **A** and in **B** the highlighted area represents the broad-spectrum region of the pMeOH/H_2O partition.

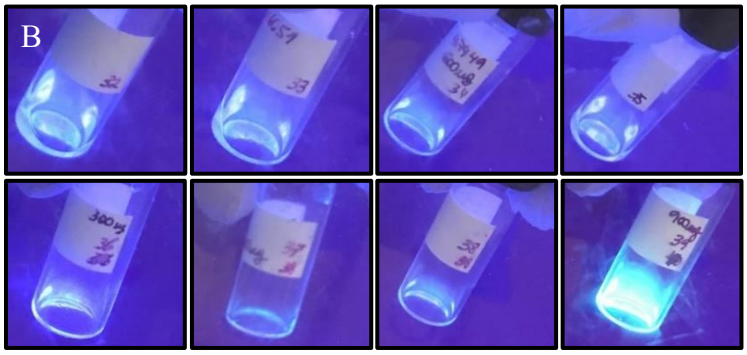


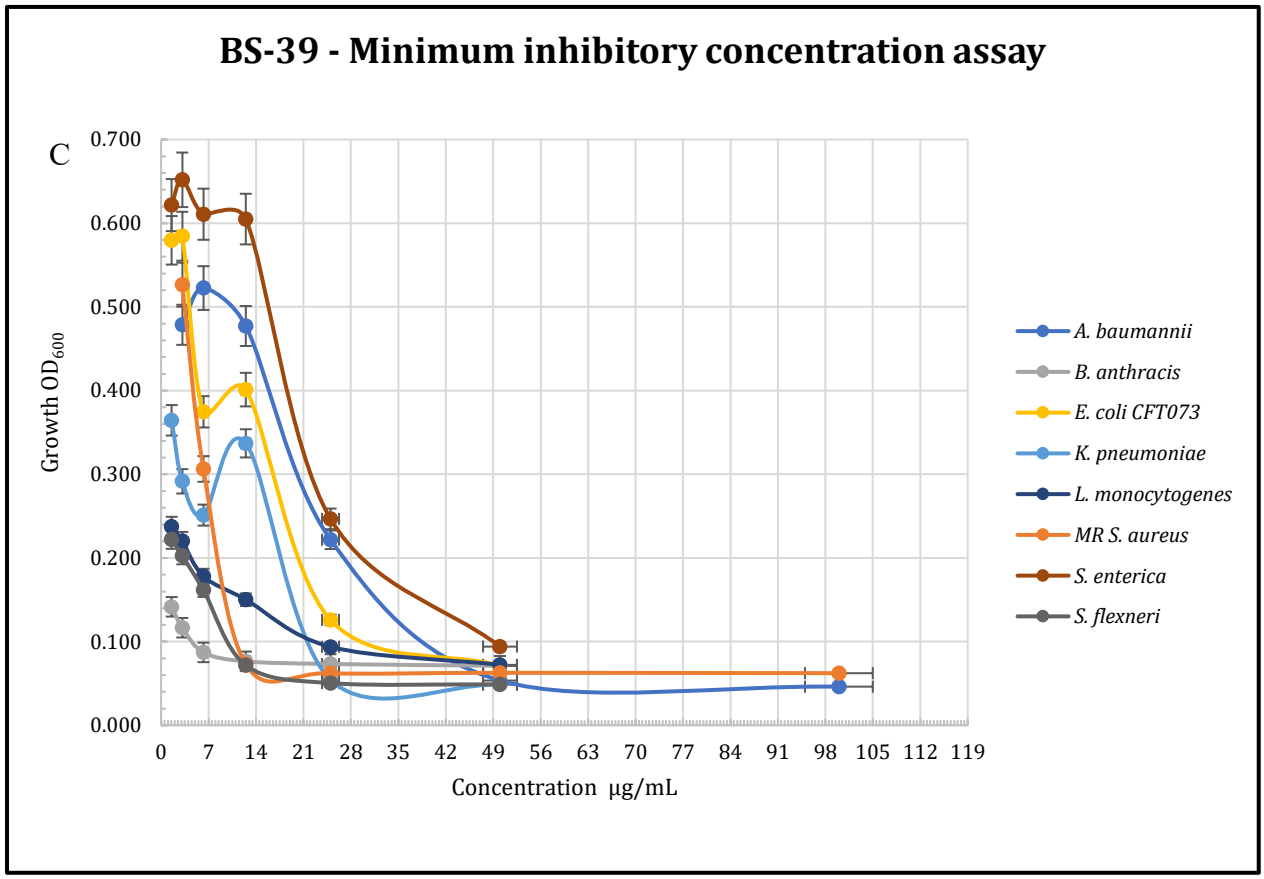
The peaks were collected from both partitions and tested for bioactivity against all pathogens. The peak obtained in the pMeOH partition showed inhibition only against *B. anthracis* (**p< 0,01; CI at 95%: 0.02619 to 0.1289), whereas in the pMeOH / H_2O partition

contained the broad-spectrum peak (BS-39) detected at a wavelength of 254 nm showing bioactivity against 8 of 9 strains tested. In addition, the bioactive peak exhibited fluorescence emission after exposed to UV light (Figure 30). Moreover, in Figure 31, the log IC_{50} values for BS-39 against all tested pathogenic strains for minimum inhibitory concentration assays using a confidence interval at 95% are shown. The statistical analysis is shown in Table 4.

Figure 28-A: Amplification of the broad-spectrum region in which the compound BS-39 represented by peak 39 is found. **B:** Fluorescence emitted by the peaks of the broad-spectrum region. **C:** Minimum inhibitory concentration assay and table containing the statistical analysis for BS-39 obtained from the broad-spectrum region against the tested microorganisms. **D:** IC_{50} calculated 95% confidence interval using Graph Pad Prism 8.0.1 software. **E:** Action of ciprofloxacin as a positive control against tested pathogenic strains. **F:** Normal growth of the tested pathogenic strains in 5% of DMSO.

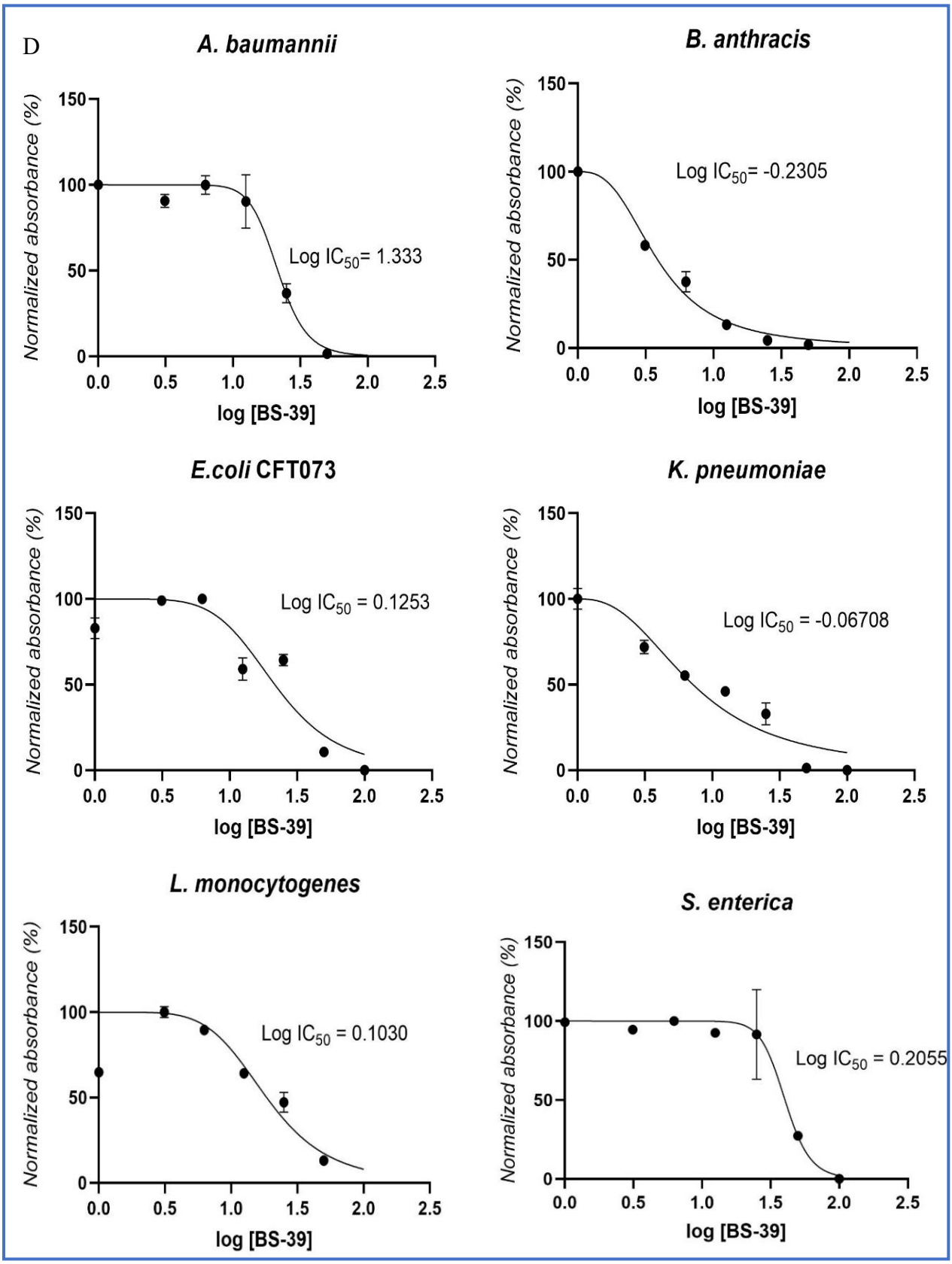


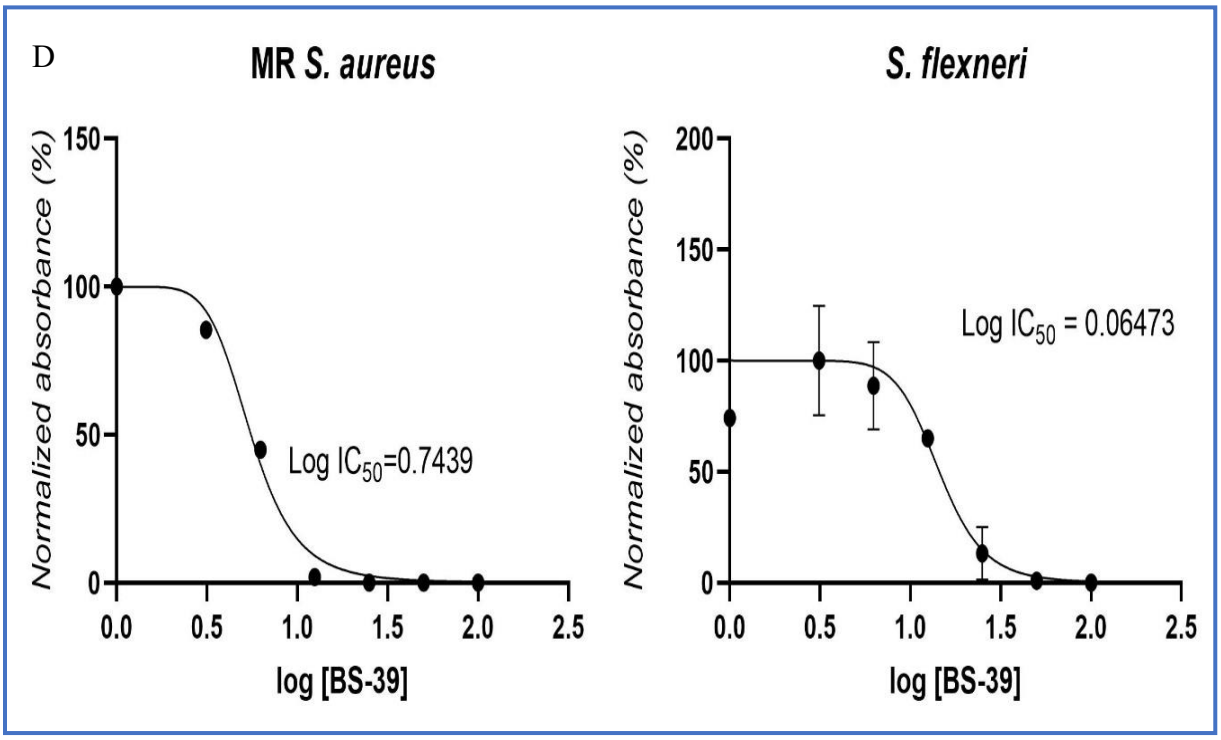


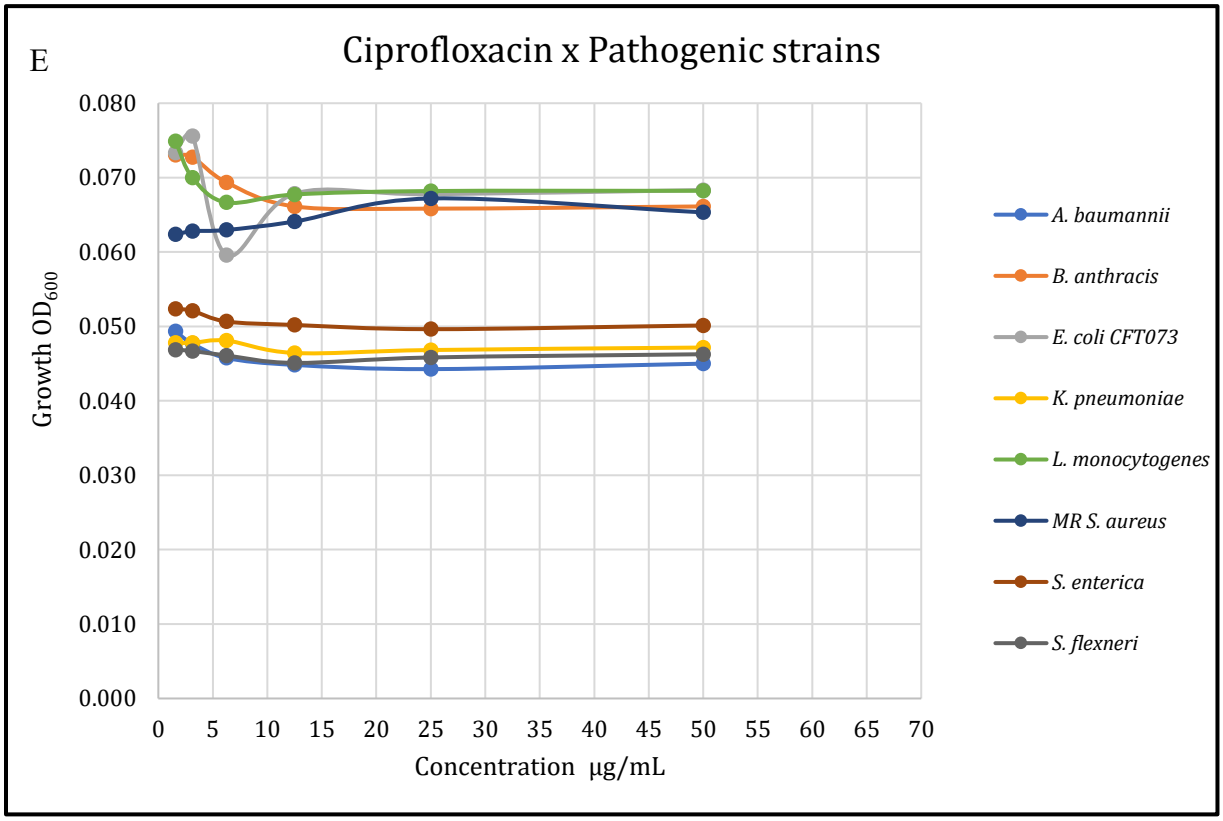


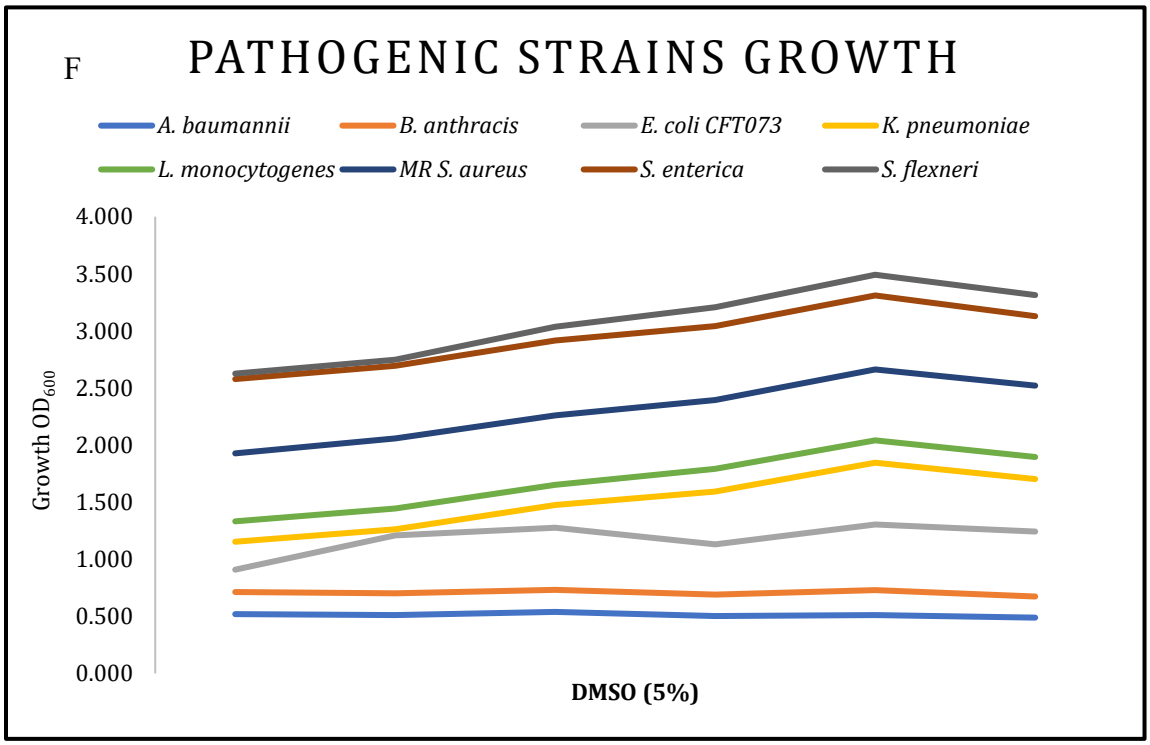
One-way ANOVA & Dunnett's multiple comparisons test for Negative control vs. BS-39

Tested strain	Mean Diff.	95% CI of diff.	Significance	Adjusted P Value
A. baumannii	0.3181	0.1018 to 0.5343	**	0.0072
B. anthracis	0.429	0.2706 to 0.5873	****	<0.0001
E. coli CFT073	0.1783	-0.06777 to 0.4244	Ns	0.1553
K. pneumoniae	0.1638	-0.1090 to 0.4365	Ns	0.2513
L. monocytogenes	0.07377	0.01935 to 0.1282	*	0.0145
MR *S. aureus*	0.5404	0.5301 to 0.5507	****	<0.0001
S. enterica	0.4732	0.2305 to 0.7158	**	0.008
S. flexneri	-0.0015	-0.07956 to 0.07656	Ns	0.9982


D
A. baumannii
Normalized absorbance (%)
Log IC50= 1.333
log [BS-39]
B. anthracis
Log IC50= -0.2305
E.coli CFT073
Log IC50 = 0.1253
K. pneumoniae
Log IC50 = -0.06708
L. monocytogenes
Log IC50 = 0.1030
S. enterica
Log IC50 = 0.2055


D
MR S. aureus
S. flexneri
Normalized absorbance (%)
Log IC50=0.7439
Log IC50 = 0.06473
log [BS-39]
log [BS-39]


E
Ciprofloxacin x Pathogenic strains
Growth OD600
Concentration µg/mL
A. baumannii
B. anthracis
E. coli CFT073
K. pneumoniae
L. monocytogenes
MR S. aureus
S. enterica
S. flexneri



In terms of broad-spectrum compounds, tetracyclines are the first important class of antibiotics and have been used since the 1940s against Gram-positive and Gram-negative pathogens, intracellular mycoplasmas, *Chlamydiae*, *Rickettsiae* and protozoan parasites [Petković et al., 2017].

The search for compounds with inhibitory activity against multi resistant pathogens is critically important. Antibiotic resistance has been recognized by the CDC (Centers for Disease Control and Prevention) as a major threat to public health. Pathogens such as MRSA show that antibiotic resistance rates are surpassing 50% in 5 out of 6 world regions of the World Health Organization (WHO) [Nair et al., 2017].

The Gram-positive *B. anthracis* is responsible for a fatal infection in which the symptoms may be similar to a common cold. This pathogen is classified by CDC as biohazard category A [Falcinelli et al., 2017].

Virulence is due to the expression of exotoxins and capsules that interfere with host cell signaling by altering the homeostasis process and inhibiting the phagocytosis of the pathogen [Falcinelli et al., 2017]. In recent years, three anti-toxin agents have received approval from the US Food and Drug Administration (FDA) for use during an outbreak of

anthrax infection including; immunoglobulin (AIG), raxibacumab and the monoclonal antibody ETI-204 [Kim et al., 2016; Xu et al., 2017]. However, in the study by Cui et al., 2017, AIG was administered in 15 people in a group of 43 patients with *B. anthracis* infection during the 2009-2010 outbreak in Scotland due to the use of contaminated heroin. Notably, the review of 43 patients in this study, including the AIG-treated group receiving AIG, showed no difference in mortality compared to AIG receptor and non-receptor patients.

Montville et al. (2006) studied the action of peptides with antimicrobial properties in *Bacillus* strains, whose the bioactivity in *B. anthracis* was detected by the compounds nisin, amide-II-magainin and the defensins HNP-I and HNP-II.

Epigallocatechin-3-gallate (EGCG) is the natural substance of the class of catechins found in abundance in green tea, which has a potent antioxidant activity. The study of Falcinelli et al. (2017) showed that the antimicrobial action of this substance was verified against two strains of *B. anthracis*.

The process of antibiotic resistance is spreading rapidly in relation to the discovery of new compounds and their introduction into clinical practice. In addition, synthetic approaches to antibiotic production have not been effective enough replace this platform [Ling et al., 2015; Chen et al., 2017]. In this perspective, Ling et al. (2015) obtained a new compound called teixobactin, isolated from the non-cultivable bacterium *Elefteria terrae*. According to the authors, teixobactin could inhibit cell wall synthesis by binding to a highly conserved region of the lipid precursors of peptidoglycan and teicoic acid in the cell wall. Furthermore, it was observed that in trials involving *S. aureus* or *Mycobacterium tuberculosis* they were not able to develop resistance to teixobactin.

The study by Li et al. (2018) isolated 27 actinobacteria strains from the rhizosphere of the reed and investigated their respective clusters of biosynthetic genes (NRPS, PKS-I and PKS-II) and the NPEs from ten isolates exhibited antimicrobial activity against *P. aeruginosa*, *S. aureus*, *B subtilis*, *C. albicans* and *F. oxysporum*.

Streptomyces nogalater-NIIST-A30 isolated by Jacob et al. (2017) showed maximum inhibition against *B. cereus*, *B. subtilis*, *E. coli*, *K. pneumoniae*, *M. smegmatis*, *P. aeruginosa*, *P. mirabilis*, *S. typhi*, *S. aureus*, *S. epidermidis* and *S. simulans*. Among the

eight different fermentation media tested, which the inorganic starch broth salts showed the best antibacterial production. Moreover, the crude extract of ethyl acetate exhibited antioxidant and non-cytotoxic properties in the L6, H9c2 and RAW 264.7 cell lines.

Nocarditocin is a thiopeptide produced by *Nocardia pseudobrasiliensis* IFM 0757. In the study by Mukai et al. (2009), the activity of nocarditocin against *Mycobacterium* and *Gordonia* species was tested, where it was highly active against the rifampicin resistant *M. tuberculosis* strains at concentrations ranging from 0.025 to 1.56 µg/µL.

4.5. Molecular mass determination of BS-39

To assure the purity of the BS-39 compound, a second purification step was performed using the same gradient solvent battery (solution B: 5% - 2 min; 25% - 47 min, 25% - 50 min; % - 50.10 min, 5% - 60 min) at a flow rate of 3.0 mL/min in 60 minutes, where a volume of 20 µL was injected (Figure 32). The results of LC-MS are shown in figure 33 (Supplementary Figure 1).

Figure 29: Second purification step for BS-39 compound on HPLC.

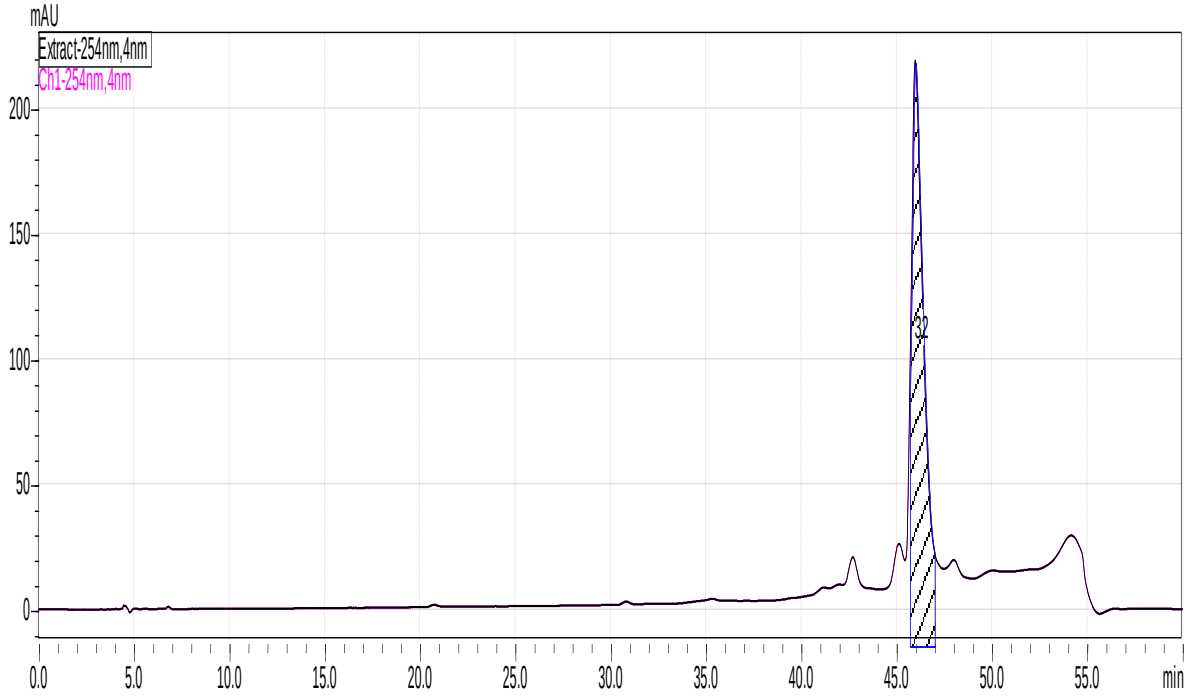
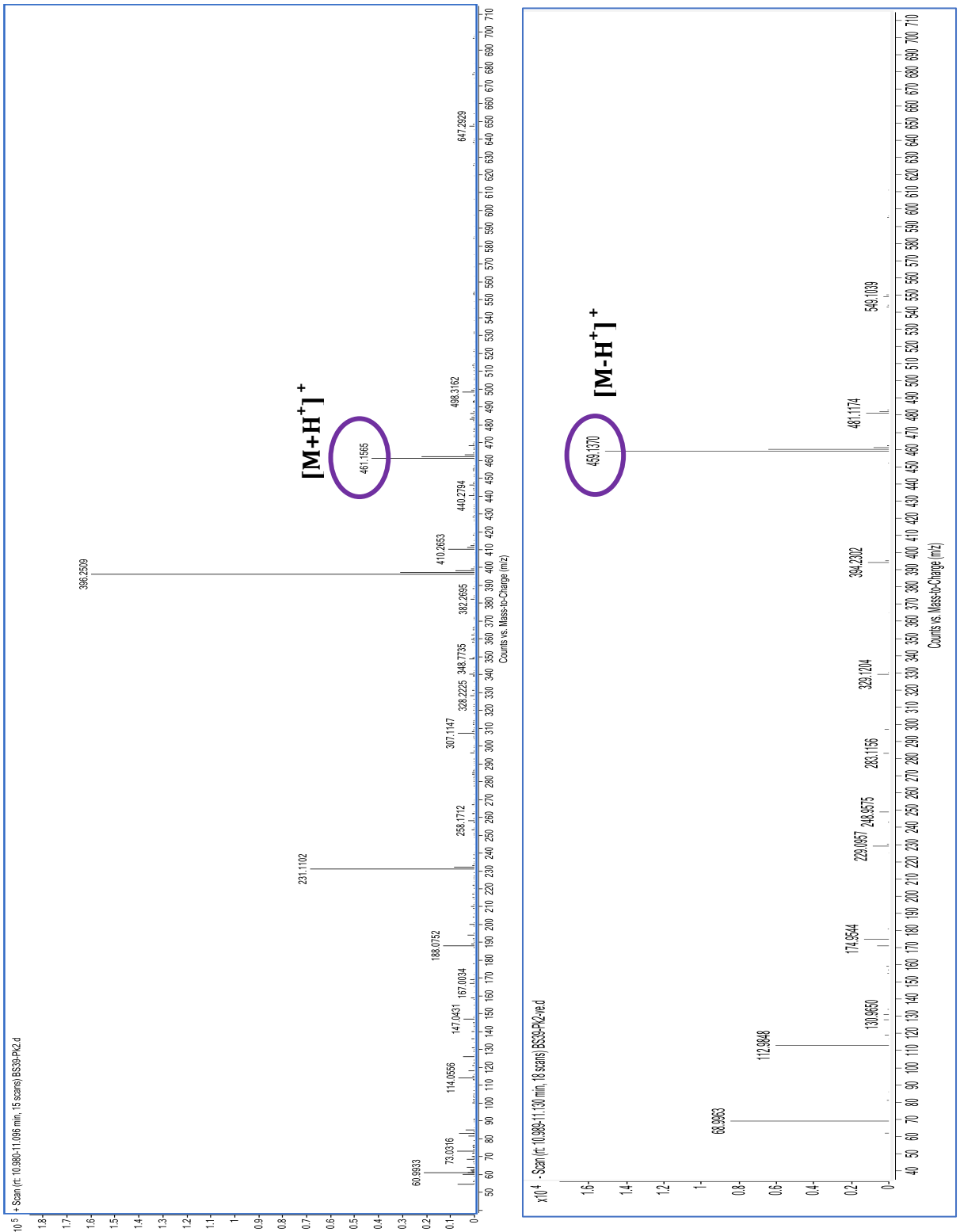


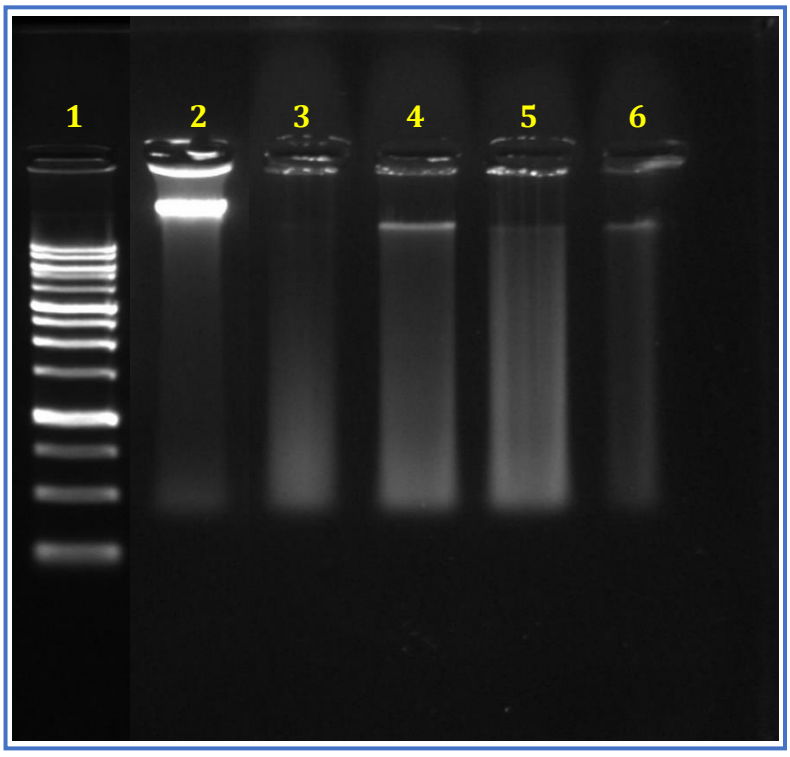
Figure 30: ESI-MS chromatogram of the purified BS-39 where the molecular ion is 461.1565 in ESI+ m/z, whereas 459.1370 in ESI$^-$ m/z.



4.6. DNA isolation

In this study, were able optimize the DNA isolation protocol for the isolate Rizo Pp Ac-11. The DNA isolation of *Streptomyces* spp. is difficult to perform because of resistance to cell lysis. Most methods utilize lysozyme and sodium dodecyl sulfate (SDS) for cell disruption, however, to increase lysis, glycine is also commonly used incorporated into the medium to minimize peptidoglycan cross-linking and muramidases such as mutanolysin or grinding of mycelia [Nikodinovic et al., 2003]. Furthermore, in some *Streptomyces* strains, DNAse activity can occur due to the production of secondary metabolites that can strongly bind to DNA [Kieser et al., 2000] (Figure 34).

Figure 31: Control of the DNA isolation in different conditions (July.18, 2018) using the new protocol (1 – Ladder, 2 – Pv55 and 3 – Rizo Pp Ac-11 grown in YEME and treated with NaCl-Tris-HCl + lysozyme; 4 - Rizo Pp Ac-11 grown in ISP_2 and treated with Tris-sucrose + lysozyme, 5 - Rizo Pp Ac-11 grown in ISP_2 and treated with Tris-HCl + lysozyme; and 6 - Rizo Pp Ac-11, 5 - grown in YEME and treated with Tris-sucrose + lysozyme).



Conclusions

5. Conclusions

Results presented in this study showed for the first time the investigation of the biotechnological potential of the microbiome associated to *Polygala* plants.

Regarding results of enzymatic screening, the most abundant activities were pectate lyase, protease and amylase. In addition, it detected a multi enzymes produced by the same bacteria.

The fungi NPEs showed discrete bioactivity against human pathogens. Thus, an optimization of culture conditions using the OSMAC approach is crucial in the investigation of new molecules.

The natural products extracts produced by the actinomycetes Rizo Pp Ac 10, Rizo Pp Ac 11, Rizo Pp Ac 19 and Rizo Pp Ac 28-1 isolated from rhizoplane of white-flower *Polygala* sp. showed potent inhibition against multi-resistant pathogens. However, we focused in Rizo Pp Ac-11 considering the potency of activity and time for production of crude extract. The investigation showed the detection of a broad-spectrum molecule. However, only 2 fractions did not active, which suggests the presence of more bioactive compounds. In addition, we optimized a protocol for lysis of cell wall of actinomycetes.

Therefore, *Polygala* spp. as well the microbiome associated to these plants consists in an unexplored source for biotechnological investigation.

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Appendix

7. Appendix

Supplementary table 1

Results of enzymatical tests in bacterial isolates from purple-flower *Polygala* sp.

Purple-flower *Polygala* sp.							
Enzymatical activity Screening - Rhizosphere							
Isolate	**Amylase**	**Cellulase**	**Protease**	**Pectin pH 5,0**	**Pectin pH 8,0**	**Lipase**	**Esterase**
I. Rizo 75	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 95	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 96	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 82	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 88	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PvP 94	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 101	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 92 verde	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 159	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 85	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PvP 83	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 81	*Negative*	*Positive*	*Positive*	*Negative*	*Positive*	*Positive*	*Negative*
Rizo PvP 22	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 21	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*
Rizo PvP 24	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PvP 23	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 27	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PvP 25	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo PvP 28	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 29	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 30	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PvP 109	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*
Rizo PvP 102	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 105	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 106	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 107	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PvP 108	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Positive*	*Negative*
Rizo PvP 110	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 158	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*

I. Rizo 98 ocre	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 156	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 174	*Positive*	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*
I. Rizo 172	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
I. Rizo 70	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 68	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 63	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 93	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 62	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 61	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Rizo 262	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Positive*
IV. Rizo 263	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 80	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*
Rizo PvP 86	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PvP 87	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PvP 89	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 104	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Positive*	*Negative*
Rizo PvP 103	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo PvP 08	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 09	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 10	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*
Rizo PvP 26	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 90	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 91	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*
Rizo PvP 92	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Positive*	*Negative*
Rizo PvP 01	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PvP 02	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PvP 03	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 05	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 07	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 124	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 123	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PvP 19	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PvP 20	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 17	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PvP 31	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 32	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 33	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 35	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 37	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PvP 38	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*

Rizo PvP 39	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 80	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PvP 11	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 12	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 18	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 36	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 78	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PvP 79	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Rizo 143	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Rizo 261	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*
I. Rizo 59	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Positive*	*Negative*
I. Rizo 79	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Rizo 58	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 56	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
I. Rizo 78	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 76	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 71	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 72	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 51	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
II. Rizo 87	*Negative*	*Positive*	*Positive*	*Negative*	*Positive*	*Positive*	*Negative*
I. Rizo 167	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 170A	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Rizo 170B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 163	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 169	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 166	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
I. Rizo 164	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 162	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 173	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
I. Rizo 161	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
I. Rizo 60	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 71	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Rizo 157	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PvP 4A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PvP 4B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
I. Rizo 165	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Positives	*11*	*6*	*43*	*10*	*29*	*43*	*19*
Positives (%)	***10.4***	***5.7***	***40.6***	***9.4***	***27.4***	***40.6***	***17.9***

Enzymatical activity Screening – Roots							
Isolate	**Amylase**	**Cellulase**	**Protease**	**Pectin pH 5,0**	**Pectin pH 8,0**	**Lipase**	**Esterase**
I. Raíz 38	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 99	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 97	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 100	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Raíz 44	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 41	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 45	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 32	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Raíz 35	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
III. Raíz 247	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 10	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 34	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 33	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 31	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
I. Raíz 256	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*
I. Raíz 09	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 05	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 03	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 51	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 06	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
II. Raíz 246	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 68	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 133	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PvP 60	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 25	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 59	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 130	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 26	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Raíz 27	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 24	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 23	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 22	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Raíz 21	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Raíz 29	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 30	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 37	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 01	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 48	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*

I. Raíz 47	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 46	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 45	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 44	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 43	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 42	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 41	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 15	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 16	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 13	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 14	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 11	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 12	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 17	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
III. Raíz 257	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 28	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 04	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Raíz 111	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 02	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 39	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 47	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 56	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 62	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 116	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 127	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 131	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Raíz PvP 134	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 63	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 61	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Raíz PvP 65	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 64	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PvP 66	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 67	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 69	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 52	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 53	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 54	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 57	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 58	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 43	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PvP 44	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*

Raíz PvP 42	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 45	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 129	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 128	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 111	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PvP 112	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 113	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 114	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 18	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Raíz 20	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
I. Raíz 19	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PvP 49	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 48	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PvP 50	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Raíz PvP 98	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 13	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 14	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 15	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 16	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 126	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PvP 46	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Positives	*30*	*1*	*36*	*24*	*42*	*4*	*0*
Positives (%)	***30***	***1***	***36***	***24***	***42***	***4***	***0***

Enzymatical activity Screening – Branches

Isolate	**Amylase**	**Cellulase**	**Protease**	**Pectin pH 5,0**	**PectinpH 8,0**	**Lipase**	**Esterase**
IV. Caule 291	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
IV. Caule 191A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 195	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 196	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Caule PvP 137	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*
IV. Caule 177	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 178	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 179	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 137	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 132	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
III. Caule 135	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 221	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 222	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 192	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Caule PvP 121	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*

Caule PvP 120	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Positive*
Caule PvP 143	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
III. Caule 119	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 191	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Caule PvP 119	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 139	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*
I. Caule 231	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 190	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 183	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 187	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Caule 228	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 197	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 226	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Caule 226A	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
III. Caule 200	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 201	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
IV. Caule 205	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 203	*Negative*		*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 196A	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 197A	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
III. Caule 138	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 180	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 185	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 116	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 199	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 198	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
IV. Caule 194	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
IV. Caule 193	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Caule 224	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 117	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Caule PvP 118	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Positive*	*Negative*
Caule PvP 71	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Caule PvP 122	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 70	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 74	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Caule PvP 75	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 72	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Caule PvP 73	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 176	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Caule 175	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 135	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*

Caule PvP 136	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 76	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 141	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Caule PvP 142	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*
Caule PvP 140	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 138	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
III. Caule 134	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 121	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 122	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 123	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 124	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 125	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 216	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 129	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Caule 130	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 144	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 210	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 220	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 219	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 218	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 217	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 213	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Caule 211	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PvP 77	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
I. Caule 214	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Positives	*5*	*14*	*8*	*1*	*25*	*7*	*4*
Positives (%)	***6.2***	***17.3***	***9.9***	***1.2***	***30.9***	***8.6***	***4.9***

Enzymatical activity Screening – Leaves

Isolate	**Amylase**	**Cellulase**	**Protease**	**Pectin pH 5,0**	**Pectin pH 8,0**	**Lipase**	**Esterase**
II. Folha 253	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
II. Folha 250	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PvP 153	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
II. Folha 251	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
II. Folha 254	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Folha 235	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Folha 234	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Folha 237	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
II. Folha 248	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
IV. Folha 264	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PvP 154	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*

Folha PvP 147	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Folha PvP 148	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Folha PvP 149	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*
Folha PvP 150	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
II. Folha 249	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Folha 241	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PvP 155	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Folha 236	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
I. Folha 238	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PvP 151	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Folha PvP 152	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PvP 145	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PvP 146	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Positives	*3*	*3*	*3*	*1*	*9*	*0*	*0*
Positives (%)	***12.5***	***12.5***	***12.5***	***4.2***	***37.5***	***0.0***	***0.0***

Enzymatical activity Screening – Flowers

Isolate	**Amylase**	**Cellulase**	**Protease**	**Pectin pH 5,0**	**PectinpH 8,0**	**Lipase**	**Esterase**
I. Flor 243	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
II. Flor 260	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Flor PvP 163	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Flor PvP 160	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Flor PvP 162	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
I. Flor 242	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Flor PvP 159	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Flor PvP 164	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Flor PvP 157	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Flor PvP 158	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
III. Flor 259	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Positives	*2*	*0*	*4*	*1*	*8*	*0*	*0*
Positives (%)	***18.2***	***0.0***	***36.4***	***9.1***	***72.7***	***0.0***	***0.0***

Supplementary table2

Results of enzymatical tests in bacterial isolates from white-flower *Polygala* sp.

White-flower *Polygala* sp.							
Enzymatical activity Screening - Rhizosphere							
Isolate	**Amylase**	**Cellulase**	**Protease**	**Pectin pH 5,0**	**PectinpH 8,0**	**Lipase**	**Esterase**
Rizo PpP 187	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 186	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 185	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 184	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 183	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 181	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 42	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo PpP 45	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PpP 219	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 195	*Negative*	*Positive*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PpP 182	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 215A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 214	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 21	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PpP 205	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 40	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 212	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 200	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 221	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 210A	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PpP 167	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PpP 169	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PpP 26	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 175	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 09	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo PpP 03	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PpP 172	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 166	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 218	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 176	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 193	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 163	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*
Rizo PpP 162	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 171	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*

Rizo PpP 206	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 189	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PpP 01A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 207C	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 207B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 208	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 204	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 11B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 11A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 179	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 178	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 207A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 30	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 197	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*
Rizo PpP 188A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 201	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 01C	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 199	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 196	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 209	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Rizo PpP 24	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 22	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 23	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo PpP 27	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 29	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 31	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 32	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 33	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 28	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo PpP 203	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 16	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 202	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 194	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 192	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo PpP 43	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PpP 39	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo PpP 38	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 01 azul	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Positives	***4***	***7***	***23***	***4***	***9***	***8***	***3***
Positives (%)	***5.6***	***9.7***	***31.9***	***5.6***	***12.5***	***11.1***	***4.2***

Enzymatical activity Screening - Roots							
Isolate	**Amylase**	**Cellulase**	**Protease**	**Pectin pH 5,0**	**PectinpH 8,0**	**Lipase**	**Esterase**
Raíz PpP 117	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 282	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 112	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PpP 113	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 128	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 293	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PpP 111	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 297	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 300	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PpP 152	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 292	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 93	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 275	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 277	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 278	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 156	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PpP 158	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 159	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 276	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 143	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Raíz PpP 279	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 150	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 97	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 129	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 280	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 273B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 139	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 133	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 86	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PpP 99	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 98	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 92	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 87	*Negative*	*Positive*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PpP 296B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 114	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PpP 90B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 124	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Raíz PpP 137	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 94A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*

Raíz PpP 94B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 144	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 273A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 96	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 119	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 131 (bolhosa)	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PpP 274A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 157	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*
Raíz PpP 107	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PpP 125	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Raíz PpP 126	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 142	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 136	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 290	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Raíz PpP 121	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 122	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 123	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 135	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Raíz PpP 283	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 109	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 284	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 291	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 274B	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*
Raíz PpP 132	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 130	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 89A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 395	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 294	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 120	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Raíz PpP 145	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 88	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 118	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 299	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 100	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 127	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 148	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 84A	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 151	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 271	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 85A	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*

Raíz PpP 89B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 84B	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz Pp04 Frg	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*
Raíz Pp06 Frg	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz Pp03 Frg	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz Pp rosa brlt	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Positives	***4***	***5***	***24***	***4***	***13***	***5***	***2***
Positives (%)	***4.7***	***5.9***	***28.2***	***4.7***	***15.3***	***5.9***	***2.4***

Enzymatical activity Screening - Branches

Isolate	Amylase	Cellulase	Protease	Pectin pH 5,0	PectinpH 8,0	Lipase	Esterase
Caule PpP 70	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 73	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 74	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 75	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*
Caule PpP 76	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*
Caule PpP 79	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 260	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 261	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 262	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 264	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 265	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 266	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 257	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 258	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Caule PpP 80	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 247	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Positive*	*Positive*
Caule PpP 249	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Caule PpP 250	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 251	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 252	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 253	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 254	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 255	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 62	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 63	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 64	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 65	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 66	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 67	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 241	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Caule PpP 243	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*

Caule PpP 244	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 256	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 61	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Caule PpP 68	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 69	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Caule PpP 242	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Positive*	*Positive*
Caule PpP 245	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Caule PpP 246	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Caule PpP 305	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Positives	***2***	***3***	***15***	***8***	***8***	***11***	***3***
Positives (%)	***5.0***	***7.5***	***37.5***	***20.0***	***20.0***	***27.5***	***7.5***

Enzymatical activity Screening - Leaves

Isolate	**Amylase**	**Cellulase**	**Protease**	**Pectin pH 5,0**	**PectinpH 8,0**	**Lipase**	**Esterase**
Folha PpP 312	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP 313	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP 315	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Folha PpP 48	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP 58	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP 59	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP 230	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP 234	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP 235	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP 236	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP rosa 01	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*
Folha PpP rosa 02	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*
Folha PpP rosa 03	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Positive*	*Negative*
Folha PpP rosa 04	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Folha PpP rosa 05	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Folha PpP rosa 06	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*
Folha PpP rosa 08	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Positives	***2***	***4***	***2***	***0***	***5***	***3***	***0***
Positives (%)	***11.8***	***23.5***	***11.8***	***0.0***	***29.4***	***17.6***	***0.0***

Supplementary table 3

Bioactivity detection in NPEs produced by endophytic fungi isolated from *Polygala* spp..

Antagonistic screening produced by endophytic fungi			
Isolate	***S. aureus***	***E. coli***	***C. albicans***
*Roxo 00**	*Negative*	*Negative*	*Negative*
Roxo 01	*Positive*	*Positive*	*Negative*
Roxo 02	*Negative*	*Negative*	*Negative*
Roxo 03	*Positive*	*Positive*	*Negative*
Roxo 07	*Negative*	*Negative*	*Positive*
Roxo 08	*Negative*	*Negative*	*Negative*
Roxo 09	*Negative*	*Negative*	*Negative*
Roxo 12	*Negative*	*Negative*	*Negative*
Roxo 14	*Negative*	*Negative*	*Negative*
Roxo 14'	*Negative*	*Negative*	*Negative*
Roxo 15	*Negative*	*Negative*	*Negative*
Roxo 24	*Negative*	*Negative*	*Negative*
Roxo 28	*Negative*	*Negative*	*Negative*
GLRF 23	*Negative*	*Negative*	*Negative*
GLRF 24	*Positive*	*Positive*	*Positive*
I Caule Pv - Fungo 1	*Positive*	*Positive*	*Negative*
I Caule Pv - Fungo 2	*Positive*	*Positive*	*Negative*
Caule Pv - Fungo 2	*Negative*	*Negative*	*Negative*
Caule Pv - Fungo 7	*Negative*	*Negative*	*Negative*
Caule Pv - Fungo D	*Negative*	*Negative*	*Negative*
Caule Pv - Fungo E	*Negative*	*Negative*	*Negative*
II Folha Pv - Fungo 1	*Negative*	*Negative*	*Negative*
Folha Pv - Fungo 10	*Negative*	*Negative*	*Negative*
Folha Pv - Fungo A	*Negative*	*Negative*	*Negative*
Folha Pv - Fungo B	*Positive*	*Positive*	*Negative*
Folha Pv - Fungo F	*Negative*	*Negative*	*Negative*
Flor Pv - Fungo 4	*Positive*	*Positive*	*Negative*
Flor Pv - Fungo 5	*Negative*	*Negative*	*Negative*
Flor Pv - Fungo C	*Negative*	*Negative*	*Negative*

Supplementary table 4

Overlay assay results for bioactivity against pathogens in actinobacteria collection.

Actinomycetes collection - Overlay Assay							
Endophytic group							
Isolate	**Source**	***S. aureus***		***E. coli***		***C. albicans***	
I. Caule 227	*P. violacea*						
II. Folha 255	*P. violacea*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
III. Raíz 247	*P. violacea*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 288	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Raíz PpP 16	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Raíz PpP 298	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo PpP 37	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Positive*
Rizo PpP 220	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rhizosphere - White-flower *Polygala* sp.							
Isolate	**Source**	***S. aureus***		***E. coli***		***C. albicans***	
Rizo Pp Ac 01	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 02	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 05	*P. paniculata*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 06	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 09	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 11	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*
Rizo Pp Ac 12	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 12 rugoso	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 12 liso	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 16	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*
Rizo Pp Ac 18 branco	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 19	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 24 rugoso	*P. paniculata*	*Positive*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 25	*P. paniculata*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 26-1	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 27	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 28	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*	*Negative*
Rizo Pp Ac 34	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 34 cinza	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 37	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 41 escuro	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 41 claro	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 42 branco	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*

Rizo Pp Ac 42 cinza	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 43	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo Pp Ac 44	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 45	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 51	*P. paniculata*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 52	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 54	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo Pp-3 Ac A	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp-3 Ac H1	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo Pp-3 Ac H2 cinza	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac I	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 02-1	*P. paniculata*	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 03	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 03-1	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 03-2	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 07	*P. paniculata*						
Rizo Pp Ac 07-1	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 07-2	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 08	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 09	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 10	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 10-1	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 12	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 12-1	*P. paniculata*						
Rizo Pp Ac 15	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 18 branco	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 19	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 20	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 20-1	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 21	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 22	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 22-1	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 24	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 24-1	*P. paniculata*						
Rizo Pp Ac 26 cinza	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 27	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 28	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*
Rizo Pp Ac 28-1	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo Pp Ac 30' branco	*P. paniculata*	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 30' branco 1	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 30''	*P. paniculata*	*Negative*	*Negative*	*Positive*	*Positive*	*Positive*	*Negative*

Rizo Pp Ac 30" branco	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*	*Positive*
Rizo Pp Ac 31	*P. paniculata*	*Positive*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pp Ac 34 branco	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 41 borda 1	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 41 borda 2	*P. paniculata*	*Positive*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 42	*P. paniculata*	*Positive*	*Negative*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo Pp Ac 43	*P. paniculata*	*Positive*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*
Rizo Pp Ac 44	*P. paniculata*	*Positive*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 45	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 48	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp Ac 49	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pp-3 Ac H2	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pp Ac 18	*P. paniculata*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*

Rhizosphere Purple-flower *Polygala* sp.

Isolate	**Source**	***S. aureus***		***E. coli***		***C. albicans***	
Rizo Pv Ac 01	*P. violacea*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pv Ac 01 branco	*P. violacea*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pv Ac 01 branco 1	*P. violacea*	*Positive*	*Positive*	*Negative*	*Negative*	*Positive*	*Negative*
Rizo Pv Ac 02	*P. violacea*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pv Ac 02 cinza	*P. violacea*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pv Ac 03	*P. violacea*	*Negative*	*Negative*	*Negative*	*Negative*	*Positive*	*Positive*
Rizo Pv Ac 03?	*P. violacea*	*Positive*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*
Rizo Pv Ac 03?1	*P. violacea*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pv Ac 05	*P. violacea*	*Positive*	*Positive*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pv Ac ?	*P. violacea*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
Rizo Pv Ac ?1	*P. violacea*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*

Supplementary figure 1:

ESI-MS chromatogram of the purified BS-39 .