VILNIUS UNIVERSITY
LIFE SCIENCES CENTRE

AKVILĖ VILPIŠAUSKAITĖ

Molecular Biotechnology
The 4th Semester student

Master Thesis

BACTERIOCINS ACTIVE AGAINST PLANT PATHOGENIC BACTERIA

Supervisor dr. Arnoldas Kaunietis

Vilnius, 2023

TABLE OF CONTENT

ABBREVIATIONS

AMP – antimicrobial peptides

BLB – bacterial leaf blight

CBB – cassava bacterial blight disease

EPS – extracellular polymeric substance

FDA – Food and Drug Administration

GRAS – generally recognized as safe

LAB – lactic acid bacteria

LAP – linear azole-containing peptides

MRSA – methicillin-resistant Staphylococcus aureus

PGPR – plant growth-promoting rhizobacteria

RiPP – ribosomally synthesized and post-translationally modified peptides

Th17 – thuricin 17

TOMM – thiazole/oxazole-modified microcins

VRE – vancomycin-resistant enterococci

VRE – vancomycin-resistant Enterococcus

WHO – World Health Organization

Xam – *Xanthomonas axonopodis* pv. *manihotis*

Xcc – *Xanthomonas campestris* pv. *campestris*

Xcm – *Xanthomonas campestris* pv. *malvacearum*

Xcv – *Xanthomonas campestris* pv. *vesicatoria*

Xoo – *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

The global agricultural industry faces significant challenges due to the annual loss of crops caused by diseases, insects, and weeds. The economic impact of plant diseases alone amounts to approximately $220 billion annually. Moreover, the current measures employed to control these diseases, such as the heavy use of pesticides, present additional environmental and health concerns. The agriculture industry, often considered one of the most dangerous industries, contributes to pollution in the ground, rivers, and oceans, leading to contaminated food, soil, water, and air. Additionally, the widespread use of antibiotics has resulted in the emergence of antibiotic-resistant bacteria strains, imposing a significant economic burden on the healthcare system. To address these challenges and reduce the reliance on chemically toxic agents, alternative strategies are being explored. One such strategy involves the use of bacteriocins and bioactive peptides, derived from prokaryotic cells, which exhibit antimicrobial properties. They are divided into three classes: class I - small, post-translationally modified, class II - small, non-post-translationally modified, and class III - large, non-post-translationally modified bacteriocins. These naturally occurring compounds have gained considerable attention as a safer and more effective alternative to synthetic control agents.

Bacteriocins have been studied for a long time, but little is known about the use of bacteriocins in agriculture. Currently, over 500 bacteriocins have been identified and characterized, with the majority originating from rhizosphere bacteria. These antimicrobials possess potent killing action, high stability, and low toxicity to humans, making them suitable alternatives to chemically toxic agents. The full potential of bacteriocins in controlling phytopathogenic bacteria remains unexplored, and additional research is required to assess their benefits and limitations comprehensively. Further research is necessary to optimize their production and develop efficient delivery systems for agricultural applications. While bacteriocins offer a promising alternative to synthetic chemicals in agriculture, the challenges associated with their use must be addressed through further research, regulation, and evaluation of their potential efficacy.

Research aim: isolate and purify bacteriocins active against phytopathogenic bacteria.

Objectives:

- Isolate bacterial strains from soil samples.
- Perform antibacterial activity screening of bacterial isolates against phytopathogenic bacteria.
- Perform identification of bacterial isolates which shows antibacterial activity against phytopathogenic bacteria.
- Evaluate antibacterial activity of the selected bacteria isolates' supernatants.
- Optimize bacteriocin purification and evaluate its molecular weight.

1. LITERATURE REVIEW

1.1. Bacteriocins

Production of antimicrobial peptides (AMPs) is a widespread phenomenon among all forms of life, from multicellular organisms to bacterial cells. In higher organisms, AMPs contribute to innate immunity and is part of the first defence line against harmful microorganisms. Maganins, cecropins and defensins are animal and thionins are plant antimicrobial peptides (Zasloff, 1987; Torreblanca et al., 1994; Broekaert et al., 1995). In bacteria, production of AMPs provides a competitive advantage for the producer in certain ecological niches because of the peptide-mediated killing of other bacteria (Nissen-Meyer and Nes, 1997). Bacteria produce two types of AMP: those which are synthesized by ribosomes (also called bacteriocins) and AMPs that are not synthesized by ribosomes, without structural genes coding for these AMPs (Chikindas et al., 2018).

Bacteriocins are multifunctional, ribosomally produced proteinaceous substances with pronounced antimicrobial activity at certain concentrations (Chikindas et al., 2018). They are protein toxins produced by bacteria and some members of archaea to inhibit the growth of similar or closely related bacterial strains (Bemena et al., 2014). Bacteriocins can exhibit narrow spectra of activity – targeting members of the same species; whereas others display broader activity spectra – targeting other species and genera (Cotter et al., 2013). Two main features distinguish the majority of bacteriocins from classical antibiotics: bacteriocins are ribosomally synthesized and have a relatively narrow killing spectrum (Riley and Wertz, 2002). The bacteriocin family includes a diversity of proteins in terms of size, microbial target, mode of action, release, and immunity mechanisms and can be divided into the two main groups: those produced by Gram-negative and produced by Gram-positive bacteria (Gordon et al., 2007; Heng et al., 2007).

1.1.1. Bacteriocins from Gram-negative bacteria

Bacteriocins from Gram-negative bacteria arise mainly from Enterobacteriaceae. They are divided into four main classes: colicins, colicin-like, phage-tail-like bacteriocins, and microcins (Chavan and Riley, 2007).

The first bacteriocin to be described had been identified in 1925 from the Gram-negative bacterium *Escherichia coli* (Gratia, 1925). It inhibited the growth of another *E. coli* strain and later this protein was classified as a colicin (Güllüce et al., 2013). Colicins are protease-sensitive, heat-sensitive high-molecular weight (30–80 kDa) bactericidal proteins synthesized by most *E. coli* strains. Compounds of this class are the most studied and are used as model systems for studying the structures, functions, and evolution of bacteriocins (Acedo et al., 2018). Protein bacteriocins produced by other Gram-negative bacteria are classified as colicin-like due to the presence of similar

structural and functional characteristics (Michel-Briand and Baysse, 2002). The most studied colicin-like bacteriocins are klebicins from *Klebsiella* spp., S-pyocins from *Pseudomonas aeruginosa*, and alveicins from *Hafnia alvei* (Preciado et al., 2016). Two of the most studied phage-tail-like bacteriocins are the R- and F-pyocins from *P. aeruginosa* (Nakayama et al., 2000). Microcins are small peptides (<10 kDa), that can be divided into two classes: posttranslationally modified (B17, C7, J25, and D93) and unmodified (E492, V, L, H47, and 24) microcins (Gillor et al., 2004). The classic example is microcin V, of *E. coli*, which is composed of only a few peptides and exhibits stability at high temperatures (Güllüce et al., 2013). The narrow antimicrobial activity spectrum limits the use of Gram-negative bacteria bacteriocins in high-end industries. In general, the studies have been focused on more suitable types of bacteriocins from Gram-positive bacteria (Balciunas et al., 2013).

1.1.2. Bacteriocins from Gram-positive bacteria

Bacteriocins produced by Gram-positive bacteria are formed by fewer than 60 amino acids and they have a broad spectrum of action (Heng et al., 2007; Perez et al., 2014; Yang et al., 2014). The main genus producers of bacteriocins are *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leptosphaeria*, *Leuconostoc*, *Melissococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Thokchom and Joshi, 2012). Gram-positive bacteria also include other bacteriocin producer genera with biotechnological importance, such as *Aerococcus*, *Microbacterium*, *Propionibacterium*, and *Bifidobacterium* (Swetwiwathana and Visessanguan, 2015). Bacteriocins produced by Gram-positive bacteria can be widely used in the medical and food industries because they are non-toxic and the most lactic acid bacteria (LAB) are generally recognized as safe (GRAS) (Cintas et al., 2001; Cleveland et al., 2001; Balciunas et al., 2013). Bacteriocins produced by the *Bacillus* genus may be considered as the second most important after bacteriocins produced by the LAB. They are increasingly becoming more important due to their sometimes broader spectra of inhibition (as compared with most LAB bacteriocins), which may include Gram-negative bacteria, yeasts or fungi, in addition to Gram-positive species, some of which are known to be pathogenic to humans and/or animals (Abriouel et al., 2011).

1.2. Classification of bacteriocins

Due to the extensive focus on LAB bacteriocins, a number of classification schemes have been suggested which are largely applicable to other Gram-positive bacteriocins. Early attempts to classify LAB bacteriocins involved placing individual bacteriocins into one of eight groups based on their heat resistance, host range, trypsin sensitivity and the degree of cross-reactivity between various bacteriocin and host combinations (Kozak et al., 1978; Geis et al., 1983). This approach was superseded by that developed by Klaenhammer (1993), who grouped bacteriocins into four distinct

classes with further subclasses. These groupings have formed the basis of all subsequent classification schemes for bacteriocins of Gram-positive bacteria. Klaenhammer suggested the four classes of bacteriocins:

- Class I or lantibiotics: defined as small membrane-active peptides (<5 kDa) containing the unusual amino acids lanthionine or b-methyl lanthionine (hence the name lantibiotics) and dehydrated residues. Nisin is the most characterised bacteriocin of the Class I.
- Class II: defined as small heat-stable non-lanthionine containing membrane-active peptides characterised by the presence of a Gly-Gly processing site in the bacteriocin's precursor, the presence of amphiphilic helices with varying amounts of hydrophobicity and moderate-to-high heat stability. These were further divided into three subgroups:
  a) Subclass IIa: Listeria-active peptides with a consensus sequence in the N-terminal of –Tyr-Gly-Asn-Gly-Val-Xaa-Cys-.
  b) Subclass IIb: poration complexes consisting of two proteinaceous peptides for activity.
  c) Subclass IIc: thiol-activated peptides requiring reduced cysteine residues for activity.
- Class III: large heat-labile proteins, often with enzymatic activity.
- Class IV: complex proteins composed of one or more chemical moieties, either lipid or carbohydrate (Klaenhammer, 1993).

All subsequent classification schemes accept the first two major classes of LAB/Gram-positive bacteriocins, the Class I lanthionine and Class II non-lanthionine containing peptides (Klaenhammer, 1993; Diep and Nes, 2002; Pag and Sahl, 2002; Heng et al., 2007). By conducting further research on bacteriocins, it has become clear that this class of ribosomally synthesized and post-translationally modified peptides that show antibacterial activity are much more varied (Arnison et al., 2013). This perception necessitated the re-evaluation of the classification of bacteriocins in Gram-positive bacteria and expanded the types of class I bacteriocins (Alvarez-Sieiro et al., 2016). According to Alvarez-Sieiro et al. (2016) classification of bacteriocins, there are three classes of bacteriocins **(Table 1.1)**:

- Class I (<10 kDa): this class encompasses all the peptides that undergo enzymatic modification during biosynthesis, which provides molecules with uncommon amino acids and structures that have an impact on their properties (e.g., lanthionine, heterocycles, head-to-tail cyclization, glycosylation). They consist of a leader peptide which serves for enzyme recognition, transport, and keeping the peptide inactive, which is fused to a core peptide (Arnison et al., 2013; Alvarez-Sieiro et al., 2016;).
- Class II (<10 kDa): this class groups bacteriocins that do not contain unusual modifications. Thus, they do not require enzymes for their maturation other than a leader peptidase and/or a transporter (Alvarez-Sieiro et al., 2016).

- Class III (>10 kDa): these are unmodified bacteriocins larger than 10 kDa with bacteriolytic or non-lytic mechanism of action (Alvarez-Sieiro et al., 2016).

Table 1.1 Classification of bacteriocins. Adapted from Hernández-González et al., 2021; Bhattacharya et al., 2022.

Class Type	Characteristic Features	Sub-classes	Major Examples of Bacteriocins
Class I	Small, heat-stable bacteriocins (<10 kDa), have a post-translational modification, resulting in the formation of atypical amino acids lanthionine and methyllanthionine.	Lantibiotics (lanthipeptides)	Nisin (Delves-Broughton et al., 1996)
		Lipolantins	Microvionin (Ovchinnikov et al., 2016)
		Thiopeptides	GE2270A (Tan et al., 2019)
		Bottromycins	Bottromycin A and B (Nakamura et al., 1965)
		Linear azole-containing peptides (LAPs)	Streptolysin S (Cox et al., 2015)
		Sactibiotics (sactipeptides)	Subtilosin A (Babasaki et al., 1985)
		Lasso peptides	Lariatin A (Iwatsuki et al., 2006)
		Cyclic bacteriocins with a “head-to-tail” connection	Enterocin AS-48 (Sánchez-Barrena et al., 2003)
		Glycocins	Sublancin (Oman et al., 2011)
Class II	Small and flexible bacteriocins (<10 kDa), with an amphiphilic helical structure. These peptides do not contain modified amino acid residues and are pH and heat resistant.	YGNG-motif containing bacteriocins	Leucocin A (Fregeau Gallagher et al., 1997)
		Linear two-peptide bacteriocins	Lactococcin G (Moll et al., 1998)
		Leaderless bacteriocins	Enterocin L50 (Luis M. Cintas et al., 1998)
		Other linear bacteriocins	Divergycin A (Rather et al., 2017)
Class III	Large (>10 kDa) unmodified bacteri ocins with bacteriolytic or non-lytic mechanism of action.	Bacteriolysins	Lysostafin (Schindler and Schuhardt, 1964)
		Non-lytic bacteriocins	Helveticin J (Joerger and Klaenhammer, 1990)
		Tailocins (phage tail-like bacteriocins)	Diffocins (Gebhart et al., 2012)

1.2.1. Class I: small post translationally modified peptides

Class I bacteriocins are divided into sub-classes **(Table 1.1)**: lantibiotics (lanthipeptides), lipolantins, thiopeptides, bottromycins, linear azole-containing peptides (LAPs), sactibiotics (sactipeptides), lasso peptides, cyclic bacteriocins with a "head-to-tail" connection and glycocins.

Lantibiotics (lanthipeptides) are peptides possessing unusual amino acids, such as lanthionine and/or (methyl)lanthionine (Arnison et al., 2013). Based on the posttranslational modification enzymes involved in the maturation process, lanthipeptides can be divided into four types, but only types I (LanBC-modified) and II (LanM-modified) can be considered lantibiotics. Types III and IV have no known antimicrobial activity (Knerr and van der Donk, 2012; Alvarez-Sieiro et al., 2016). A great number of different lantibiotics are produced by LAB. Among them, nisin, a type I lantibiotic produced by *Lactococcus lactis*, is the best studied (Delves-Broughton et al., 1996). This bacteriocin was given a GRAS designation and has been used as a natural food preservative for many years.

Lipolantins is a newly discovered variant of lantipeptides, which is characterized by the presence of avionin residue and the N-terminal guanidino fatty acid. The key representative of the group, microvionin is a bacteriocin derived from culture extracts of *Microbacterium arborescens* 5913, active against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus pneumonia* (Ovchinnikov et al., 2016). Lipolanthins' biosynthesis is a hybrid of the biosynthetic pathways of ribosomal peptide synthesis and fatty acid or polyketides biosynthesis. The role of lipolanthines is still being investigated (Wiebach et al., 2018).

Thiopeptides are thiazole-containing peptide antibiotics of ribosomal origin with complex structures (Arnison et al., 2013). There are three main components to thiopeptides—a central pyridine ring, a core macrocyclic ring, and a tail. The tail extends from the central pyridine ring while the macrocyclic ring is connected by the central pyridine. The macrocyclic ring is decorated with various amino acids as well as thiazole, oxazole, and thiazoline substituents. Some thiopeptides have a secondary side ring with unique chemical moieties (Chan and Burrows, 2021). Thiopeptides have multiple biological activity – antibacterial, antiviral, antiparasitic, and immunosuppressive (Tymoszewska et al., 2017). Thiopeptides are usually active in the nanomolar range, but their poor water solubility and low bioavailability make it difficult to use them in clinical application, despite their high activity. Thiopeptide derivative GE2270A is currently the only bacteriocin of this type undergoing clinical trials in the treatment of gastrointestinal infections caused by *Clostridium difficile* (Tan et al., 2019).

Bottromycins are modified thiazole/oxazole-microcins, similar in structure to thiopeptides. Their distinctive features include the presence of macrocyclic amidine, decarboxylated C-terminal thiazole, and several rare β-methylated amino acid residues (Baquero et al., 2019). Bottromycins were originally isolated from the fermentation broth of *Streptomyces bottropensis* in the late 1950s and

described as peptides' natural products with antibacterial activity against Gram-positive pathogens (Waisvisz et al., 1957; Franz et al., 2021). The botromycins discovered to date are produced by bacteria of the genus *Streptomyces* spp. and are potent agents against multidrug-resistant microorganisms, such as MRSA and vancomycin-resistant *enterococci* (VRE). Botromycins also inhibit protein synthesis by interacting with the bacterial 50S ribosomal subunit (Baquero et al., 2019; de Castro et al., 2020).

Linear azole-containing peptides (LAPs) are peptides possessing various combinations of heterocyclic rings of thiazole and (methyl)oxazole, which are derived from cysteine, serine, and threonine residues *via* ATP-dependent cyclodehydration and subsequent flavin mononucleotide-dependent dehydrogenation (Melby et al., 2011). They belong to a family of ribosomally synthesized and post-translationally modified peptides (RiPPs) referred to as thiazole/oxazole-modified microcins (TOMMs), along with the other bacteriocin classes, thiopeptides and bottromycins (Haft et al., 2010). The most relevant LAB-produced LAP is streptolysin S (Cox et al., 2015). Other two compounds of this group – plantazolicin and goadsporin – have been structurally characterized. Plantazolicin is a metabolic byproduct of *Bacillus amyloliquefaciens* FZB42, it shows selective antibacterial activity against closely related strains of the genus *Bacillus*. Goadsporin obtained from *Streptomyces* sp. TP-A0584, also has a narrow spectrum of action, which is limited to members of the genus. The mechanism of action of LAPs has not been studied (Makarova et al., 2019).

Sactibiotics (sactipeptides) are sulphur-to-α-carbon-containing peptides (Arnison et al., 2013; Mathur et al., 2015). Sactibiotics are produced by representatives of the genus *Bacillus* and, in addition to antibacterial properties, can also have spermicidal and hemolytic properties (Silkin et al., 2008; Huang et al., 2009). The first and best studied example of this subclass, subtilosin A secreted by *Bacillus subtilis*, was identified more than 30 years ago (Babasaki et al., 1985).

Lasso peptides are a group of RiPPs that shows as a main characteristic the presence of an amide bond between the first amino acid in the core peptide chain and a negatively charged residue in positions +7 to +9, generating a ring that embraces the C-terminal linear part of the polypeptide (Arnison et al., 2013; Hegemann et al., 2015). Currently, the structures of three lasso peptide bacteriocins produced by Gram-positive bacteria have been characterized: lariatin A produced by *Rhodococcus jostii* (Iwatsuki et al., 2006), streptomonomycin produced by *Streptomonospora alba* (Metelev et al., 2015), and svicenin produced by *Streptomyces sviceus* (Li et al., 2015). Lasso peptides are extremely stable structures that are resistant to the action of enzymes and high temperatures. During linearization, lasso peptides lose their properties and therefore, chemical synthesis of active bacteriocins of this type is not possible (Woraprayote et al., 2016). Moreover, lasso peptides display diverse activities which range from antimicrobial to putative antiviral or anticancer (Maksimov et al., 2012).

Cyclic bacteriocins with a “head-to-tail” connection are a group of RiPPs whose N- and C-termini are linked by a peptide bond, thereby rendering a circular molecule. All of them contain either four or five alpha helical segments (Montalbán-López et al., 2012; Lohans et al., 2013; Himeno et al., 2015; Acedo et al., 2018).

Glycocins are bacteriocins containing glycosylated residue(s) (Arnison et al., 2013). Sublancin, produced by *Bacillus subtilis* 168, is the first glycocin characterized experimentally (Oman et al., 2011). Currently, at least 10 different glycocins have been experimentally identified (Norris and Patchett, 2016; Ren et al., 2018). These peptides have antimicrobial activity against several pathogenic bacteria, including *Streptococcus pyogenes*, MRSA and food-spoilage bacteria *Listeria monocytogenes*. Glycocins exhibit immunostimulatory properties and make a promising source of new antibiotics and food preservatives familiar to Nisin (Wang et al., 2018).

1.2.2. Class II: unmodified bacteriocins

Class II bacteriocins are divided into sub-classes **(Table 1.1)**: YGNG-motif containing bacteriocins, linear two-peptide bacteriocins, leaderless bacteriocins and other linear bacteriocins.

YGNG-motif containing bacteriocins, often referred in the literature as type IIa or pediocin-like peptides, have several defining structural characteristics, such as a conservative N-terminal YGNG motif, at least one disulfide bridge, an amphipathic α-helix, and a common cationic charge. To date, a large number of bacteriocins of this class have been identified, but three-dimensional structures have been determined for only a few of them. The most well-studied compounds are leucocin A produced by *Leuconostoc gelidum* UAL 187, carnobacteriocin B2 produced by *Carnobacterium piscicola* LV17, sacacin P produced by *Lactobacillus sake* Lb706, curvacin A produced by *Lactobacillus curvatus* LTH1174, and enterocin HF produced by *Enterococcus faecium* M3K31 (Ekblad et al., 2016; Adedire O. M. and Odeniyi O. A., 2017).

Linear two-peptide bacteriocins consist of two very different peptides, and full activity requires the presence of both peptides in about equal quantities (Nissen-Meyer et al., 2010). They are produced as precursors containing double N-terminal glycine-type leader peptides and active center peptides with GXXXG and/or GXXXG-like motifs, where glycine can be replaced by alanine/serine. The gene cluster responsible for the synthesis of bacteriocin encodes two precursor peptides, immunity protein, ABC transporter, and auxiliary protein (Andryukov et al., 2018).

Leaderless bacteriocins are unique as they are synthetized without an N-terminal leader peptide, which usually functions as a recognition sequence for secretion and modification and maintains the bacteriocin inactive inside the producer cell. They usually contain a formylated N-terminal methionine (Liu et al., 2011; Masuda et al., 2012). Currently, there are at least 20 known leaderless

bacteriocins. One of the best studied and characterized leaderless bacteriocins is the plasmid-encoded two-peptide enterocin L50 from *E. faecium* L50 (Luis M. Cintas et al., 1998).

The group of other linear bacteriocins includes single-peptide non-pediocin-like peptides. Lactococcins A and B, enterocin B, carnobacteriocin A, divergycin A, and lactococcin 972 are the most well-studied members of the group. A typical tertiary structure consists of 2-3 stranded antiparallel β-sheets in a β-sandwich conformation with a predominance of aromatic residues at one terminus. These bacteriocins have a variety of antibacterial spectra, gene cluster organization, primary sequences, secretion mechanisms, and modes of action typical for other groups of this class (Rather et al., 2017).

1.2.3. Class III

Class III bacteriocins are divided into sub-classes **(Table 1.1)**: bacteriolysins, non-lytic bacteriocins and tailocins (phage tail-like bacteriocins).

Bacteriolysins are large polypeptides with a molecular weight of 27 to 35 kDa, which are characterized by sensitivity to higher temperatures and the ability to lyse the cell walls of target bacteria. Bacteriolysins consist of two key domains connected by a linker helix: the N-terminal catalytic domain and the recognition domain at the C-terminus. The catalytic domain is characterized as a hydrolytic protease that targets peptides and peptidoglycan cross-links. In addition to its main function, the substrate recognition domain is also an anchor for the catalytic domain movement along the peptidoglycan chain. The specificity and spectrum of action of bacteriolysins depends on the ability to hydrolyze various peptidoglycan sites (Rather et al., 2017). The most well-known bacteriolysins produced by Gram-positive bacteria are lysostafin produced by *Staphylococcus simulans* (Schindler and Schuhardt, 1964), zoocin A produced by *Streptococcus zooepidemicus* 4881 (Simmonds et al., 1996), millericin B produced by *Streptococcus milleri* NMSCC 061 (Beukes et al., 2000), and enterolysin A produced by *Enterococcus faecalis* LMG 2333 (Nilsen et al., 2003).

Non-lytic bacteriocins are similar to bacteriolysins in that they are large, thermolabile polypeptides, but their mechanism of function is not focused on cell wall lysis (Acedo et al., 2018). Examples of such bactericins are helveticin J produced by *Lactobacillus helveticus* 481 (Joerger and Klaenhammer, 1990), casecin 80 produced by *Lactobacillus casei* (Rammelsberg et al., 1990), and dysgalacticin produced by *Streptococcus dysgalactiae* subsp. *equisimilis* (Heng et al., 2006).

Tailocins (phage tail-like bacteriocins) are larger protein structures (20–100 kDa) consisting of 8-14 different polypeptide subunits that expose similarities to the bacteriophage tail modules (Ghequire and De Mot, 2015). Bacteriocins of this subclass are divided into two groups – R and F. R-type tailocins, which are evolutionarily related to the tails of phages in the family of Myoviridae and form a long shell-encircled tube, at one end of which is a complex basal plate with receptor-

binding proteins (RBP) (Sun et al., 2018). F-type bacteriocins belonging to the tails of phages in the Siphoviridae family do not have a shell. The mechanism of fuction of tailocins is not fully understood, and presumably it involves compression of the shell and penetration of the nucleus through the cell wall, which leads to the formation of a channel or pore that violates the membrane potential of the target cell (Scholl, 2017; Tracanna et al., 2017; Acedo et al., 2018).

1.3. Applications of bacteriocins

Bacteriocins have many positive properties that have made them particularly interesting for various applications. LAB bacteriocins are inherently tolerant of high thermal stress and are known for their activity over a wide pH range. These antimicrobial peptides are also colourless, odourless, and tasteless, which further improves their potential usability. They are also easily degraded by proteolytic enzymes due to their proteinaceous nature. Consequently, bacteriocins do not live long in the human body or in the environment, which minimizes the chance of target strains interacting with degraded fragments (Perez et al., 2014). Some of the most widely studied applications of bacteriocins – food industry, livestock industry and medicine.

1.3.1. Food industry

Food and beverage spoilage is always a concern in food industry, as it may destroy the taste of the food and beverages, as well as cause some food-borne illnesses in humans (Villalobos-Delgado et al., 2019). Chemical additives have been used widely to preserve food, but they can cause a lot of human health problems due to their toxicity. This concern has led to the high demand of natural and chemical-free products used to preserve food in the market in order to avoid health problems (Zanetti et al., 2018). The use of bacteriocins in food industry has been extensively investigated, particularly in dairy products, eggs, vegetables, and meat products (Zacharof and Lovitt, 2012). So far, nisin and pediocin PA-1 are bacteriocins licensed as food preservatives (Vijay Simha et al., 2012).

Nisin is one of the bacteriocins that was given a GRAS designation and has been approved by the United States Food and Drug Administration (FDA) and World Health Organization (WHO) to be applied in food production (Delves-Broughton, 2005). Nisin is used in over 50 countries and Nisaplin^{TM} is sold as a natural food protectant. It is effective in several food systems, inhibiting the growth of a wide range of Gram-positive bacteria, including many important food-borne pathogens, such as *Listeria monocytogenes*. It is mainly used in canned food and dairy products, and is especially effective in the production of processed cheese and spreads, whereas it protects against heat-resistant spore-forming organisms such as those of the genera *Bacillus* and *Clostridium*. This is particularly important in the case of prevention of *Clostridium botulinum* infection as there can be serious consequences due to toxin formation by this species (Deegan et al., 2006).

Pediocin PA-1 is a broad-spectrum bacteriocin produced by LAB such as *Pediococcus* spp. and is commercially available under the name Alta 2341^{TM} or Microgard^{TM} (Garsa et al., 2014). This bacteriocin exhibits particularly strong activity against *L. monocytogenes* and has been shown to be more effective than nisin against some food-borne pathogens such as *S. aureus* and Gram-negative organisms such as *Pseudomonas* and *E. coli* (L.M Cintas et al., 1998; Rodríguez et al., 2002; Jamuna and Jeevaratnam, 2004). The potential application of pediocins to dairy products is further enhanced by its stability in aqueous solutions, its wide pH range, and high resistance to heating or freezing (Sobrino-López and Martín-Belloso, 2008).

1.3.2. Livestock industry

Livestock are domesticated animals that are raised agriculturally to provide labour and commodities, such as milk, meat, eggs, furs and leathers. Livestock, as food sources, provide nutrients needed by humans every day, such as proteins, fats and vitamins. It is important to have proper feeding and hygiene to sustain the livestock health and enhance the economics *via* maximized production. However, animals on the farm are still easily infected by viruses and bacteria. The examples of infectious diseases caused by bacteria in livestock are mastitis, post-weaning diarrhea, meningitis, arthritis, endocarditis, pneumonia and septicemia (Varijakshapanicker et al., 2019). Mastitis is a common disease that can be found in dairy cattle. It refers to the inflammation of the mammary gland and under tissue as the results of bacterial infection, chemical or thermal injury. Mastitis is normally caused by the contamination of the milking machine or hands with pathogens, such as *Staphylococcus aureus*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*, leading to damage in the milk-secreting tissues and ducts, and can be fatal for severe cases without proper treatments (Zadoks et al., 2011). To solve this problem, there are limitations in using antibiotic treatments, such as gentamicin, which may lead to the emergence of pathogens that are resistant to antibiotics (Munita and Arias, 2016). As an alternative to antibiotics, many research studies have investigated the use of bacteriocin that exert inhibitory ability to inhibit or kill the pathogens in livestock.

The use of nisin as preventive medicine and as a remedy for mastitis in cattle has been investigated in the livestock industry. Nisin-based injectable drugs have been reported to control nearly 99.9% of bacteria that cause mastitis, such as *S. aureus* and *S. dysgalactiae* after drug administration (Kitazaki et al., 2010). Another widely tested bacteriocin, that has been used in treatment of mastitis in lactating dairy cows, is lacticin 3147, extracted from *L. lactis* sub-specie *Lactis* DPC3147. It inhibits the growth of *S. dysgalactiae* and *S. aureus*, thereby significantly reducing the probability of mastitis (Pieterse and Todorov, 2010; Suda et al., 2012).

1.3.3. Medicine

First antibiotic penicillin was discovered in 1928 by Professor of Bacteriology Alexander Fleming and antimicrobial effects against a wide range of pathogens were considered as a big contribution in the medical field (Joerger, 2003; Adedeji, 2016). However, the findings of new antibiotics began decreasing after 1985, since the discoveries of bacteria that were resistant to antibiotics increased significantly (Munita and Arias, 2016). This problem alerted humans to find out alternative antimicrobial agents that can be used in killing or inhibiting pathogens. Bacteriocin, with its proteinaceous nature, was recommended by many researchers to replace antibiotics for the treatment of infectious disease due to its low toxicity. Many research studies have been done to investigate bacteriocin, which can be used to solve human health's and device-associated infections problems.

Lantibiotics exhibit potent activity against Gram-positive bacteria including clinically relevant multidrug resistant species such as MRSA, VRE, vancomycin intermediate *S. aureus*, *S. pneumoniae*, *Listetria*, *Bacillus* sp. and *C. difficile*. Several lantibiotic peptides displaying outstanding *in vivo* activity have been put forward clinically for the treatment of potentially fatal bacterial diseases (Field et al., 2015). Furthermore, the lantibiotic mutacin B-Ny266 displays activity against Gram-negative strains of *Neisseria* and *Helicobacter* (Ghodhbane et al., 2015). The use of bacteriocins in combination with antibiotics has shown efficacy at reducing the concentration of antibiotic needed (chloramphenicol amongst others) to inhibit bacterial cell growth of *E. faecalis*. Nisin, for example, displays potent anti-biofilm activity against *E. faecalis* in combination with penicillin, ciprofloxacin, and chloramphenicol (Tong et al., 2014; Ongey and Neubauer, 2016). Findings also demonstrate the anti-biofilm activity of nisin in combination with polymyxins against *P. aeruginosa* (Mathur et al., 2017). The formation of biofilms on medical devices including catheters is extremely problematic where they constitute the most frequent cause of nosocomial septicemia. Bacteriocins Pep 5 and epidermin produced by *Staphylococcus epidermidis* both demonstrated an inhibitory action against the adhesion of *Staphylococcus* species to the surfaces of silicon catheters (Field et al., 2015). Additionally, nisin has been reported to possess biofilm penetrating abilities potentially making it a useful tool in preventing or eradicating biofilm communities on invasive medical devices (Mathur et al., 2018).

Several bacteriocins have shown anticancer activities by selectively acting against cancer cells (Papo and Shai, 2005; Hoskin and Ramamoorthy, 2008; Kaur and Kaur, 2015). Bacteriocins produced by Gram-negative bacteria, such as microcin E492 and colicins (A, D, E1, E2, E3), or by Gram-positive bacteria, including nisin, have demonstrated cytotoxic effects against malignant human cell lines (Lancaster et al., 2007; Lagos et al., 2009; Joo et al., 2012; Kamarajan et al., 2015). The cytotoxicity of bacteriocins against cancer cells is caused by the induction of apoptosis and/or

depolarization of the cell membrane leading to permeability changes (Kaur and Kaur, 2015). It is important to note that the majority of studies relating to the anticancer properties of bacteriocins have been of an *in vitro* nature and, thus, there is a need for *in vivo* validation (Soltani et al., 2021).

While the benefits of bacteriocins in treating infectious disease appear evident, it must also be noted that some Gram-positive bacteria can utilize bacteriocins as virulence factors increasing their pathogenicity (Dicks et al., 2018). For instance, pathogenic *Streptococcus* strains produce various bacteriocin virulence factors including hemolysins, intermedilysin and streptolysin S, involved in an invasive group of *Streptococcus* infection (Tabata et al., 2019).

1.4. Bacteriocins as biocontrol agents in agriculture

It is estimated, that between 25% and 31% of all crops produced worldwide are lost annually because of diseases, insects and weeds. These losses do not include losses caused by environmental factors such as freezes, droughts, air pollutants, nutrient deficiencies, and toxicities. It has been estimated that of the 28% average of total losses, 11% are caused by diseases, 8% by insects, and 9% by weeds **(fig. 1.1)**. Considering that 11% of the crops are lost of plant diseases alone, the total annual worldwide crop loss of plant diseases is about $220 billion. The need for measures to control plant diseases limits the amount of land available for cultivation each year, limits the kinds of crops that can be grown in fields already contaminated with certain microorganisms, and annually necessitates the use of millions of kilograms of pesticides for treating seeds, fumigating soils, spraying plants, or the postharvest treatment of fruits and vegetables (Agrios, 2005a).

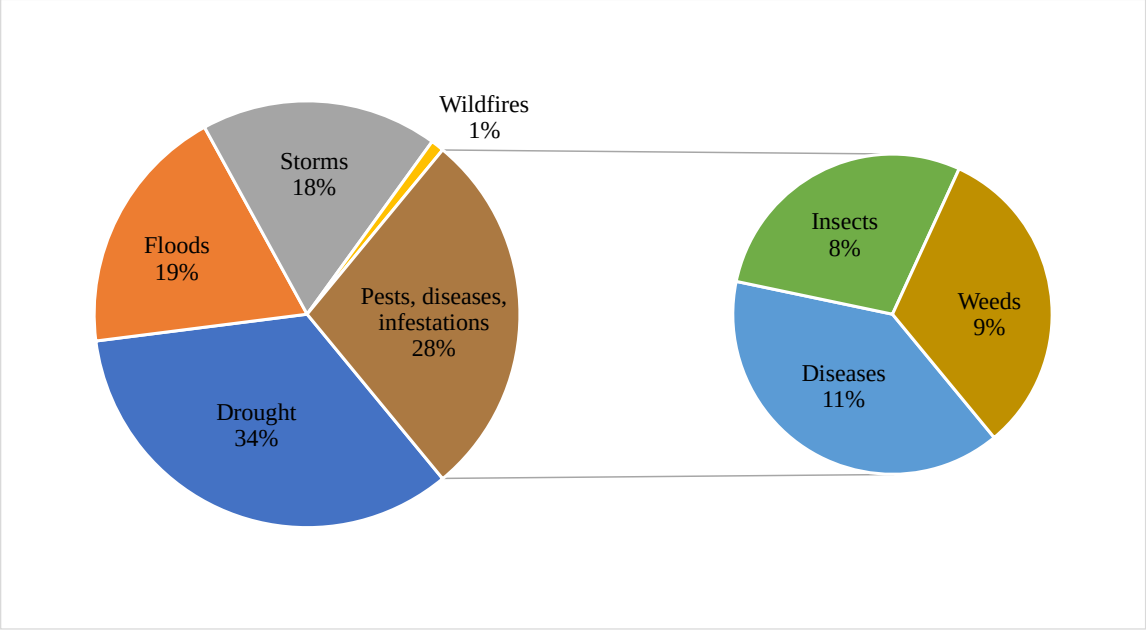

Fig. 1.1 Estimated annual crop losses percentage to drought, floods, storms, wildfires and pests, diseases, infestations worldwide. Based on Agrios, 2005a; *The impact of disasters and crises on agriculture and food security*, 2021.

The absence of plant diseases control measures generates a significant global issue, since agriculture industry has been considered among one of the most dangerous industries, responsible for polluting the ground, river, and ocean water, leading to polluted food, soil, water, and air. The extended use of synthetic fertilizers and pesticides including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators, and antibiotics not only pose danger to the consuming public but is of high threat to farmers and workers in the field handling these chemicals. Additionally, the widespread use of antibiotics has led to stronger, more antibiotic-resistant strains of bacteria which is significantly increasing the economic burden on the entire healthcare system. One strategy to combat or overcome the hazards is to consider an alternative to supplement or replace these chemically toxic agents with more safe and effective antimicrobial agents. On this matter, bacteriocins and bioactive peptides, that originated from prokaryotic cells, with potential antimicrobial activity have been explored vastly as an alternative strategy to replace the chemically used control agents (Cintas et al., 2001; Zhang et al., 2009; Mojgani, 2017). A wide range of rhizosphere- and plant-associated bacteria have been identified as potential bacteriocin producers demonstrating a wide range of inhibitory spectrum toward economically important plant pathogens (Mojgani, 2017). Bacteriocins can be applied to the plant as a foliar spray or seed treatment, providing a natural alternative to synthetic pesticides. Moreover, bacteriocins can be used as biocontrol agents to control the growth of unwanted bacteria in soil and water. They can be applied as a soil amendment or a water treatment, providing a natural alternative to synthetic chemicals. Bacteriocins produced by *Bacillus* spp., such as subtilosin and amylocyclicin, have been shown to be effective against a range of soil and waterborne pathogens, including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Khatoon et al., 2020).

To date, more than 500 bacteriocins have been identified and characterized of which the majority is produced by rhizosphere bacteria. These antimicrobials characterized as highly potent toxins with powerful killing action, high stability, and low toxicity to humans have been considered as a viable option and a suitable alternative to chemically toxic agents used in many industrial applications. However, there are several challenges associated with bacteriocin use in agriculture that need to be addressed. One major challenge is the narrow spectrum of activity, which limits their effectiveness against phytopathogens. Additionally, high production costs can make bacteriocins an expensive option for farmers. Further research is needed to optimize the production of bacteriocins and develop effective delivery systems for their application in agriculture. Another challenge is the lack of specific regulations governing the use of bacteriocins in agriculture. This can make it difficult for producers to obtain approval for their use, further hindering their adoption. Furthermore, the potential of bacteriocins in controlling phytopathogenic bacteria in agriculture has not been fully evaluated. The lack of research limits the understanding of the benefits and limitations of bacteriocins

and requires further investigation. While bacteriocins offer a promising alternative to synthetic chemicals in agriculture, the challenges associated with their use must be addressed through further research, regulation, and evaluation of their potential efficacy (Mojgani, 2017).

1.4.1. Phytopathogenic bacteria

About 100 species of bacteria cause diseases in plants (Agrios, 2005b). The main symptoms of bacterial diseases are wilting, necrosis, chlorosis, rot, overgrowth (galls), and scab (Nazarov et al., 2020). Based on scientific and economic importance, the most significant plant pathogens are *Pseudomonas syringae* pathovars, *Ralstonia solanacearumi*, *Agrobacterium tumefaciens*, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), *Xanthomonas campestris* pathovars, *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya* (*dadantii* and *solani*) and *Pectobacterium carotovorum*. Other significant phytopathogenic bacteria are *Streptomyces europaeiscabiei* and *Streptomyces scabies* (Mansfield et al., 2012).

Pseudomonas syringae is a rod-shaped, Gram-negative phytopathogenic bacterium that causes diseases of monocots, herbaceous dicots, and woody dicots, worldwide (Lamichhane et al., 2014). *P. syringae* belongs to the genus *Pseudomonas* (Mulet et al., 2010). *P. syringae* is one of the best-studied plant pathogens and serves as a model for understanding bacterial pathogenicity, molecular mechanisms of plant-microbe interactions as well as microbial ecology and epidemiology (Hirano and Upper, 2000). So far, more than 60 pathovars have been identified in the species, with each pathovar infecting a characteristic group of host plant species. Collectively, the pathovars of *P. syringae* infect almost all economically important crop species, making *P. syringae* one of most common pathogens on plants. In addition, new disease outbreaks, caused by *P. syringae* isolates, continue to threaten global crop production (McCann et al., 2013).

Ralstonia solanacearumi is an aerobic non-spore-forming, Gram-negative, plant pathogenic bacterium, belonging to the genus *Ralstonia*. Probably, *R. solanacearum* is the most destructive plant pathogenic bacterium worldwide. One of the reasons is that *R. solanacearum* species is composed of a large group of strains varying in their geographical origin, host range and pathogenic behavior (Denny, 2006; Genin, 2010). This heterogeneous group is nowadays recognized as a complex of species which has been divided into four main phylotypes, all of them causative agents of bacterial wilt (Hayward, 1991; Fegan and Prior, 2005). *R. solanacearum* is a water and soil borne pathogen that infects plants *via* wounds, root tips or cracks at the sites of lateral root emergence (Álvarez et al., 2008, 2010). It is considered a quarantine bacterium and a pest of economic and environmental importance in the European Union (EFSA Panel on Plant Health (EFSA PLH Panel) et al., 2019). The management of disease remains limited and is hampered by the ability of the pathogen to survive

for years in wet soil, water ponds, on plant debris or in asymptomatic weed hosts, which act as inoculum reservoirs (Mansfield et al., 2012).

Agrobacterium tumefaciens (recently reclassified as *Rhizobium radiobacter*) is a rod-shaped, Gram-negative soil bacterium, belonging to the genus *Agrobacterium* (Smith and Townsend, 1907). Bacterium is closely related to nitrogen-fixing bacteria which dwell at root nodules in legumes. Unlike the most other soil-dwelling bacteria, it infects the roots of plants to cause Crown Gall Disease (Jin et al., 1990). *A. tumefaciens* targets dicots and causes economic damage to various plants, for instance, walnuts, grape vines, stone fruits, nut trees, sugar beets, horse radish, rhubarb and other various crop species worldwide (Cubero et al., 2006). However, *A. tumefaciens* possesses a very rare feature: the ability for conducting horizontal genetic exchange between organisms of different phylogenetic kingdoms, thus making it a potential vector in the production of transgenic plants (Gelvin, 2012). Bacterium contains a plasmid, the tumor-inducing or Ti plasmid, a segment of which, called T-DNA, integrates into the host plant chromosomes causing a cancerous proliferation of the stem tissue often around the junction of the root and shoot (crown gall) (Hull et al., 2021). Scientists have exploited this ability of this bacteria to put DNA into its host to create transgenic plants. *A. tumefaciens* have emerged as an important molecular tool for manipulating plants and creating genetically modified crops for research and agriculture (Bundock et al., 1995; Kunik et al., 2001; Michielse et al., 2008).

Xanthomonas is a large genus of plant-associated Gram-negative bacteria. These yellow-pigmented bacteria are generally rod shaped with a single polar flagellum, are obligate aerobes (Bradbury, 1984). The genus comprises 27 species that cause serious diseases in almost 400 plants (124 monocots and 268 dicots) including a wide variety of important crops such as rice, citrus, cabbage and pepper (Jun et al., 2010; Ryan et al., 2011). Pathogenic species of *Xanthomonas* show a high degree of host plant specificity and species can be further differentiated into pathovars that are defined by characteristic host range and/or tissue specificity (Ryan et al., 2011; Jacques et al., 2016). Among the most notable of these pathogens are *X. campestris* pv. *campestris* (*Xcc*), the causal agent of black rot of crucifers that affects all cultivated brassicas, *X. campestris* pv. *vesicatoria* (*Xcv*), now reclassified as *X. euvesicatoria*, the causal agent of bacterial spot of pepper and tomato, and *X. campestris* pv. *malvacearum* (*Xcm*, now *X. axonopodis* pv. *malvacearum*), which causes angular leaf spot of cotton (Mansfield et al., 2012).

Xanthomonas oryzae pv. *oryzae* (*Xoo*) is a rod-shaped, Gram-negative bacterium, belonging to the genus *Xanthomonas* (Bradbury, 1984). It produces a yellow soluble pigment, called xanthomonadin, and extracellular polymeric substance (EPS). EPS is important in the protection of bacteria from desiccation and for the attenuation of wind- and rain-borne dispersal (Swings et al., 1990). *Xoo* cause bacterial leaf blight (BLB) of rice (*Oryza sativa*) (Mew, 1987). Bacterium infects

the rice leaf typically through hydathodes at the leaf tip, broken trichomes, leaf margins and wounds in the leaves or roots (Noda and Kaku, 1999; Nozue et al., 2011). BLB is efficiently controlled using resistant rice cultivars. However, because *Xoo* has the capacity to express effectors that suppress some host defense responses, often this resistance is eventually overcome (Verdier et al., 2012). Control of the disease with copper compounds, antibiotics and other chemicals has not proven to be effective, due to variation in sensitivity of pathogenic strains toward applied chemicals (George et al., 1997). The use of *Pseudomonas* and *Bacillus* strains have been reported for the biocontrol of rice pathogens such as *Xoo*, *Magnaporthe oryzae*, and *Rhizoctonia solani* (Ji et al., 2008; Spence et al., 2014). Plant growth promoting *Bacillus* spp. were found to suppress BLB in rice under greenhouse conditions (Chithrashree et al., 2011). Rhizospheric antagonistic *P. aeruginosa* have been documented as beneficial biocontrol agents against *Xoo* (Yasmin et al., 2016).

Another phytopathogen, belonging to the genus *Xanthomonas*, is *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). *Xam* is the causal agent of cassava bacterial blight (CBB) disease and generates losses of up to 100% under the appropriate climatic conditions (Lozano, 1974). Cassava (*Manihot esculenta Crantz*) is a tuberous woody shrub in *Euphorbiaceae* family, which could be annual or perennial, and is grown because of its starchy roots. It is one of the most important staples in the tropics eaten by 800 million people around the globe (Lebot, 2009). *Xam* induces a wide combination of symptoms, including angular leaf lesions, blight, wilt, stem exudates and stem canker in Cassava. *Xam* is considered as a quarantine organism in all countries that grow cassava (Verdier et al., 2004).

Erwinia amylovora a Gram-negative bacterium in the genus *Erwinia* (Paulin, 2000). *E. amylovora* causes fire blight disease of apple, pear, quince, blackberry, raspberry and many wild and cultivated rosaceous ornamentals (Thomson, 2000). Fire blight not only can greatly reduce crop yield and marketability in the season by infecting blossoms and killing of fruit spurs, but also cause the loss of entire trees and orchards (Zhao et al., 2019). The management of fire blight is based on sanitation, cultural practices and the use of a limited number of bactericides and biological control products, mainly to combat blossom blight. Furthermore, streptomycin remains the most effective control material in areas in which sensitive strains of *E. amylovora* are present. However, in many areas, resistant strains are prevalent or regulations against the use of antibiotics in plant agriculture preclude the use of streptomycin (Norelli et al., 2003). Many experiments with antagonistic bacteria have been performed to control the fire blight. Extensive field trials have been conducted mainly with strains of *Pseudomonas agglomerans* and *Pseudomonas fluorescens* (Johnson and Stockwell, 1998).

Xylella fastidiosa is an aerobic, Gram-negative bacterium of the genus *Xylella*. It is a plant pathogen and is transmitted exclusively by xylem fluid-feeding sap insects (Homoptera, Cicadellidae) (Redak et al., 2004). *X. fastidiosa* broad host range includes both monocots and dicots, and bacterium is associated with several important plant diseases, including Pierce's disease of grapevine, citrus

variegated chlorosis and almond leaf scorch disease. Elm, oak, oleander, maple, sycamore, coffee, peach, mulberry, plum, periwinkle, pear and pecan are also other host species of the bacterium (European Food Safety Authority (EFSA), 2016). Because there are no effective control measures that target the bacterium itself, the most widely used methods of control are severe pruning and rouging of infected vines, and control of the insect vector *via* insecticide applications (Daugherty et al., 2015). Current efforts are turning towards the natural chemistries produced by grapevine-associated microbes as potential control measures for Pierce's disease infected grapevines (Aldrich et al., 2015).

The genus *Dickeya* was formed in 2005 by the reclassification of former *Erwinia chrysanthemi* into six species as *Dickeya chrysanthemi*, *D. dadantii*, *D. diffenbachiae*, *D. dianthicola*, *D. zeae* and *D. paradisiaca* (Samson et al., 2005). All *Dickeya* spp. cause economically important diseases on different plant hosts worldwide, including 10 monocot and 16 dicot families (Ma et al., 2007; Samson et al., 2005). *D. dadantii* is a Gram-negative plant pathogenic bacterium that causes soft-rot, stem wilt, and blight diseases on a wide range of economically important crops including potato, carrots, and cabbage (Nelson, 2009). *D. solani* is a Gram-negative bacterium able to cause disease symptoms on a variety of crop and ornamental plants worldwide, mostly known for causing blackleg and soft rot in potato crops (Toth et al., 2011). Currently there are no effective chemical controls for *Dickeya* spp. The most important practices involve lowering the prevalence of disease by proper sanitation of materials, exclusion of infected materials, and avoiding environments conducive to disease. The most important of disease management is the exclusion, because *D. dadantii* and *D. solani* can move through vegetative propagated tissues asymptomatically. Therefore, it is important to have a certified disease-free stock (Toth et al., 2011).

Pectobacterium (formerly *Erwinia*) *carotovorum* is a Gram-negative plant-specific pathogen of the genus *Pectobacterium*. Bacterium cause soft rot disease of various plant hosts, and blackleg in potato by degradation of the plant cell wall (Aizawa, 2014). Application of chemical bactericides for controlling soft rot bacteria is not favoured because of their non-persistence, side toxic effects, high cost as well as development of resistance in bacterial populations. Therefore, biological control may be one of the good crop protection methods for controlling bacterial soft rot disease by application of *Trichoderma* spp., *Bacillus* spp. or *Pseudomonas* spp., which widely applied as biological agents against many soil-borne pathogens (Wulff et al., 2003; Alabouvette et al., 2006).

Potato common scab, caused by several *Streptomyces* species, is a serious potato disease causing significant economic problems worldwide (Sarwar et al., 2019). *Streptomyces europaeiscabiei* and *Streptomyces scabies* are Gram-positive bacterial pathogens that causes common scab disease to several crops, particularly in the potato (Bouchek-Mechiche et al., 2000). Because of the limited understanding of the genetic diversity of *S. scabies* and the genetic differences in various

potato cultivars, developing effective control strategies for potato common scab is challenging (Dees and Wanner, 2012). Traditional control methods such as soil amendment/chemistry to lower soil pH, soil fumigation with chloropicrin, pre-sowing treatment of seed tubers with fluazinam or flusulfamide, and crop rotation are usually not efficacious and may harm the environment (Wilson et al., 1999; Larkin et al., 2011; Dees and Wanner, 2012). Research in biological control as an alternative approach is emerging. Several studies have used biocontrol agents, including non-pathogenic *Streptomyces* spp., *Pseudomonas* spp., and *Bacillus* spp., to combat potato common scab (Eckwall and Schottel, 1997; Han et al., 2005; St-Onge et al., 2011; Meng et al., 2012; Wanner et al., 2014; Arseneault et al., 2015).

1.4.2. Bacteriocin producing rhizobacteria

Rhizosphere is densely populated by a diverse group of microorganisms among which the Gram-positive bacteria are dominant. The bacteria inhabiting the rhizosphere are usually termed as rhizobacteria, which based on their effect on plant growth are grouped into:

- The beneficial bacteria responsible for plant growth and development and termed as plant growth-promoting rhizobacteria (PGPR).
- The deleterious rhizobacteria responsible for plant disease.
- Neutral group (Kloepper et al., 2004).

The PGPR exert their beneficial effect either by providing hormones or by producing antagonistic substances like antibiotics and bacteriocins (Glick and Bashan, 1997). Bacteriocin excretion provides producer strains with an advantage, through significant reduction of direct competitor populations, allowing improved performance and survival of the producer strain. PGPR producing bacteriocins benefit from this competitive ability to inhibit closely related strains and thus clearing the niche space for themselves (Riley and Wertz, 2002). A bacteriocin that also promotes plant growth and development through mechanisms such as a decrease in the population of root associated plant-bacterial pathogens, would result in more vigorous plants (Subramanian and Smith, 2015). Several *Bacillus* and *Pseudomonas* spp. well known as PGPR are also potential bacteriocin producers (Podile and Kishore, 2006).

Several bacteriocins isolated, identified, and characterized from *Bacillus* species include cerein7 and 8A produced by *B. cereus* (Oscáriz and Pisabarro, 2001; Bizani et al., 2005); Bac-GM17 from rhizosphere-derived *B. clausii* GM17; subtilisin H4, IH7, and Bac14B from *B. subtilis* strains (Compaoré et al., 2013); and thuricin 17 and thuricin Bn1 from *B. thuringiensis* strains (Gray et al., 2006). The bacteriocin cerein7, from *B. cereus* with a mass of 3.94 kDa was the first to be isolated from this species (Oscáriz et al., 1999). Cerein 8A isolated from *B. cereus* 8A, interferes with cell membrane integrity and causes cell wall damage, which is the mode of action of many bacteriocins

(Bizani et al., 2005). Bacteriocin Bac-GM17 from the rhizosphere bacteria *B. clausii* GM17 is a 5.158 kDa monomer protein and have bactericidal effect on *A. tumefaciens* C58 and fungistatic effect on *Candida tropicalis* R2 CIP203 (Mouloud et al., 2013). *B. subtilis* strain IH7 produces a bacteriocin Bac IH7 which is reported to have a plant growth promoting activity. Tomato and muskmelon treated with Bac IH7 showed enhanced germination percentage and increased shoot weight and height, and root lengths. It also served as a biocontrol for *Alternaria solani* and other seed borne pathogens (Hammami et al., 2011). Bac14B produced by *B. subtilis* strain 14B was isolated from the rhizosphere of healthy almond plant in Turkey. Bac14B showed significant antibacterial activity against *A. tumefaciens*, the causal agent of crown gall disease (Hammami et al., 2009). Amylocyclin, a small peptide bacteriocin produced by *B. amyloliquefaciens* spp., is a circular bacteriocin with high antibacterial and antifungal activity (Scholz et al., 2014). Amylocyclin was found active against *R. solanacearum*, the causal agent of capsicum bacterial wilt, and *X. campestris*, the causal agent of black rot disease in cruciferous plants (Hu et al., 2010). The 30-kDa lectin-like bacteriocin putidacin, produced by *Pseudomonas putida* strain BW11M1, was isolated from banana root. This bacteriocin contains regions that resemble mannose-binding domains of lectins in monocotyledonous plants (Parret et al., 2003). Putidacin has inhibitory activity against strains of a number of *Pseudomonas* species, including pathovars of *P. syringae* (Parret et al., 2005).

The only bacteriocin studied extensively for plant growth promotion is thuricin 17 (Th17). Th17, isolated from *B. thuringiensis* strain NEB17, is a low molecular weight peptide of 3.162 kDa, stable across a pH range of 1.0-9.25, highly heat resistant and is inactivated by treatment with proteolytic enzymes. To understand the antimicrobial activity of Th17, a range of *Bacillus* and non-*Bacillus* species were studied. Results indicated no inhibitory effect on nodulating rhizobia and other PGPR strains. However, Th17 acts as inhibitor to *E. coli*, a unique finding regarding this peptide since it is uncommon for Gram-positive bacteria to inhibit Gram-negative bacteria. The mode of action of the bacterial peptide is both bactericidal and bacteriostatic (Gray et al., 2006). Th17 increases plant growth through direct and indirect mechanisms. Indirect mechanisms of action include induction of plant disease resistance and inhibition of susceptible pathogenic strains by binding to receptors or the cell membrane/wall, leading to an increase in ecological niche space for producer strains or nodulation of associated plants (Gray and Smith, 2005; Mabood et al., 2014). Treatment with Th17 enhanced production of phenolics, phenylalanine ammonia lyase activity (lignification-related enzymes), and also the levels of peroxidase and superoxide dismutase enzymes (antioxidative enzymes) in 2-week old soybean plants, indicates that it provoked defence system responses (Jung et al., 2008, 2011). Direct stimulation takes place when this compound binds to receptors in leaf or root tissues, and acts as a pseudo-stress signal leading to triggering of various metabolic pathways, resulting in enhancement of photosynthetic rates (Gray et al., 2006). Generally, plants elevate

photosynthetic rates under pathogen or insect challenge, to compensate for decreased photosynthesis in damaged tissues (Nowak and Caldwell, 1984). In the case of Th17, the response has been induced without any stress to counteract, resulting in a net increase in growth (Gray and Smith, 2005). When Th17 was root-drench-applied nodule number root, shoot, and total biomass of soybean was increased. Foliar application also enhanced leaf area and leaf greenness (Lee et al., 2009). Research on Th17 has demonstrated its promising role as a plant growth promoter under stressful conditions. As an example, Th17 treated soybean plants showed a reduced impact by water deficit stress. Application of Th17 to soybean roots plus inoculation with N_2-fixing *B. japonicum* increased root and nodule biomass by 37% and 55%, respectively and increased leaf area, photosynthetic rate, and abscisic acid levels in roots under water deficit stress (Prudent et al., 2015). A proteomic study indicated more rapid and efficient mobilization of carbon, nitrogen, and storage proteins of soybean seeds treated with Th17, resulting in enhanced germination under salt stress (Subramanian et al., 2016). Collectively, these findings highlight the role of Th17 as a biocontrol and plant growth promoting bacteriocin in agriculture.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Media used for isolation and cultivation of microorganisms

Isolated bacteria were grown in Petri dishes with Nutrient Broth (NB), 10:NB (10× diluted NB), Brain Heart Infusion (BHI), Luria-Bertani (LB) and selective Ashby's Mannitol medium supplemented with 15 g/L agar (Fisher Scientific) when needed **(Table 2.1)**. The sterilization of media was performed in an autoclave at 121°C for 20 min.

Table 2.1 Media used for isolation and cultivation of microorganisms.

Name of the medium	**Composition of the medium**
Nutrient Broth (Difco)	10 g/L peptone 1 g/L beef extract 2 g/L yeast extract 5 g/L NaCl
Brain Heart Infusion (LAB M)	17.5 g/L Brain-Heart Infusion Solids 10 g/L tryptose 2 g/L glucose 5 g/L NaCl 2.5 g/L Na_2HPO_4
Luria-Bertani (Carl Roth)	10 g/L peptone 5 g/L yeast extract 10 g/L NaCl
Ashby's Mannitol medium	10 g/L mannitol (Merck) 0.2 g/L NaCl (Carl Roth) 0.2 g/L KH_2PO_4 (Merck) 5 g/L $CaCO_3$ (Merck) 0.2 g/L $MgSO_4 \times 7H_2O$ (Merck) 0.1 g/L $CaCl_2 \times 2H_2O$ (Merck)

2.1.2. Microorganisms used in this study

Bacterial strains used in this study are presented in **Table 2.2**. Cultures were maintained and grown on LB agar medium in a thermostat or in liquid LB medium in a thermoshaker at 30°C using 180 rpm.

Table 2.2 Bacterial strains (continued in 27 page). *Marked non-phytopathogenic species.

Species	**Strain**	**Obtained from**
Xanthomonas vesicatoria	DSM 22252	Leibniz Institute, DSMZ – German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany)
Xanthomonas campestris	DSM 3586	
**Agrobacterium radiobacter*	DSM 30147	
Pseudomonas syringae	DSM 10604	
Pseudomonas syringae	DSM 50315	
Pectobacterium atrosepticum	DSM 18077	
Pectobacterium carotovorum	DSM 30168	
Dickeya solani	DSM 28711	
Streptomyces scabiei	DSM 41658	
Streptomyces europaeiscabiei	DSM 41802	
Enterococcus faecalis	DSM 2570	
**Bacillus subtilis*	ATCC 6633	
Candida albicans	ATCC 10231	

Staphylococcus aureus subsp. *aureus*	ATCC 25923	
**Staphylococcus epidermidis*	ATCC 12228	

2.2. Methods

2.2.1. Sample collection

Soil samples were randomly collected from a potato, strawberry and rye field located in the village of Laukininkėliai, Varėna district (54°15'15.2" N 24°14'25.2" E). Soil samples were taken under similar environmental conditions in October 2021. All samples were carefully collected by scraping the soil surface with a sterile scoop and transferred to the sterile sample tubes.

2.2.2. Isolation of bacteria strains from the soil

One gram of each soil sample was suspended in 9 mL of sterile 0.9% NaCl (Carl Roth) solution. The mixture was shaken for 30 min. in a shaker at room temperature (21°C). Further, samples were diluted 10^3 10^4, 10^5 times in sterile 0.9% NaCl solution and 100 μL of each dilution were spread on Petri dishes with NB, 10:NB, BHI and LB agar medium. The Petri dishes were incubated at 30°C for 48 h, and colonies were further sub-cultured and spread on a new and fresh agar plates for pure bacterial strain isolation based on morphological differences. The isolated bacterial strains were further inoculated in liquid LB medium and after overnight growth in a thermoshaker glycerol solution was added to a final concentration of 20% and stored at –75°C. The growth media used in the work and their composition are described in **Table 2.1**.

2.2.3. Screening for bacterial isolates active against phytopathogens

The isolated strains and phytopathogens were refreshed and grown in LB medium at 30°C in a thermoshaker for 24 h. The prepared cultures were used to screen isolates active against phytopathogens by using a spot on a lawn assay (Schillinger and Lücke, 1989). Melted LB agar medium was inoculated (5% v/v) with phytopathogenic culture, mixed and poured into Petri plates. When the agar was solid, 10 μL of the bacteria isolate culture was placed onto a Petri dish with an indicator strain of phytopathogen and left to dry. A well diffusion assay was performed by using a sterile pipette tip to cut wells in the agar medium with an indicator strain and 50 μL of the sample was poured into the wells and left for 10 min. for samples to absorb. After that, the plates were incubated in a thermostat at 30°C for 24 hours. After incubation, the inhibition zones were evaluated.

2.2.4. Identification by 16S rRNA gene sequencing

Genomic DNA was extracted from bacteria isolates by using a GeneJET Genomic DNA Purification (Thermo Fisher Scientific) kit. For PCR to amplify the 16S rRNA region was used primer 1492R (5’- TACGGYTACCTTGTTACGACTT- 3’) and primer 27F (5’- AGAGTTTGATC

MTGGCTCAG-3'). The PCR was performed using PfuX7 polymerase (Nørholm, 2010), 10×Taq buffer (Thermo Fisher Scientific), dNTPs (2 mM each, Thermo Fisher Scientific), $MgCl_2$ (25 mM, Thermo Fisher Scientific), primers (0.5 μM each, Metabion) and genomic DNA according to the manufacturer's recommendations. PCR products were purified using the GeneJet PCR purification kit (Thermo Fisher Scientific). The PCR products were sequenced at BaseClear (Leiden, Netherlands).

2.2.5. DNA electrophoresis

PCR products were visualized using 1% (w/v) agarose gel electrophoresis. The system was filled with 1×TAE buffer solution (Thermo Fisher Scientific). DNA electrophoresis was performed at voltage of 80 V, for 45 min. Gels were visualized and analysed using the transilluminator MiniBIS Pro (DNA Bio-Imaging System). DNA fragment length standard GeneRuler (Thermo Fisher Scientific) was also run on the gel to estimate PCR product length.

2.2.6. Phylogenetic analysis

DNA sequences were analysed using 16S-based ID tool (BLASTn analysis) in EzBioCloud software: https://www.ezbiocloud.net. Multiple cluster alignment and phylogenetic analysis were performed on MEGA software (v. 11) based on the Neighbour Joining method using the p-distance method and a 1000 repetition bootstrap to evaluate statistical support.

2.2.7. SDS-PAGE protein electrophoresis

First, chloroform-methanol extraction of supernatant proteins was performed according to Wessel and Flügge (1984) protocol. Then, Tris-Glycine-SDS-PAGE protein electrophoresis was performed according to the protocol of Rosenberg (1996). Protein separation was performed in SDS-polyacrylamide 4% concentrating and 12% separating gels. Tricine-SDS-PAGE protein electrophoresis was performed according to the protocol of Schägger (2006). Protein separation was performed in SDS-polyacrylamide 4% concentrating gel, 10% spacer gel and 16% separating gels. After electrophoresis, gels were fixed for 30 min. in a fixing solution containing 20% propanol and 10% acetic acid. After fixation, gels were washed 3 times for 10 min. in deionized water. After washing, one gel was stained with PageBlue Protein Staining Solution (Thermo Fisher Scientific) according to the manufacturer's recommendations. Another gel was used for antibacterial activity evaluation. After fixation and washing with deionized water, the gel was transferred to a Petri dish and poured with melted LB agar medium inoculated with phytopathogenic indicator strain. After the agar solidified, the plate was incubated in thermostat overnight at 30°C. After incubation, zones of growth inhibition were evaluated.

2.2.8. Bacteriocin extraction by amberlite XAD-16N

Bacteria culture was inoculated (1%) into 100 mL LB media and incubated in thermoshaker at 30 °C. After reaching certain optical density the culture was centrifuged at 5000×g for 20 min at 4°C. The supernatant was collected and filtered by 0.22 μm (Durapore) filter. The filtered supernatant was divided into 3 equal parts for different bacteriocin purification methods.

The amberlite XAD-16N resin (Sigma-Aldrich) was equilibrated in water (1% w/v) for 30 minutes. Then the water was decanted and the resin soaked in MeOH (1% w/v) for 30 minutes. After that MeOH was decanted and the resin was mixed with water (50% w/v) and washed from MeOH by mixing. The filtered supernatant was mixed with amberlite XAD-16N resin and shaken at 200 rpm for 1 h at 4°C. The mixture was poured into column to separate resin and the supernatant. The proteins were eluted from the resin using 100% MeOH by shaking at 200 rpm for 1 h at 4°C. MeOH with eluted proteins were collected and evaluated for antibacterial activity using a spot on a lawn or a well diffusion assay.

2.2.9. Bacteriocin extraction by C18-SPE

CHROMABOND C18 (45 μm, 6 mL/500 mg) SPE column (Macherey-Nagel) was activated by loading 6 mL 100% acetonitrile, then washed with 6 mL water. The filtered supernatant was loaded on the column and then the column was washed with 3 mL water to remove unbounded proteins. The analytes were eluted with 6 mL 100% acetonitrile and evaluated for antibacterial activity using a well diffusion assay.

2.2.10. Bacteriocin extraction by ammonium sulphate

Ammonium sulphate (Sigma-Aldrich) was gradually added to the supernatant up to 80% saturation and stirred for 1 h at 4°C. Then the sample was centrifuged at 10000×g for 1 h at 4°C. The pellets were resuspended in 20 mM Tris-HCl, pH 8, buffer and dialyzed using a dialysis membrane (Membra-Cel, MD25/8-14 kDa, Carl Roth) in the same or other buffer to remove the salt. A well diffusion assay was performed to evaluate antibacterial activity.

2.2.11. Ion-exchange chromatography

For anion-exchange chromatography UNO Q1 or UNO Q6 columns (Bio-rad) were used, and for cation-exchange chromatography UNO S1 column (Bio-rad) was used. Protein chromatography was performed using the BioLogic DuoFlow medium-pressure chromatography system (Bio-rad). The samples were equilibrated in binding buffers (A) and loaded on the column, which was pre-equilibrated in the same buffer. Then the column was washed from unbounded proteins using 5-10

column volumes of binding buffers and then proteins were eluted in an increasing linear gradient of NaCl using 10-20 column volumes of elution buffers (B). All buffers are listed in **Table 2.3**.

Table 2.3 Buffers used for anion-, cation-exchange and hydrophobic interaction chromatography.

Ion-exchange chromatography	**Starting buffer**		**Elution buffer**	
Anion	A1	20 mM TRIS-HCl, pH 8 (Carl Roth)	B1	20 mM TRIS-HCl, pH 8 (Carl Roth) + 500 mM NaCl
Cation	A2	20 mM MES, pH 6.1 (Carl Roth)	B2	20 mM MES, pH 6.1 (Carl Roth) + 1000 mM NaCl
	A3	20 mM citric acid, pH 3.1 (Carl Roth)	B3	20 mM citric acid, pH 3.1 (Carl Roth) + 1000 mM NaCl
Hydrophobic interaction chromatography	A4	20 mM TRIS-HCl, pH 8 (Carl Roth) + 2000 mM NaCl	B4	20 mM TRIS-HCl, pH 8 (Carl Roth)

A spot on a lawn assay was performed to evaluate collected flow through and elution fractions for antibacterial activity.

2.2.12. Hydrophobic interaction chromatography

For hydrophobic interactions chromatography (HIC) Bio-Scale column (Bio-Rad) filled with 5 mL Macro-Prep Methyl HIC resin (Bio-rad) was used. Chromatography was performed using BioLogic DuoFlow medium-pressure chromatography system (Bio-rad). Sample was equilibrated in buffer A4 and loaded on columns pre-equilibrated in the same buffer. Unbounded proteins were washed using A4 buffer. Proteins from the column were eluted in a decreasing linear gradient of NaCl using B4 buffer. A spot on a lawn assay was performed to evaluate flow through and elution fractions activity after purification.

3. RESULTS AND DISCUSSION

3.1. Isolation and screening of bacterial strains active against plant pathogenic bacteria

Three samples of soil from a potato, strawberry and rye field were subjected to bacteria isolation on agar medium: NB, 10:NB, BHI and LB. As a result, 51 morphologically different colonies were isolated **(Supplement 1)**: 29 strains from potato field, 11 strains from rye field and 11 strains from strawberry field. Further, to identify bacterial isolates active against plant pathogenic bacteria, a spot on a lawn assay was used **(Table 3.1)**. After screening of 51 bacterial strains, it was revealed that 11 of them (BL1, BL5, BL11, BL17, BL25, BL26, RL2, RL6, RL7, RL8, BR2) inhibited growth of 10 phytopathogenic strains: *X. vesicatoria* DSM 22252, *X. campestris* DSM 3586, *A. radiobacter* DSM 3014710, *P. syringae* DSM 10604, *P. syringae* DSM 50315, *P. atrosepticum* DSM 18077, *P. carotovorum* DSM 30168, *D. solani* DSM 28711, *S. scabiei* DSM 41658, *S. europaeiscabiei* DSM 41802. A few examples of isolates (BL5, RL2, RL6, RL8, BR2) active against phytopathogenic bacteria are presented in **Figure 3.1.** The largest number (6) of active isolates was isolated from the potato field. 10 isolates were active against more than 1 phytopathogen and BL5, BR2 isolates showed the broadest antibacterial spectrum.

Table 3.1 Screening of antibacterial activity of a potato, strawberry and rye field isolates against plant pathogenic bacteria. Legends: – (no zone of inhibition), + (small zone of inhibition), ++ (large zone of inhibition).

Phytopathogen	**Active isolates**										
	BL1	BL5	BL11	BL17	BL25	BL26	RL2	RL6	RL7	RL8	BR2
X. vesicatoria DSM 22252	–	+	+	+	+	–	++	++	–	++	++
X. campestris DSM 3586	++	+	++	–	–	+	++	++	+	–	++
A. radiobacter DSM 30147	–	–	–	–	–	–	+	+	+	+	++
P. syringae DSM 10604	–	+	–	–	–	–	–	+	–	+	+
P. syringae DSM 50315	–	+	–	–	–	–	–	+	–	–	+
P. atrosepticum DSM 18077	–	++	–	+	–	+	–	+	–	–	+
P. carotovorum DSM 30168	+	+	–	–	–	+	–	–	–	–	+
D. solani DSM 28711	–	+	–	–	–	+	–	–	–	–	+
S. scabiei DSM 41658	–	++	–	–	–	–	–	+	+	–	–
S. europaeiscabiei DSM 41802	–	–	–	–	–	–	–	–	–	–	–

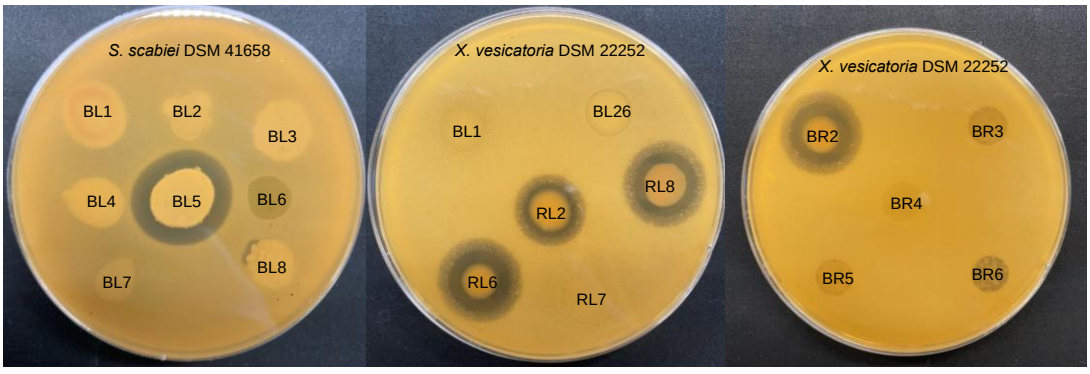

Figure 3.1 BL5 isolate active against *S. scabiei* DSM 41658, RL2, RL6, RL8 and BR2 isolates active against *X. vesicatoria* DSM 22252.

In addition, the capability of isolates to fixate nitrogen was assessed. Nitrogen-fixing bacteria are colonized in plants and can effectively provide nitrogen to plants without need to form specific nodules, thus enchanting plant growth and productivity (Mahmud et al., 2020). Ashby's Mannitol Agar is a selective growth medium used to culture and identify nitrogen-fixing bacteria. In this medium, mannitol is the source of carbon and atmospheric nitrogen is the source of nitrogen. Bacteria isolates were grown on selective Ashby's Mannitol Agar in thermostat at 30°C for 72 h. It was revealed that among the isolates, showing antimicrobial activity, isolates RL2, RL6 and BR2 are capable to grow on selective medium and to fixate nitrogen from the environment **(Figure 3.2)**. In addition to antibacterial activity against phytopathogens, the RL2, RL6 and BR2 were able to fix atmospheric nitrogen and could be applied in agriculture not only as preventive measure against phytopathogenic bacteria, but also as plant growth enhancers.

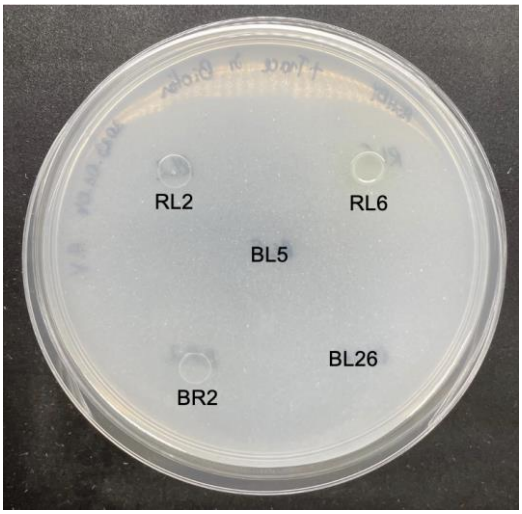

Figure 3.2 Growth of BL26, BL5, BR2, RL2 and RL6 isolates on selective Ashby's Mannitol Agar medium.

3.2. Identification of isolates active against phytopathogens

Bacteria isolates showing antibacterial activity were further identified by 16S DNA sequencing. The alignment (BLASTn analysis) of 16S RNA using the 16S-based ID tool in EzBioCloud software showed that isolates BL1, BL5, BL11, BL17, BL25, BL26, and RL7 belong to *Bacillus* genus, and isolates BR2, RL2 and RL6 belong to *Pseudomonas* genus **(Supplement 2)**. The results were summarized and are presented in **Table 3.2**.

The analysis of 16S rRNA sequences using the BLASTn algorithm indicated that BL1 isolate is closely related to *B. toyonensis* BCT-7112 and *B. mobilis* 0711P9-1 species, sharing 99.3% identity **(Supplement 2, Table 2)**. BL5 isolate 16S rRNA has highest similarity to *B. velezensis* CR-505 sharing 99.7% identity **(Supplement 2, Table 3)**. The BLASTn algorithm indicated that 16S rRNA of BL11, BL17, BL25 isolates have highest similarity to *B. toyonensis* BCT-7112, sharing a maximum 99.5%, 99.9%, 99.8% identity respectively **(Supplement 2, Tables 4 – 6)**. 16S rRNA

sequence of BL26 isolate has highest similarity to *B. altitudinis* 41KF2b strain, sharing 100% identity **(Supplement 2, Table 7)**. Analysis of 16S rRNA sequence indicated RL2 isolate to be closely related to *P. lini* CFBP 5737 strain, sharing 99.9% identity **(Supplement 2, Table 9)**. Both BR2 and RL6 isolates are closely related to *P. silesiensis* A3 strain, sharing 99.8% identity **(Supplement 2, Table 8, 10)**. RL7 isolate is closely related to *B. wiedmanii* FSL W8-0169, sharing 99.8% identity **(Supplement 2, Table 11)**.

Further, a phylogenetic tree analysis of 16S DNA sequences demonstrates the relatedness between the strains and nearest neighbour of each strain, as portrayed in **Supplement 3**. BL5 isolate is related to *B. tequilensis* bacteria species **(Supplement 3, Figure 2)**. BL26 in the phylogenetic tree was clustered with *B. altitudinis* type strains **(Supplement 3, Figure 6)**. A phylogenetic tree demonstrates that BR2, RL2 and RL6 isolates belong to *P. lini* clade **(Supplement 3, Figure 7-9)**. BL1, BL11, BL17, BL25 and RL7 isolates were grouped in the same clade, which is closely related to pathogenic *B. cereus* and *B. anthracis* **(Supplement 3, Figure 1, Figures 3 – 5)**. They were assigned to *B. cereus* group – group of spore-forming, aerobic, facultative anaerobic, rod-shaped bacteria and is comprised of at least eight closely related species: *B. anthracis*, *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis*, *B. cytotoxicus*, and *B. toyonensis*. It is important to note that while some strains within the *B. cereus* group can be pathogenic, many strains are not harmful to humans and have beneficial applications in various fields, such as agriculture and biotechnology. The pathogenicity of these bacteria can vary depending on the specific strain, its genetic characteristics, and the conditions of infection or exposure, for example *B. anthracis* is the causative agent of anthrax. Some *B. cereus* strains are commonly recognized as food poisoning agents, but strains can also cause localized wound and eye infections as well as systemic disease. Certain *B. thuringiensis* strains are entomopathogens and have been commercialized for use as biopesticides, while some strains have been reported to cause infection in immunocompromised individuals. (Liu et al., 2015). *B. toyonensis* is a bacterial species that has been associated with various potential benefits and risks. *B. toyonensis* are known for their plant growth-promoting properties. They can produce substances that facilitate nutrient uptake, protect plants against pathogens, and improve overall plant health. However, it is important to note the possibility that some strains may have the potential to cause infections or adverse effects, especially in individuals with weakened immune systems. This risk, however, requires further investigation and clarification (Santoyo et al., 2016). Since identification based on the 16S rRNA is very limited at species level and due to risks associated with *B. cereus* group, it was decided not to use BL1, BL11, BL17, BL25 and RL7 isolates in further research.

Based on BLASTn and phylogenetic tree analysis, it can be concluded that isolate BL5 is closely related to *B. tequilensis* bacteria species. BL26 isolate is closely related to *B. altitudunis*

41KF2b strain. A phylogenetic tree demonstrated that BR2, RL2 and RL6 isolates belong to *P. lini* clade: *P. lini* has been shown to perform nitrogen fixation, thus promoting plant growth (Padda et al., 2019). *B. tequilensis* GYUN-300 is known to exhibit antagonistic activity against the fungal pathogen, *Colletotrichum acutatum*, which causes anthracnose that manifests primarily as a fruit rot in red pepper (Kwon et al., 2022). The endophytic *B. altitudinis* has a notable influence on plant growth and researchers claim that *B. altitudinis* could be used as a favourable candidate source to enhance plant growth in sustainable agriculture (Zhang et al., 2021).

Table 3.2 Summarized 16S rRNA analysis results.

Isolate name	Genus	Closely related species*
BL1	*Bacillus*	*Bacillus toyonensis* (*B.cereus* group)
BL5	*Bacillus*	*Bacillus velezensis*
BL11	*Bacillus*	*Bacillus toyonensis* (*B.cereus* group)
BL17	*Bacillus*	*Bacillus toyonensis* (*B.cereus* group)
BL25	*Bacillus*	*Bacillus toyonensis* (*B.cereus* group)
BL26	*Bacillus*	*Bacillus altitudinis*
BR2	*Pseudomonas*	*Pseudomonas silesiensis*
RL2	*Pseudomonas*	*Pseudomonas lini*
RL6	*Pseudomonas*	*Pseudomonas silesiensis*
RL7	*Bacillus*	*Bacillus wiedmanii* (*B.cereus* group)

*based on EzBioCloud 16S database analysis

3.3. Antibacterial activity in supernatants

Next, production of antibacterial substances of *Bacillus* sp. BL5, BL26 strains, and *Pseudomonas* sp. BR2, RL2, RL6 strains was evaluated in liquid medium. Strains were grown in liquid LB medium at 30°C for 97 h and antibacterial activity using the spot on a lawn assay was evaluated in the supernatants of the cultures. Samples were taken in different growth phases periodically. Phytopathogens, which were previously affected by isolates, were used as indicator strains **(Table 3.1)**.

Results showed that no growth inhibition zones of indicator strains were detected using the supernatants of *Bacillus* sp. BL26 strain and *Pseudomonas* sp. BR2, RL2 strains. Supernatant of *Pseudomonas* sp. RL6 strain inhibited growth of *X. campestris* DSM 3586 and *P. syringae* DSM 10604 phytopathogens **(Figure 3.3, 3.4)**. The production of antibacterial substances against *X. campestris* DSM 3586 were detected in exponential and stationary growth phases, after 5 h of growth and remained until 50 h of growth. The production of antibacterial substances against *P. syringae* DSM 10604 were also detected in exponential and stationary growth phases, after 5 h of growth and remained until 72 h of growth, however, the activity appeared to be very low, since growth inhibition zones were not very clear.

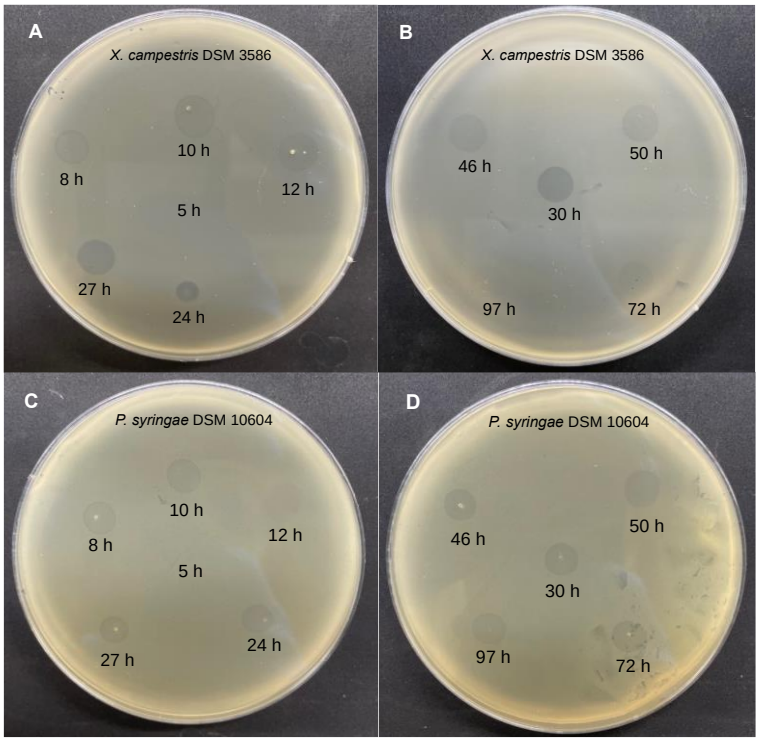

Figure 3.3 *Pseudomonas* sp. RL6 strain's supernatants antibacterial activity against phytopathogens *X. campestris* DSM 3586 (A and B) and *P. syringae* DSM 10604 (C and D) evaluation by spot on a lawn assay. Different sample collection times are indicated in the figure.

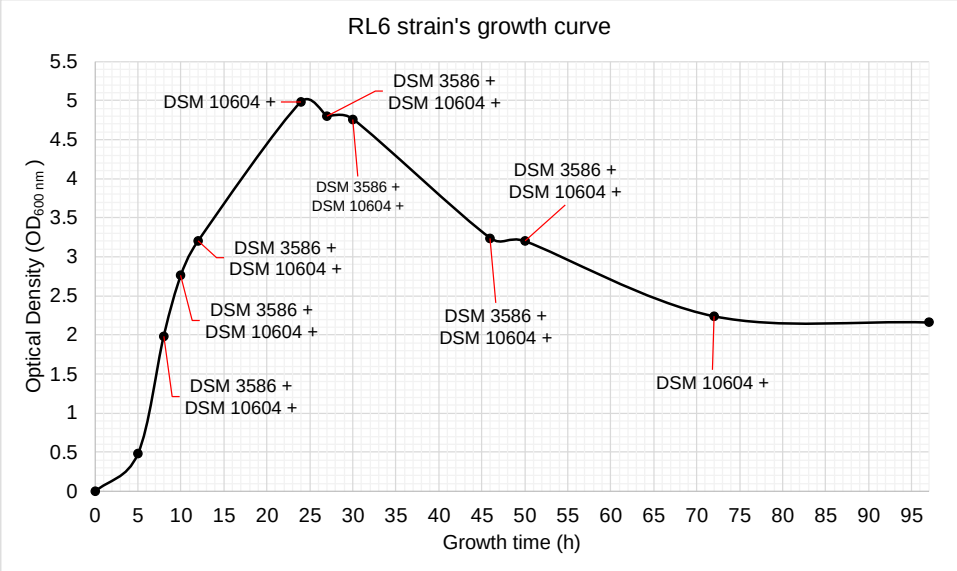

Figure 3.4 *Pseudomonas* sp. RL6 strain's growth curve. Activity against phytopathogenic strain at certain growth time is market in the graph. Legends: + (small zone of inhibition), ++ (large zone of inhibition).

Bacillus sp. BL5 strain supernatant inhibited growth of *S. scabiei* DSM 41658 and *P. syringae* DSM 50315 phytopathogens. Samples collected in different periods revealed that growth inhibition was visible after 8 h of growth and remained until 30 h of growth against *S. scabiei* DSM 41658 and *P. syringae* DSM 50315 phytopathogens **(Figure 3.5)**. Growth curve indicates that supernatant antibacterial activity is present only in exponential growth phase at 8 h, 10 h, 12 h, 27 h and 30 h until $OD_{600\ nm}$ 4.16 **(Figure 3.6)**.

Decreased antimicrobial activity after prolonged incubation of RL6 and BL5 strains could be attributed to proteolytic degradation by extracellular proteases.

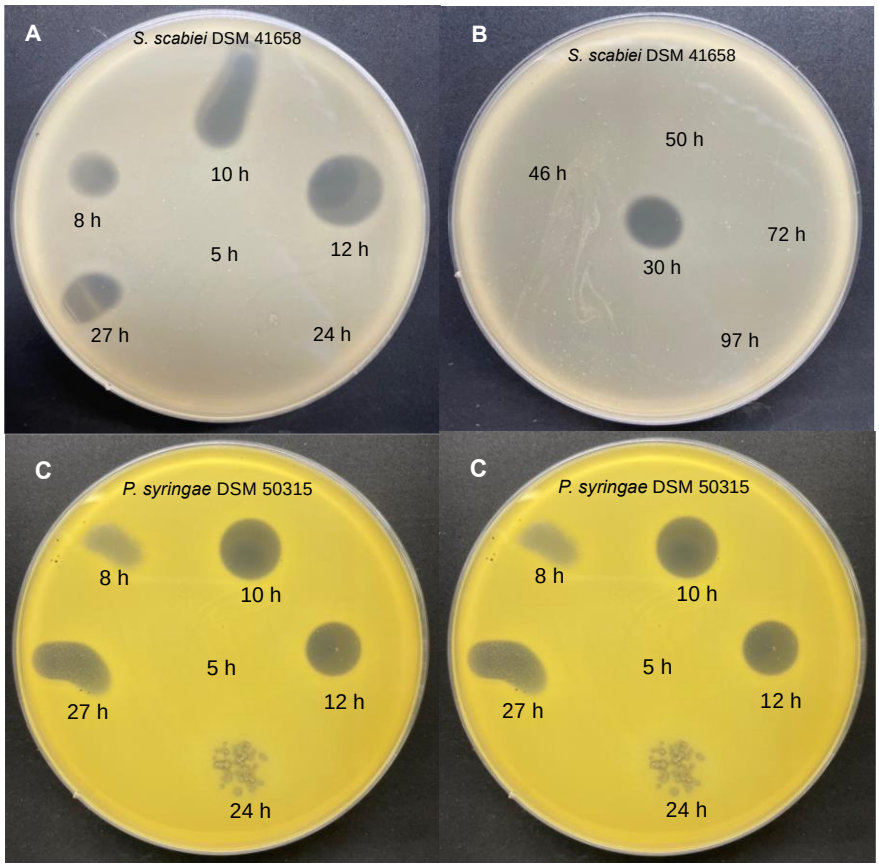

Figure 3.5 *Bacillus* sp. BL5 strain's supernatants antibacterial activity against phytopathogens *S. scabiei* DSM 41658 (A and B) and *P. syringae* DSM 50315 (C and D) evaluation by the spot on a lawn assay. Different sample collection times are indicated in the figure.

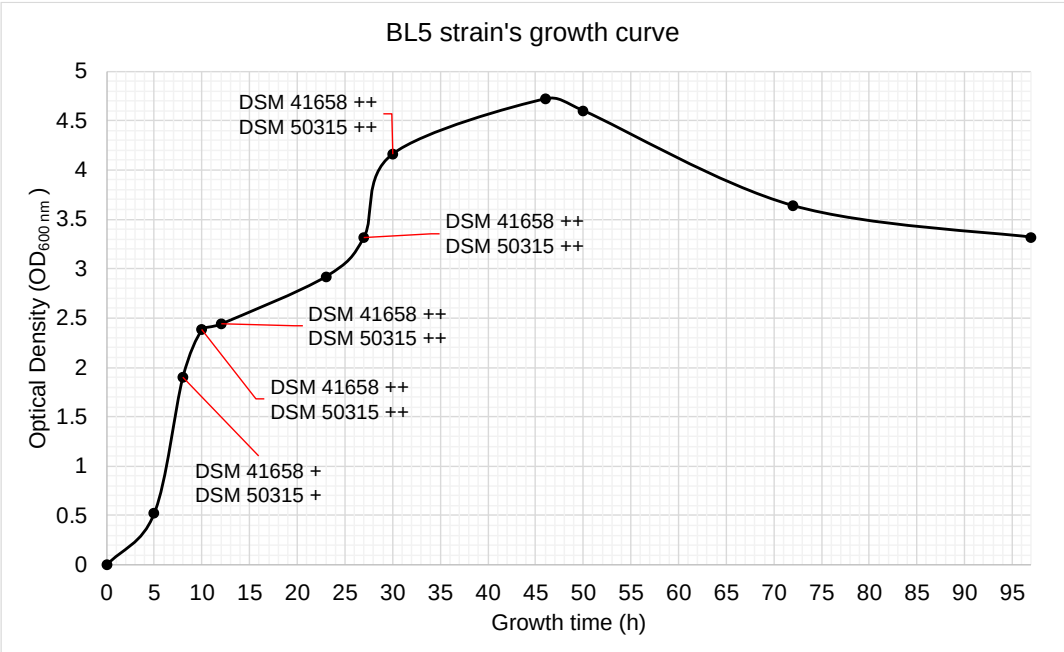

Figure 3.6 *Bacillus* sp. BL5 strain's growth curve. Activity against phytopathogenic strain at certain growth time is market in the graph. Legends: + (small zone of inhibition), ++ (large zone of inhibition).

3.4. SDS-PAGE analysis of supernatants

The collected supernatants of *Pseudomonas* sp. RL6 and *Bacillus* sp. BL5 strains were concentrated with the chloroform-methanol protein extraction method and further analysed by Tris-Glycine-SDS-PAGE and Tricine-SDS-PAGE protein electrophoresis. To evaluate molecular weight of antibacterial proteins, after the electrophoresis gels were overlayed with agar medium inoculated with indicator strains. No bands of *Pseudomonas* sp. RL6 and *Bacillus* sp. BL5 strains produced peptides in Tris-Glycine-SDS-PAGE protein electrophoresis gels were visible, however analysis of *Bacillus* sp. BL5 strain's peptides with *S. scabiei* DSM 41658 as an indicator strain revealed an inhibition zone at the position of ~3.5 kDa of the molecular weight marker band **(Figure 3.7)**, suggesting that peptide with antibacterial activity has a molecular weight of ~3.5 kDa. Therefore, the antibacterial substances produced by *Bacillus* sp. BL5 strain may be classified as a Class I or Class II bacteriocin based on molecular weight, since class I and class II include bacteriocins with a molecular weight of less than 10 kDa. Production of antibacterial peptide was detected between 8 h and 30 h of growth. Inhibition zones were visible in all fractions, however the highest activity of antibacterial peptide was visible in supernatant collected after 30 h of growth, when $OD_{600\ nm}$ reached 4.16 **(Figure 3.7)**.

Since peptide with antibacterial activity produced by *Bacillus* sp. BL5 strain has a molecular weight of ~3.5 kDa, we opted to conduct the same analysis using the tricine-SDS-PAGE protein electrophoresis, which is the preferred electrophoretic system for the resolution of proteins smaller than 30 kDa (Schägger, 2006) **(Figure 3.8)**. Growth inhibition zone of *S. scabiei* DSM 41658 indicator strain was revealed in the similar position of ~3.5 kDa. Largest inhibition zone appeared at 12 h marking in the gel, suggesting that the highest production of antibacterial peptide occurs after 12 h of *Bacillus* sp. BL5 strain's growth, when $OD_{600\ nm}$ reaches 2.44. No protein bands were visible in stained gels, because peptide of interest may be very diluted in the supernatant and therefore does not yield a clearly visible band. Overall results suggest that the highest production of ~3.5 kDa molecular weight antibacterial peptide occurs between 12 h and 30 h of *Bacillus* sp. BL5 strain's growth ($OD_{600\ nm}$ between 2.44 and 4.16).

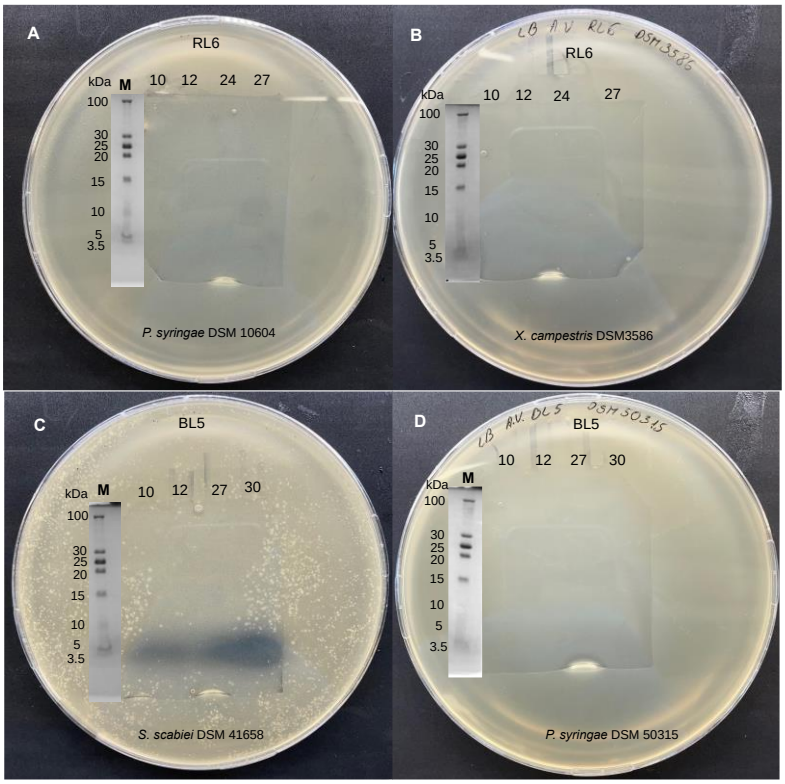

Figure 3.7 Overlayed Tris-Glycine-SDS-PAGE gels after analysis of *Pseudomonas* sp. RL6 and *Bacillus* sp. BL5 strains' supernatants. A and B – analysis of *Pseudomonas* sp. RL6 strain's supernatants, C and D –analysis of *Bacillus* sp. BL5 strain's supernatants. A – agarized LB medium inoculated with indicator *P. syringae* DSM 10604 strain, B – agarized LB medium inoculated with indicator *X. campestris* DSM 3586 strain, C – agarized LB medium inoculated with indicator *S. scabiei* DSM 41658 strain, D – agarized LB medium inoculated with indicator *P. syringae* DSM 50315 strain. Line M – protein marker, lines 10 –30 indicate growth time (h) when the supernatants were collected.

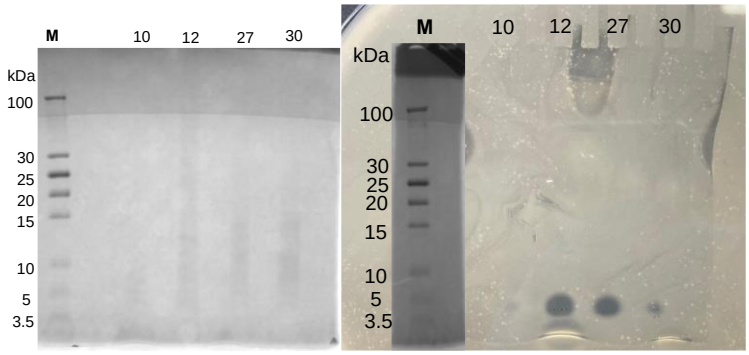

Figure 3.8 Tricine-SDS-PAGE protein electrophoresis analysis of *Bacillus* sp. BL5 strain's supernatants. Agarized LB medium inoculated with indicator *S. scabiei* DSM 41658 strain. Line M – protein marker, 10 – 30 indicate growth time (h) when the supernatants were collected.

3.5. Crude extraction of bacteriocin

The antimicrobial substances detected in the supernatants using the spot on a lawn assay were further purified by amberlite XAD-16N (20-60 mesh) resin, a C18-SPE column and ammonium sulphate precipitation method. To reach the optimal yield of extracted bacteriocin and detect whether other antibacterial peptides are produced, the *Bacillus* sp. BL5 strain was grown until $OD_{600\ nm}$ reached 2.1 and 3.46. The obtained culture supernatant was further submitted for bacteriocin

extraction and purification using different methods. To evaluate antibacterial activity, and during purification stages, *S. scabiei* DSM 41658 was used as the indicator strain.

Bacillus sp. BL5 strain was grown until $OD_{600\ nm}$ reached 2.1. After purification with amberlite XAD-16N resin and a C18-SPE column, no antibacterial activity was observed in elution fractions. The lack of activity was consistent in both the purified peptide fraction and the flow through samples. The absence of activity in the flow through fractions suggests that the protein may not have been desorbed and eluted from the resin or it lost its activity during the procedure. In contrast, purification with ammonium sulfate was successful **(Figure 3.9)**. Samples obtained after ammonium sulfate precipitation and dialyzed in 20 mM TRIS-HCl, pH 8 buffer exhibited antibacterial activity against indicator *S. scabiei* DSM 41658 strain. This indicates that the ammonium sulfate precipitation and subsequent dialysis, which remove small molecules and contaminants, likely contributed to concentrating and enhancing the antibacterial components in the sample. Based on these findings, the ammonium sulfate precipitation method was chosen for further experiments.

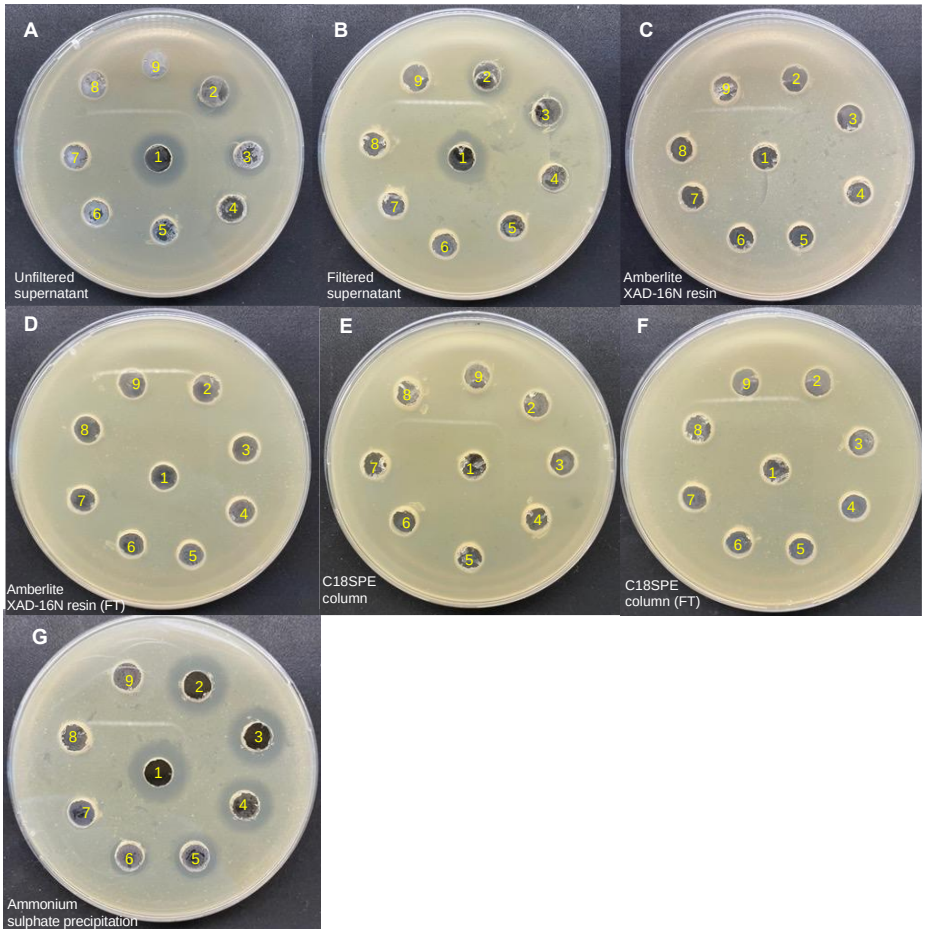

Figure 3.9 Well diffusion assay of crude extraction of bacteriocin from *Bacillus* sp. BL5 strain's supernatants. LB agar medium inoculated with *S. scabiei* DSM 41658. A – unfiltered supernatants before purification, B – filtered supernatants before purification, C – samples after purification with amberlite XAD-16N resin, D – flow through after purification with amberlite XAD-16N resin, E – samples after purification with C18-SPE column, F – flow through after purification with C18-SPE column, G – dialyzed samples after ammonium sulphate precipitation. 1 – undiluted fraction, 2 – 9 represents two-fold dilutions of the sample.

To reach optimal yield of extracted bacteriocin and detect whether other antibacterial peptides are produced, *Bacillus* sp. BL5 strain was grown until $OD_{600\ nm}$ reached 3.46. Prior to crude extraction and after ammonium sulfate precipitation, the peptide displayed no antibacterial activity against *P. syringae* DSM 50315, suggesting that no other antibacterial peptide was produced during longer cultivation. Comparing cells grown to $OD_{600\ nm}$ 2.1 and 3.46 and evaluating inhibition zones according to sample dilutions, it was observed that the activity of antibacterial peptide decreases, since the activity was mainly observed up to a dilution of 10 times, with only slight inhibition zones visible at a dilution of 10^2 times in the case of the filtered supernatant **(Figure 3.10)**.

In conclusion, based on the results obtained, the ammonium sulfate precipitation method was selected as the optimal purification method for extracting the bacteriocin from the *Bacillus* sp. BL5 strain's supernatants. Additionally, by comparing ammonium sulfate precipitation method results, when *Bacillus* sp. BL5 strain was grown until $OD_{600\ nm}$ 2.1 and 3.46, no antibacterial peptide active against *P. syringae* DSM 50315 was produced during longer cultivation and highest antibacterial peptide yield is obtained when optical density reaches 2.1.

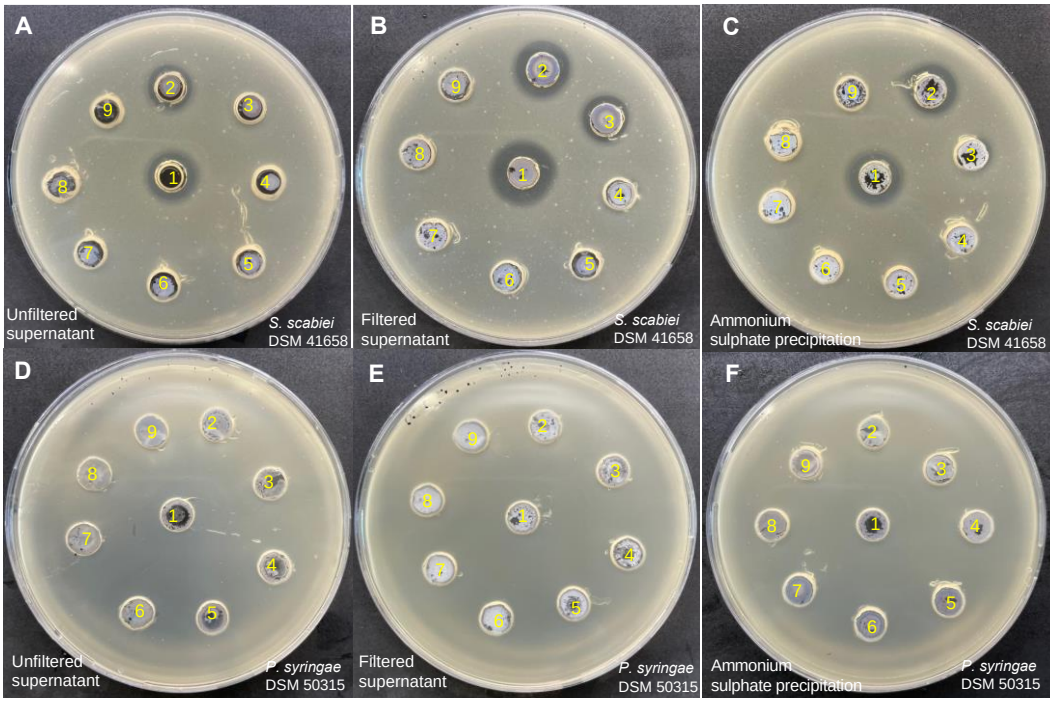

Figure 3.10 Well diffusion assay of crude extraction of bacteriocin from *Bacillus* sp. BL5 strain's supernatants. A, B, C – LB agar medium inoculated with *S. scabiei* DSM 41658, D, E, F – LB agar medium inoculated with *P. syringae* DSM 50315. A and D – unfiltered supernatants before purification, B and E – filtered supernatants before purification, C and F – dialyzed samples after ammonium sulphate precipitation. 1 – undiluted fraction, 2 – 9 represents two-fold dilutions of the sample.

3.6. Purification of bacteriocin using protein chromatography

The obtained crude bacteriocin extract from *Bacillus* sp. BL5 strain's supernatant using ammonium sulphate precipitation was subsequently purified by anion-exchange, cation-exchange

and hydrophobic interaction chromatography methods. To optimize the purification of the bacteriocin by liquid chromatography system, several different strategies were tested in this work **(Figure 3.11)**.

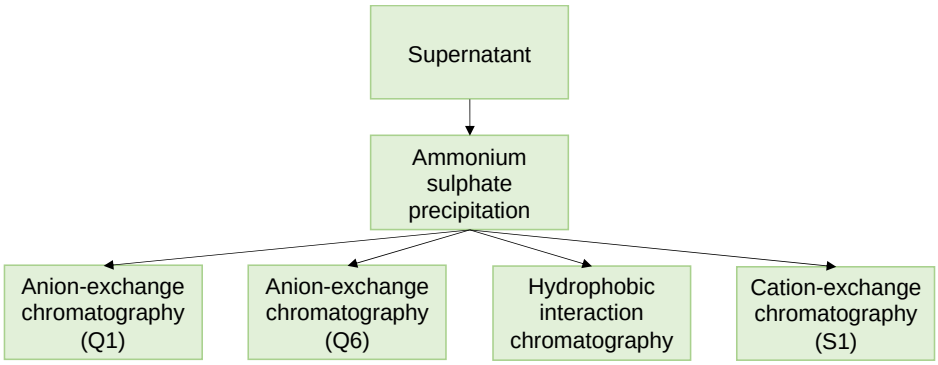

Figure 3.11 Bacteriocin purification from supernatant by liquid chromatography systems. Q1 and Q6 – columns used for anion-exchange chromatography, S1 column used for cation-exchange chromatography.

First, anion-exchange chromatography was performed. Sample after ammonium sulphate precipitation was dialyzed in 20 mM TRIS-HCl, pH 8 buffer. The same buffer and Q1 column (1.3 mL volume) were used in anion-exchange chromatography **(Supplement 4, Figure 11)**. After peptide elution from the column, elution fractions were analyzed using the spot on a lawn assay and tricine-SDS-PAGE protein electrophoresis. Tricine-SDS-PAGE protein electrophoresis gel was overlayed with LB agar medium inoculated with indicator strain *S. scabiei* DSM 41658 **(Figure 3.12)**. The anion-exchange chromatography results displayed multiple peaks instead of a singular peak, indicating impurities in the peptide sample. Additionally, the spot on a lawn assay revealed the highest activity in elution fractions 7 to 10 and the flow through fraction, suggesting inefficient binding of the antibacterial peptide. In the stained tricine-SDS-PAGE protein electrophoresis gel protein bands at positions of ~3.5 kDa and above were visible only in supernatant and ammonium sulphate precipitation fractions. The overlayed gel showed similar results – highest activity was visible in supernatant and ammonium sulphate fractions at a position of ~3.5 kDa, with activity significantly decreasing in anion-exchange chromatography fractions.

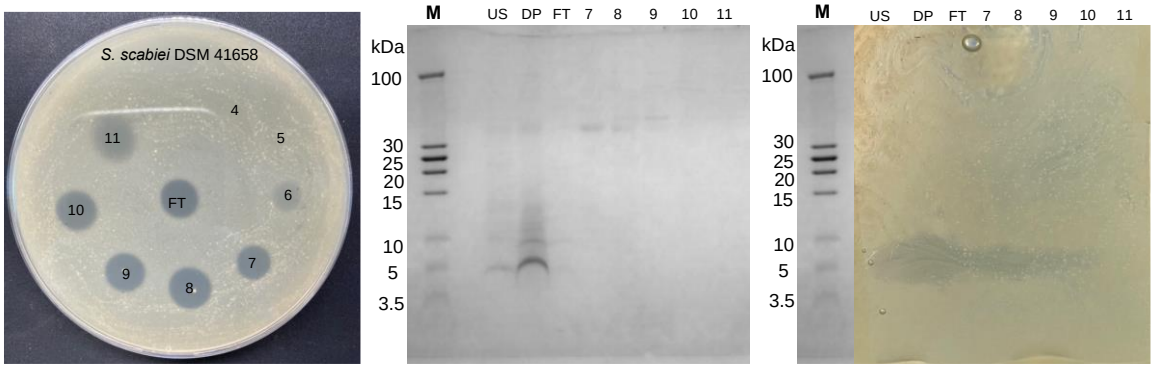

Figure 3.12 The spot on a lawn assay and Tricine-SDS-PAGE protein electrophoresis analysis of anion-exchange chromatography fractions. Agarized LB medium inoculated with indicator *S. scabiei* DSM 41658 strain. US – unfiltered supernatant, DP – dialyzed sample after ammonium sulphate precipitation, FT – flow through.

Then, to obtain more efficient absorption of antimicrobial peptide on the column, purification procedure was repeated using a bigger volume (6 mL) anion-exchange chromatography Q6 column **(Supplement 4, Figure 12)**. Dialyzed sample after ammonium sulphate precipitation was loaded 3 times repeatedly on the Q6 column. Elution fractions were further analyzed by using the spot on a lawn assay. No antibacterial activity was observed in the flow through sample, however because of a chromatography system malfunction, the chromatogram displayed inappropriate absorbance peaks **(Figure 3.13)**. Despite that, it was observed that antibacterial peptide eluted widely in different elution fractions and bacteriocin was not effectively separated. The absence of protein precipitation in a single fraction after anion-exchange chromatography could be attributed to several factors. It is possible that the protein of interest did not bind strongly to the anion-exchange resin and therefore did not elute with a single fraction. Additionally, protein may form aggregates, thus preventing protein precipitation in a single fraction (Ng and Haddad, 2000). However, this strategy appeared to be more effective for sample absorption.

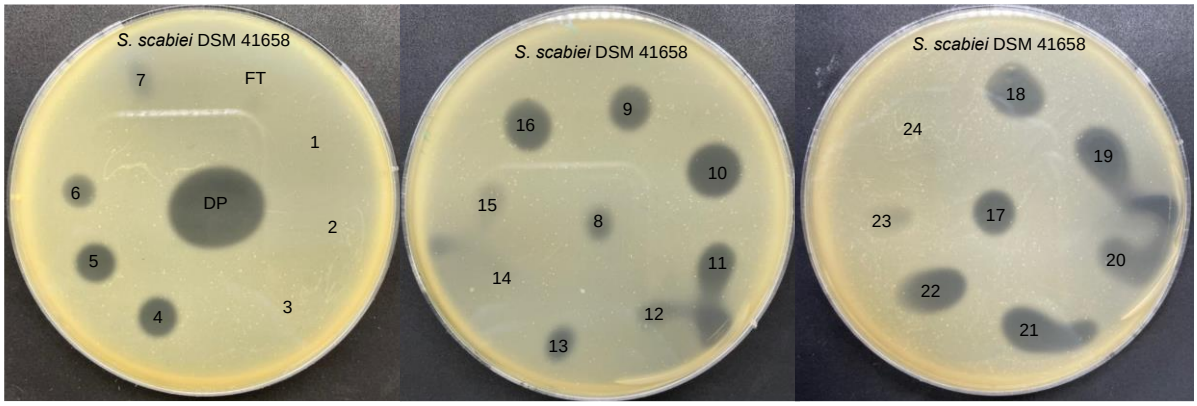

Figure 3.13 The spot on a lawn assay of anion-exchange chromatography fractions. LB agar medium inoculated with *S. scabiei* DSM 41658. DP – dialyzed sample after ammonium sulphate precipitation, FT – flow through.

In the next stage, purification of antibacterial peptide using cation-exchange chromatography was evaluated. After ammonium sulphate precipitation, proteins were dialyzed in two buffers: 20 mM MES, pH 6.1 and 20 mM citric acid pH, 3.1. After dialyzing sample in 20 mM citric acid, pH 3.1 buffer, unfortunately, the sample formed sediment. To remove sediments, the sample was filtered using a 0.22 μm filter. Unfortunately, the antibacterial activity was lost in the filtered sample, and it was eliminated from the study. The sample dialyzed in 20mM MES, pH 6.1 buffer was loaded on cation-exchange column S1. After elution, fractions were further analyzed using the spot on a lawn assay. No growth inhibition zones of *S. scabiei* DSM 41658 appeared in LB agar medium plate, neither in flow throw fraction, nor in elution fractions. It indicated that bacteriocin is probably not stable under these conditions and this protocol is not suitable for purification.

Lastly, to separate molecules based on their hydrophobicity, hydrophobic interaction chromatography (HIC) was performed **(Supplement 4, Figure 13)**. After ammonium sulphate

precipitation, the sample was dialyzed in 20 mM TRIS-HCl, pH 8, supplemented with 2000 mM NaCl and loaded 3 times repeatedly on HIC column pre-equilibrated in the same buffer (20 mM TRIS-HCl, pH 8 + 2000 mM NaCl). After elution, fractions were further analyzed by using the spot on a lawn assay. HIC results showed that zones of growth inhibition were visible in flow through and elution fractions 7 to 18, meaning that the bacteriocin does not properly bind to the column and its desorption is spread widely in elution fractions **(Figure 3.14)**. This methodology was also determined to be ineffective. Dialyzed sample was also supplemented with ammonium sulphate up to 2000 mM. The salt in the buffer reduces the solvation of sample solutes and exposes the hydrophobic regions along the surface of the protein molecule. This facilitates the adsorption of these hydrophobic regions to the hydrophobic areas of the HIC column (Jennissen and Heilmeyer, 1975). However, after supplementing the sample with ammonium sulphate, the sample formed sediment and could no longer been used for HIC.

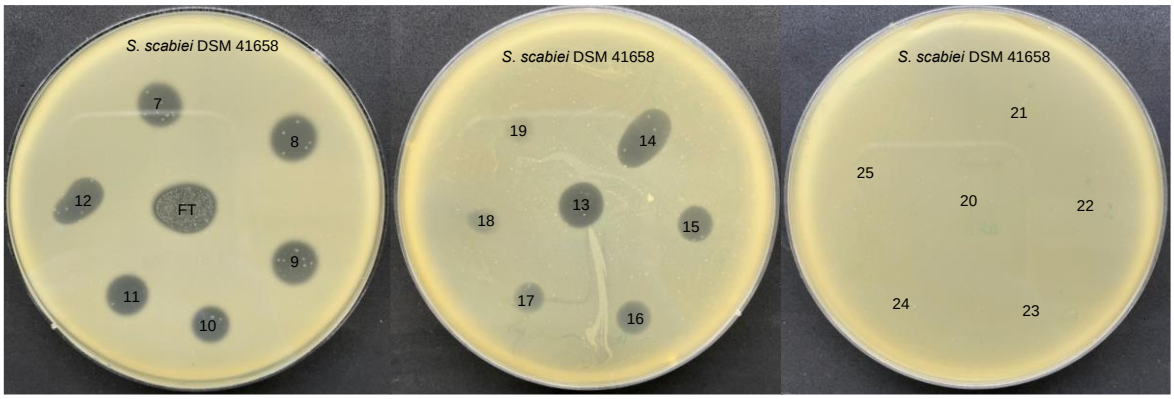

Figure 3.14 The spot on a lawn assay of hydrophobic interaction chromatography fractions. LB agar medium inoculated with *S. scabiei* DSM 41658. FT – flow through.

In conclusion, the results obtained from the protein chromatography techniques indicated that the bacteriocin exhibited affinity to the anion-exchange chromatography column. However, upon elution, it was found to be dispersed among multiple fractions and not effectively separated from other proteins. Therefore, an additional stage is required to achieve efficient separation and concentration of the bacteriocin. Furthermore, the cation-exchange chromatography results revealed possible loss of activity during purification, suggesting potential instability of the bacteriocin under the utilized conditions. Similarly, when subjected to HIC, the bacteriocin eluted across numerous fractions. Moreover, HIC demonstrated inefficient binding of the bacteriocin to the column.

In the future, it is recommended to consider purifying elution fractions obtained after anion-exchange chromatography using RP-HPLC (Reverse Phase High-Performance Liquid Chromatography). RP-HPLC is valuable for protein purification as it offers high resolution. It is commonly used in combination with other chromatographic techniques or as a final purification step to obtain highly purified proteins or peptides for further characterization or downstream applications (Josic and Kovac, 2010).

CONCLUSIONS

1. 51 morphologically different colonies were isolated from the soil, and it was determined that 11 bacterial strains: BL1, BL5, BL11, BL17, BL25, BL26, RL2, RL6, RL7, RL8, BR2 were active against tested plant pathogenic bacteria.
2. 16S rRNA BLASTn and phylogenetic analysis indicated that BL1, BL5, BL11, BL17, BL25, BL26, and RL7 isolates belongs to *Bacillus* genus, and BR2, RL2 and RL6 isolates belongs to *Pseudomonas* genus.
3. *Bacillus* sp. BL5 strain's supernatant inhibited growth of *S. scabiei* DSM 41658 and *P. syringae* DSM 50315 phytopathogens. Supernatant of *Pseudomonas* sp. RL6 strain inhibited growth of *X. campestris* DSM3586 and *P. syringae* DSM 10604 phytopathogens.
4. Antibacterial peptide produced by *Bacillus* sp. BL5 strain is ~3.5 kDa size and based on its molecular size it may be classified as class I or class II bacteriocin.
5. Crude extraction of antibacterial peptide from *Bacillus* sp. BL5 strain's supernatant was achieved by using ammonium sulphate precipitation method. Further purification by anion-exchange chromatography was partially successful, but additional purification steps were needed to separate and concentrate bacteriocin more efficiently.

VILNIUS UNIVERSITY
LIFE SCIENCES CENTRE

Akvilė Vilpišauskaitė
Master Thesis

BACTERIOCINS ACTIVE AGAINST PLANT PATHOGENIC BACTERIA

SUMMARY

The global agricultural industry faces significant challenges due to the annual loss of crops caused by diseases. Current measures employed to control these diseases, such as the heavy use of pesticides and antibiotics, present additional environmental and health concerns. To address these challenges and reduce the reliance on chemically toxic agents, alternative strategies are being explored. One such strategy involves the use of bacteriocins and bioactive peptides, which exhibit antimicrobial properties. Bacteriocins have been studied for a long time, but little is known about the use of bacteriocins in agriculture. These antimicrobials possess potent killing action, high stability, and low toxicity to humans, making them suitable alternatives to chemically toxic agents. While bacteriocins offer a promising alternative to synthetic chemicals in agriculture, the challenges associated with their use must be addressed through further research, regulation, and evaluation of their potential efficacy. Further research is necessary to optimize their production and develop efficient delivery systems for agricultural applications.

The aim of this study was to isolate and purify bacteriocin active against phytopathogenic bacteria. To isolate bacterial strains from the soil, they were grown on NB, 10:NB, BHI and LB medium, at 30°C. 51 isolates were obtained and 11 isolates with antibacterial activity were identified by applying the spot on a lawn assay. By culturing isolates on selective Ashby's Mannitol Agar medium, it was determined that RL2, RL6, and BR2 isolates can use atmospheric nitrogen as a nitrogen source. 16S rRNA BLASTn and phylogenetic analysis indicated that BL1, BL5, BL11, BL17, BL25, BL26, and RL7 isolates belongs to *Bacillus* genus, and BR2, RL2 and RL6 isolates belongs to *Pseudomonas* genus. The spot on a lawn assay revealed that *Bacillus* sp. BL5 strain's supernatant inhibited growth of *S. scabiei* DSM 41658 and *P. syringae* DSM 50315 phytopathogens. Analysis of *Bacillus* sp. BL5 isolate's supernatant indicated that peptide with antibacterial activity has a molecular weight of ~3.5 kDa. Crude extraction of antibacterial peptide from *Bacillus* sp. BL5 strain's supernatant was achieved by using ammonium sulphate precipitation method. To isolate bacteriocin from bacteria, different liquid chromatography systems were used. Purification by anion-exchange chromatography was partially successful, but additional purification steps were needed.

REFERENCE LIST

Abriouel, H., Franz, C.M.A.P., Ben Omar, N., Gálvez, A., 2011. Diversity and applications of Bacillus bacteriocins. FEMS Microbiol Rev 35, 201–232. https://doi.org/10.1111/j.1574-6976.2010.00244.x

Acedo, J.Z., Chiorean, S., Vederas, J.C., van Belkum, M.J., 2018. The expanding structural variety among bacteriocins from Gram-positive bacteria. FEMS Microbiology Reviews 42, 805–828. https://doi.org/10.1093/femsre/fuy033

Adedeji, W.A., 2016. THE TREASURE CALLED ANTIBIOTICS. Ann Ib Postgrad Med 14, 56–57.

Adedire O. M., Odeniyi O. A., 2017. Antimicrobial Activities of Bacteriocin-Like Extracellular Metabolites Produced by Soil Bacteria. PBJ 47–54. https://doi.org/10.20510/ukjpb/5/i4/166545

Agrios, G.N., 2005a. INTRODUCTION, in: Plant Pathology. Elsevier, pp. 3–75. https://doi.org/10.1016/B978-0-08-047378-9.50007-5

Agrios, G.N., 2005b. PLANT DISEASES CAUSED BY PROKARYOTES: BACTERIA AND MOLLICUTES, in: Plant Pathology. Elsevier, pp. 615–703. https://doi.org/10.1016/B978-0-08-047378-9.50018-X

Aizawa, S.-I., 2014. Pectobacterium carotovorum — Subpolar Hyper-Flagellation, in: The Flagellar World. Elsevier, pp. 58–59. https://doi.org/10.1016/B978-0-12-417234-0.00018-9

Alabouvette, C., Olivain, C., Steinberg, C., 2006. Biological Control of Plant Diseases: The European Situation. Eur J Plant Pathol 114, 329–341. https://doi.org/10.1007/s10658-005-0233-0

Aldrich, T.J., Rolshausen, P.E., Roper, M.C., Reader, J.M., Steinhaus, M.J., Rapicavoli, J., Vosburg, D.A., Maloney, K.N., 2015. Radicinin from Cochliobolus sp. inhibits Xylella fastidiosa , the causal agent of Pierce's Disease of grapevine. Phytochemistry 116, 130–137. https://doi.org/10.1016/j.phytochem.2015.03.015

Álvarez, B., Biosca, E.G., López, M.M., 2010. On the life of Ralstonia solanacearum, a destructive bacterial plant pathogen. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology 267–279.

Álvarez, B., Vasse, J., Le-Courtois, V., Trigalet-Démery, D., López, M.M., Trigalet, A., 2008. Comparative Behavior of *Ralstonia solanacearum* Biovar 2 in Diverse Plant Species. Phytopathology® 98, 59–68. https://doi.org/10.1094/PHYTO-98-1-0059

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., Kuipers, O.P., 2016. Bacteriocins of lactic acid bacteria: extending the family. Appl Microbiol Biotechnol 100, 2939–2951. https://doi.org/10.1007/s00253-016-7343-9

Andryukov, B.G., Mikhaylov, V.V., Besednova, N.N., Zaporozhets, T.S., Bynina, M.P., Matosova, E.V., 2018. The Bacteriocinogenic Potential of Marine Microorganisms. Russ J Mar Biol 44, 433–441. https://doi.org/10.1134/S1063074018060020

Arnison, P.G., Bibb, M.J., Bierbaum, G., Bowers, A.A., Bugni, T.S., Bulaj, G., Camarero, J.A., Campopiano, D.J., Challis, G.L., Clardy, J., Cotter, P.D., Craik, D.J., Dawson, M., Dittmann, E., Donadio, S., Dorrestein, P.C., Entian, K.-D., Fischbach, M.A., Garavelli, J.S., Göransson, U., Gruber, C.W., Haft, D.H., Hemscheidt, T.K., Hertweck, C., Hill, C., Horswill, A.R., Jaspars, M., Kelly, W.L., Klinman, J.P., Kuipers, O.P., Link, A.J., Liu, W., Marahiel, M.A., Mitchell, D.A., Moll, G.N., Moore, B.S., Müller, R., Nair, S.K., Nes, I.F., Norris, G.E., Olivera, B.M., Onaka, H., Patchett, M.L., Piel, J., Reaney, M.J.T., Rebuffat, S., Ross, R.P., Sahl, H.-G., Schmidt, E.W., Selsted, M.E., Severinov, K., Shen, B., Sivonen, K., Smith, L., Stein, T., Süssmuth, R.D., Tagg, J.R., Tang, G.-L., Truman, A.W., Vederas, J.C., Walsh, C.T., Walton, J.D., Wenzel, S.C., Willey, J.M., van der Donk, W.A., 2013. Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature. Nat Prod Rep 30, 108–160. https://doi.org/10.1039/c2np20085f

Arseneault, T., Goyer, C., Filion, M., 2015. *Pseudomonas fluorescens* LBUM223 Increases Potato Yield and Reduces Common Scab Symptoms in the Field. Phytopathology® 105, 1311–1317. https://doi.org/10.1094/PHYTO-12-14-0358-R

Babasaki, K., Takao, T., Shimonishi, Y., Kurahashi, K., 1985. Subtilosin A, a new antibiotic peptide produced by Bacillus subtilis 168: isolation, structural analysis, and biogenesis. J Biochem 98, 585–603. https://doi.org/10.1093/oxfordjournals.jbchem.a135315

Balciunas, E.M., Castillo Martinez, F.A., Todorov, S.D., Franco, B.D.G. de M., Converti, A., Oliveira, R.P. de S., 2013. Novel biotechnological applications of bacteriocins: A review. Food Control 32, 134–142. https://doi.org/10.1016/j.foodcont.2012.11.025

Baquero, F., Lanza, V.F., Baquero, M.-R., del Campo, R., Bravo-Vázquez, D.A., 2019. Microcins in Enterobacteriaceae: Peptide Antimicrobials in the Eco-Active Intestinal Chemosphere. Front. Microbiol. 10, 2261. https://doi.org/10.3389/fmicb.2019.02261

Bemena, L.D., Mohamed, L.A., Fernandez, A.M., Lee, B.H., 2014. Applications of bacteriocins in food, livestock health and medicine. International Journal of Current Microbiology and Applied Sciences 3, 924–949.

Beukes, M., Bierbaum, G., Sahl, H.-G., Hastings, J.W., 2000. Purification and Partial Characterization of a Murein Hydrolase, Millericin B, Produced by Streptococcus milleri NMSCC 061. Appl. Environ. Microbiol. 66, 23–28. https://doi.org/10.1128/AEM.66.1.23-28.2000

Bhattacharya, D., Nanda, P.K., Pateiro, M., Lorenzo, J.M., Dhar, P., Das, A.K., 2022. Lactic Acid Bacteria and Bacteriocins: Novel Biotechnological Approach for Biopreservation of Meat and Meat Products. Microorganisms 10, 2058. https://doi.org/10.3390/microorganisms10102058

Bizani, D., Motta, A.S., Morrissy, J.A.C., Terra, R.M.S., Souto, A.A., Brandelli, A., 2005. Antibacterial activity of cerein 8A, a bacteriocin-like peptide produced by Bacillus cereus. Int Microbiol 8, 125–131.

Bouchek-Mechiche, K., Gardan, L., Normand, P., Jouan, B., 2000. DNA relatedness among strains of Streptomyces pathogenic to potato in France: description of three new species, S. europaeiscabiei sp. nov. and S. stelliscabiei sp. nov. associated with common scab, and S. reticuliscabiei sp. nov. associated with netted scab. International Journal of Systematic and Evolutionary Microbiology 50, 91–99. https://doi.org/10.1099/00207713-50-1-91

Bradbury, J.F., 1984. Genus II. Xanthomonas Dowson, in: Bergey's Manual of Systematic Bacteriology. Williams & Wilkins, 1984, pp. 199–210.

Broekaert, W.F., Terras, F.R., Cammue, B.P., Osborn, R.W., 1995. Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiol 108, 1353–1358. https://doi.org/10.1104/pp.108.4.1353

Bundock, P., den Dulk-Ras, A., Beijersbergen, A., Hooykaas, P.J., 1995. Trans-kingdom T-DNA transfer from Agrobacterium tumefaciens to Saccharomyces cerevisiae. The EMBO Journal 14, 3206–3214. https://doi.org/10.1002/j.1460-2075.1995.tb07323.x

Chan, D.C.K., Burrows, L.L., 2021. Thiopeptides: antibiotics with unique chemical structures and diverse biological activities. J Antibiot 74, 161–175. https://doi.org/10.1038/s41429-020-00387-x

Chavan, M.A., Riley, M.A., 2007. Molecular Evolution of Bacteriocins in Gram-Negative Bacteria, in: Riley, M.A., Chavan, M.A. (Eds.), Bacteriocins. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 19–43. https://doi.org/10.1007/978-3-540-36604-1_3

Chikindas, M.L., Weeks, R., Drider, D., Chistyakov, V.A., Dicks, L.M., 2018. Functions and emerging applications of bacteriocins. Current Opinion in Biotechnology 49, 23–28. https://doi.org/10.1016/j.copbio.2017.07.011

Chithrashree, Udayashankar, A.C., Chandra Nayaka, S., Reddy, M.S., Srinivas, C., 2011. Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by Xanthomonas oryzae pv. oryzae. Biological Control 59, 114–122. https://doi.org/10.1016/j.biocontrol.2011.06.010

Cintas, L.M., Casaus, M.P., Herranz, C., Nes, I.F., Hernández, P.E., 2001. Review: Bacteriocins of Lactic Acid Bacteria. Food sci. technol. int. 7, 281–305. https://doi.org/10.1106/R8DE-P6HU-CLXP-5RYT

Cintas, L.M, Casaus, P., Fernández, M.F., Hernández, P.E., 1998. Comparative antimicrobial activity of enterocin L50, pediocin PA-1, nisin A and lactocin S against spoilage and foodborne pathogenic bacteria. Food Microbiology 15, 289–298. https://doi.org/10.1006/fmic.1997.0160

Cintas, Luis M., Casaus, P., Holo, H., Hernandez, P.E., Nes, I.F., Håvarstein, L.S., 1998. Enterocins L50A and L50B, Two Novel Bacteriocins from *Enterococcus faecium* L50, Are Related to Staphylococcal Hemolysins. J Bacteriol 180, 1988–1994. https://doi.org/10.1128/JB.180.8.1988-1994.1998

Cleveland, J., Montville, T.J., Nes, I.F., Chikindas, M.L., 2001. Bacteriocins: safe, natural antimicrobials for food preservation. International Journal of Food Microbiology 71, 1–20. https://doi.org/10.1016/S0168-1605(01)00560-8

Compaoré, C.S., Nielsen, D.S., Ouoba, L.I.I., Berner, T.S., Nielsen, K.F., Sawadogo-Lingani, H., Diawara, B., Ouédraogo, G.A., Jakobsen, M., Thorsen, L., 2013. Co-production of surfactin and a novel bacteriocin by Bacillus subtilis subsp. subtilis H4 isolated from Bikalga, an African alkaline Hibiscus sabdariffa seed fermented condiment. International Journal of Food Microbiology 162, 297–307. https://doi.org/10.1016/j.ijfoodmicro.2013.01.013

Cotter, P.D., Ross, R.P., Hill, C., 2013. Bacteriocins — a viable alternative to antibiotics? Nat Rev Microbiol 11, 95–105. https://doi.org/10.1038/nrmicro2937

Cox, C.L., Doroghazi, J.R., Mitchell, D.A., 2015. The genomic landscape of ribosomal peptides containing thiazole and oxazole heterocycles. BMC Genomics 16, 778. https://doi.org/10.1186/s12864-015-2008-0

Cubero, J., Lastra, B., Salcedo, C.I., Piquer, J., Lopez, M.M., 2006. Systemic movement of Agrobacterium tumefaciens in several plant species. J Appl Microbiol 101, 412–421. https://doi.org/10.1111/j.1365-2672.2006.02938.x

Daugherty, M.P., O'Neill, S., Byrne, F., Zeilinger, A., 2015. Is Vector Control Sufficient to Limit Pathogen Spread in Vineyards? Environmental Entomology 44, 789–797. https://doi.org/10.1093/ee/nvv046

de Castro, I., Mendo, S., Caetano, T., 2020. Antibiotics from Haloarchaea: What Can We Learn from Comparative Genomics? Mar Biotechnol 22, 308–316. https://doi.org/10.1007/s10126-020-09952-9

Deegan, L.H., Cotter, P.D., Hill, C., Ross, P., 2006. Bacteriocins: Biological tools for bio-preservation and shelf-life extension. International Dairy Journal 16, 1058–1071. https://doi.org/10.1016/j.idairyj.2005.10.026

Dees, M.W., Wanner, L.A., 2012. In Search of Better Management of Potato Common Scab. Potato Res. 55, 249–268. https://doi.org/10.1007/s11540-012-9206-9

Delves-Broughton, J., 2005. Antimicrobial Applications. Food Australia 57, 525–527.

Delves-Broughton, J., Blackburn, P., Evans, R.J., Hugenholtz, J., 1996. Applications of the bacteriocin, nisin. Antonie van Leeuwenhoek 69, 193–202. https://doi.org/10.1007/BF00399424

Denny, T., 2006. Plant pathogenic Ralstonia species, in: Gnanamanickam, S.S. (Ed.), Plant-Associated Bacteria. Springer Netherlands, Dordrecht, pp. 573–644. https://doi.org/10.1007/978-1-4020-4538-7_16

Dicks, L.M.T., Dreyer, L., Smith, C., van Staden, A.D., 2018. A Review: The Fate of Bacteriocins in the Human Gastro-Intestinal Tract: Do They Cross the Gut–Blood Barrier? Front. Microbiol. 9, 2297. https://doi.org/10.3389/fmicb.2018.02297

Diep, D.B., Nes, I.F., 2002. Ribosomally synthesized antibacterial peptides in Gram positive bacteria. Curr Drug Targets 3, 107–122. https://doi.org/10.2174/1389450024605409

Eckwall, E.C., Schottel, J.L., 1997. Isolation and characterization of an antibiotic produced by the scab disease-suppressive Streptomyces diastatochromogenes strain PonSSII. Journal of

Industrial Microbiology and Biotechnology 19, 220–225. https://doi.org/10.1038/sj.jim.2900455
EFSA Panel on Plant Health (EFSA PLH Panel), Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jaques Miret, J.A., Justesen, A.F., MacLeod, A., Magnusson, C.S., Milonas, P., Navas-Cortes, J.A., Parnell, S., Potting, R., Reignault, P.L., Thulke, H., Van der Werf, W., Vicent Civera, A., Yuen, J., Zappalà, L., Van der Wolf, J., Kaluski, T., Pautasso, M., Jacques, M., 2019. Pest categorisation of the Ralstonia solanacearum species complex. EFS2 17. https://doi.org/10.2903/j.efsa.2019.5618
Ekblad, B., Kyriakou, P.K., Oppegård, C., Nissen-Meyer, J., Kaznessis, Y.N., Kristiansen, P.E., 2016. Structure–Function Analysis of the Two-Peptide Bacteriocin Plantaricin EF. Biochemistry 55, 5106–5116. https://doi.org/10.1021/acs.biochem.6b00588
European Food Safety Authority (EFSA), 2016. Update of a database of host plants of Xylella fastidiosa: 20 November 2015. EFS2 14. https://doi.org/10.2903/j.efsa.2016.4378
Fegan, M., Prior, P., 2005. How complex is the Ralstonia solanacearum species complex. Bacterial wilt Disease and the Ralstonia solanacearum Species Complex 449–461.
Field, D., Daly, K., O'Connor, P.M., Cotter, P.D., Hill, C., Ross, R.P., 2015. Efficacies of nisin A and nisin V semipurified preparations alone and in combination with plant essential oils for controlling Listeria monocytogenes. Appl Environ Microbiol 81, 2762–2769. https://doi.org/10.1128/AEM.00070-15
Franz, L., Kazmaier, U., Truman, A.W., Koehnke, J., 2021. Bottromycins - biosynthesis, synthesis and activity. Nat. Prod. Rep. 38, 1659–1683. https://doi.org/10.1039/D0NP00097C
Fregeau Gallagher, N.L., Sailer, M., Niemczura, W.P., Nakashima, T.T., Stiles, M.E., Vederas, J.C., 1997. Three-Dimensional Structure of Leucocin A in Trifluoroethanol and Dodecylphosphocholine Micelles: Spatial Location of Residues Critical for Biological Activity in Type IIa Bacteriocins from Lactic Acid Bacteria †. Biochemistry 36, 15062–15072. https://doi.org/10.1021/bi971263h
Garsa, A.K., Kumariya, R., Kumar, A., Lather, P., Kapila, S., Sood, S.K., 2014. Industrial cheese whey utilization for enhanced production of purified pediocin PA-1. LWT - Food Science and Technology 59, 656–665. https://doi.org/10.1016/j.lwt.2014.07.008
Gebhart, D., Williams, S.R., Bishop-Lilly, K.A., Govoni, G.R., Willner, K.M., Butani, A., Sozhamannan, S., Martin, D., Fortier, L.-C., Scholl, D., 2012. Novel High-Molecular-Weight, R-Type Bacteriocins of Clostridium difficile. J Bacteriol 194, 6240–6247. https://doi.org/10.1128/JB.01272-12
Geis, A., Singh, J., Teuber, M., 1983. Potential of lactic streptococci to produce bacteriocin. Appl Environ Microbiol 45, 205–211. https://doi.org/10.1128/AEM.45.1.205-211.1983
Gelvin, S.B., 2012. Traversing the Cell: Agrobacterium T-DNA's Journey to the Host Genome. Front. Plant Sci. 3. https://doi.org/10.3389/fpls.2012.00052
Genin, S., 2010. Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. New Phytologist 187, 920–928. https://doi.org/10.1111/j.1469-8137.2010.03397.x
George, M.L.C., Bustamam, M., Cruz, W.T., Leach, J.E., Nelson, R.J., 1997. Movement of *Xanthomonas oryzae* pv. *oryzae* in Southeast Asia Detected Using PCR-Based DNA Fingerprinting. Phytopathology® 87, 302–309. https://doi.org/10.1094/PHYTO.1997.87.3.302
Ghequire, M.G.K., De Mot, R., 2015. The Tailocin Tale: Peeling off Phage Tails. Trends Microbiol 23, 587–590. https://doi.org/10.1016/j.tim.2015.07.011
Ghodhbane, H., Elaidi, S., Sabatier, J.-M., Achour, S., Benhmida, J., Regaya, I., 2015. Bacteriocins active against multi-resistant gram negative bacteria implicated in nosocomial infections. Infect Disord Drug Targets 15, 2–12. https://doi.org/10.2174/1871526514666140522113337
Gillor, O., Kirkup, B.C., Riley, M.A., 2004. Colicins and microcins: the next generation antimicrobials. Adv Appl Microbiol 54, 129–146. https://doi.org/10.1016/S0065-2164(04)54005-4

Glick, B.R., Bashan, Y., 1997. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnology Advances 15, 353–378. https://doi.org/10.1016/S0734-9750(97)00004-9

Gordon, D.M., Oliver, E., Littlefield-Wyer, J., 2007. The Diversity of Bacteriocins in Gram-Negative Bacteria, in: Riley, M.A., Chavan, M.A. (Eds.), Bacteriocins. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 5–18. https://doi.org/10.1007/978-3-540-36604-1_2

Gratia, A., 1925. Sur un remarquable exemple d'antagonisme entre deux souches de colibacille. C. R. Soc. Biol. 93, 1041–1042.

Gray, E.J., Lee, K.D., Souleimanov, A.M., Di Falco, M.R., Zhou, X., Ly, A., Charles, T.C., Driscoll, B.T., Smith, D.L., 2006. A novel bacteriocin, thuricin 17, produced by plant growth promoting rhizobacteria strain Bacillus thuringiensis NEB17: isolation and classification. J Appl Microbiol 100, 545–554. https://doi.org/10.1111/j.1365-2672.2006.02822.x

Gray, E.J., Smith, D.L., 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biology and Biochemistry 37, 395–412. https://doi.org/10.1016/j.soilbio.2004.08.030

Güllüce, M., Karadayı, M., Barış, Ö., 2013. Bacteriocins: Promising Natural Antimicrobials. https://doi.org/10.13140/2.1.5014.5606

Haft, D.H., Basu, M.K., Mitchell, D.A., 2010. Expansion of ribosomally produced natural products: a nitrile hydratase- and Nif11-related precursor family. BMC Biol 8, 70. https://doi.org/10.1186/1741-7007-8-70

Hammami, I., Rhouma, A., Jaouadi, B., Rebai, A., Nesme, X., 2009. Optimization and biochemical characterization of a bacteriocin from a newly isolated *Bacillus subtilis* strain 14B for biocontrol of *Agrobacterium* spp . strains. Letters in Applied Microbiology 48, 253–260. https://doi.org/10.1111/j.1472-765X.2008.02524.x

Hammami, I., Triki, M.A., Rebai, A., 2011. Purification and characterization of the novel bacteriocin IH7 with antifungal and antibacterial properties. Journal of Plant Pathology 93, 443–454.

Han, J.S., Cheng, J.H., Yoon, T.M., Song, J., Rajkarnikar, A., Kim, W.G., Yoo, I.D., Yang, Y.Y., Suh, J.W., 2005. Biological control agent of common scab disease by antagonistic strain *Bacillus* sp. sunhua. Journal of Applied Microbiology 99, 213–221. https://doi.org/10.1111/j.1365-2672.2005.02614.x

Hayward, A.C., 1991. Biology and Epidemiology of Bacterial Wilt Caused by Pseudomonas Solanacearum. Annu. Rev. Phytopathol. 29, 65–87. https://doi.org/10.1146/annurev.py.29.090191.000433

Hegemann, J.D., Zimmermann, M., Xie, X., Marahiel, M.A., 2015. Lasso peptides: an intriguing class of bacterial natural products. Acc Chem Res 48, 1909–1919. https://doi.org/10.1021/acs.accounts.5b00156

Heng, N.C.K., Ragland, N.L., Swe, P.M., Baird, H.J., Inglis, M.A., Tagg, J.R., Jack, R.W., 2006. Dysgalacticin: a novel, plasmid-encoded antimicrobial protein (bacteriocin) produced by Streptococcus dysgalactiae subsp. equisimilis. Microbiology 152, 1991–2001. https://doi.org/10.1099/mic.0.28823-0

Heng, N.C.K., Wescombe, P.A., Burton, J.P., Jack, R.W., Tagg, J.R., 2007. The Diversity of Bacteriocins in Gram-Positive Bacteria, in: Riley, M.A., Chavan, M.A. (Eds.), Bacteriocins. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 45–92. https://doi.org/10.1007/978-3-540-36604-1_4

Hernández-González, J.C., Martínez-Tapia, A., Lazcano-Hernández, G., García-Pérez, B.E., Castrejón-Jiménez, N.S., 2021. Bacteriocins from Lactic Acid Bacteria. A Powerful Alternative as Antimicrobials, Probiotics, and Immunomodulators in Veterinary Medicine. Animals 11, 979. https://doi.org/10.3390/ani11040979

Himeno, K., Rosengren, K.J., Inoue, T., Perez, R.H., Colgrave, M.L., Lee, H.S., Chan, L.Y., Henriques, S.T., Fujita, K., Ishibashi, N., Zendo, T., Wilaipun, P., Nakayama, J., Leelawatcharamas, V., Jikuya, H., Craik, D.J., Sonomoto, K., 2015. Identification, Characterization, and Three-Dimensional Structure of the Novel Circular Bacteriocin,

Enterocin NKR-5-3B, from Enterococcus faecium. Biochemistry 54, 4863–4876. https://doi.org/10.1021/acs.biochem.5b00196
Hirano, S.S., Upper, C.D., 2000. Bacteria in the Leaf Ecosystem with Emphasis on *Pseudomonas syringae* —a Pathogen, Ice Nucleus, and Epiphyte. Microbiol Mol Biol Rev 64, 624–653. https://doi.org/10.1128/MMBR.64.3.624-653.2000
Hoskin, D.W., Ramamoorthy, A., 2008. Studies on anticancer activities of antimicrobial peptides. Biochimica et Biophysica Acta (BBA) - Biomembranes 1778, 357–375. https://doi.org/10.1016/j.bbamem.2007.11.008
Hu, C.-B., Malaphan, W., Zendo, T., Nakayama, J., Sonomoto, K., 2010. Enterocin X, a novel two-peptide bacteriocin from Enterococcus faecium KU-B5, has an antibacterial spectrum entirely different from those of its component peptides. Appl Environ Microbiol 76, 4542–4545. https://doi.org/10.1128/AEM.02264-09
Huang, T., Geng, H., Miyyapuram, V.R., Sit, C.S., Vederas, J.C., Nakano, M.M., 2009. Isolation of a variant of subtilosin A with hemolytic activity. J Bacteriol 191, 5690–5696. https://doi.org/10.1128/JB.00541-09
Hull, R., Head, G., Tzotzos, G.T., 2021. Technologies for crop improvement, in: Genetically Modified Plants. Elsevier, pp. 35–81. https://doi.org/10.1016/B978-0-12-818564-3.00002-0
Iwatsuki, M., Tomoda, H., Uchida, R., Gouda, H., Hirono, S., Ōmura, S., 2006. Lariatins, Antimycobacterial Peptides Produced by *Rhodococcus* sp. K01−B0171, Have a Lasso Structure. J. Am. Chem. Soc. 128, 7486–7491. https://doi.org/10.1021/ja056780z
Jacques, M.-A., Arlat, M., Boulanger, A., Boureau, T., Carrère, S., Cesbron, S., Chen, N.W.G., Cociancich, S., Darrasse, A., Denancé, N., Fischer-Le Saux, M., Gagnevin, L., Koebnik, R., Lauber, E., Noël, L.D., Pieretti, I., Portier, P., Pruvost, O., Rieux, A., Robène, I., Royer, M., Szurek, B., Verdier, V., Vernière, C., 2016. Using Ecology, Physiology, and Genomics to Understand Host Specificity in *Xanthomonas*. Annu. Rev. Phytopathol. 54, 163–187. https://doi.org/10.1146/annurev-phyto-080615-100147
Jamuna, M., Jeevaratnam, K., 2004. Isolation and partial characterization of bacteriocins from Pediococcus species. Appl Microbiol Biotechnol 65, 433–439. https://doi.org/10.1007/s00253-004-1576-8
Jennissen, H.P., Heilmeyer, L.M.G., 1975. General aspects of hydrophobic chromatography. Adsorption and elution characteristics of some skeletal muscle enzymes. Biochemistry 14, 754–760. https://doi.org/10.1021/bi00675a017
Ji, G.-H., Wei, L.-F., He, Y.-Q., Wu, Y.-P., Bai, X.-H., 2008. Biological control of rice bacterial blight by Lysobacter antibioticus strain 13-1. Biological Control 45, 288–296. https://doi.org/10.1016/j.biocontrol.2008.01.004
Jin, S.G., Prusti, R.K., Roitsch, T., Ankenbauer, R.G., Nester, E.W., 1990. Phosphorylation of the VirG protein of Agrobacterium tumefaciens by the autophosphorylated VirA protein: essential role in biological activity of VirG. J Bacteriol 172, 4945–4950. https://doi.org/10.1128/jb.172.9.4945-4950.1990
Joerger, M.C., Klaenhammer, T.R., 1990. Cloning, expression, and nucleotide sequence of the Lactobacillus helveticus 481 gene encoding the bacteriocin helveticin J. J Bacteriol 172, 6339–6347. https://doi.org/10.1128/jb.172.11.6339-6347.1990
Joerger, R., 2003. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poultry Science 82, 640–647. https://doi.org/10.1093/ps/82.4.640
Johnson, K.B., Stockwell, V.O., 1998. MANAGEMENT OF FIRE BLIGHT: A Case Study in Microbial Ecology. Annu. Rev. Phytopathol. 36, 227–248. https://doi.org/10.1146/annurev.phyto.36.1.227
Joo, N.E., Ritchie, K., Kamarajan, P., Miao, D., Kapila, Y.L., 2012. Nisin, an apoptogenic bacteriocin and food preservative, attenuates HNSCC tumorigenesis via CHAC 1. Cancer Med 1, 295–305. https://doi.org/10.1002/cam4.35
Josic, D., Kovac, S., 2010. Reversed-Phase High Performance Liquid Chromatography of Proteins. CP Protein Science 61. https://doi.org/10.1002/0471140864.ps0807s61

Jun, S.-R., Sims, G.E., Wu, G.A., Kim, S.-H., 2010. Whole-proteome phylogeny of prokaryotes by feature frequency profiles: An alignment-free method with optimal feature resolution. Proc. Natl. Acad. Sci. U.S.A. 107, 133–138. https://doi.org/10.1073/pnas.0913033107

Jung, W.-J., Mabood, F., Souleimanov, A., Smith, D.L., 2011. Induction of defense-related enzymes in soybean leaves by class IId bacteriocins (thuricin 17 and bacthuricin F4) purified from Bacillus strains. Microbiological Research 167, 14–19. https://doi.org/10.1016/j.micres.2011.02.004

Jung, W.-J., Mabood, F., Souleimanov, A., Smith, D.L., 2008. Effect of chitin hexamer and thuricin 17 on lignification-related and antioxidative enzymes in Soybean Plants. J. Plant Biol. 51, 145–149. https://doi.org/10.1007/BF03030724

Kamarajan, P., Hayami, T., Matte, B., Liu, Y., Danciu, T., Ramamoorthy, A., Worden, F., Kapila, S., Kapila, Y., 2015. Nisin ZP, a Bacteriocin and Food Preservative, Inhibits Head and Neck Cancer Tumorigenesis and Prolongs Survival. PLoS ONE 10, e0131008. https://doi.org/10.1371/journal.pone.0131008

Kaur, Sumanpreet, Kaur, Sukhraj, 2015. Bacteriocins as Potential Anticancer Agents. Front Pharmacol 6, 272. https://doi.org/10.3389/fphar.2015.00272

Khatoon, Z., Huang, S., Rafique, M., Fakhar, A., Kamran, M.A., Santoyo, G., 2020. Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. Journal of Environmental Management 273, 111118. https://doi.org/10.1016/j.jenvman.2020.111118

Kitazaki, K., Baba, T., Koga, Y., Kuwano, G., Fukuda, H., Kawada, E., Takasu, Y., Takehana, H., Koga, S., Nagatoshi, K., 2010. The use of nisin A in preventing bovine mastitis infection. Food and Food Ingredient Journal of Japan 215, 449–456.

Klaenhammer, T.R., 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev 12, 39–85. https://doi.org/10.1111/j.1574-6976.1993.tb00012.x

Kloepper, J.W., Ryu, C.-M., Zhang, S., 2004. Induced Systemic Resistance and Promotion of Plant Growth by *Bacillus* spp. Phytopathology® 94, 1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259

Knerr, P.J., van der Donk, W.A., 2012. Discovery, biosynthesis, and engineering of lantipeptides. Annu Rev Biochem 81, 479–505. https://doi.org/10.1146/annurev-biochem-060110-113521

Kozak, W., Bardowski, J., Dobrzański, W.T., 1978. Lactostrepcins--acid bacteriocins produced by lactic streptococci. J Dairy Res 45, 247–257. https://doi.org/10.1017/s0022029900016423

Kunik, T., Tzfira, T., Kapulnik, Y., Gafni, Y., Dingwall, C., Citovsky, V., 2001. Genetic transformation of HeLa cells by *Agrobacterium*. Proc. Natl. Acad. Sci. U.S.A. 98, 1871–1876. https://doi.org/10.1073/pnas.98.4.1871

Kwon, H.-T., Lee, Y., Kim, J., Balaraju, K., Kim, H.T., Jeon, Y., 2022. Identification and Characterization of Bacillus tequilensis GYUN-300: An Antagonistic Bacterium Against Red Pepper Anthracnose Caused by Colletotrichum acutatum in Korea. Front. Microbiol. 13, 826827. https://doi.org/10.3389/fmicb.2022.826827

Lagos, R., Tello, M., Mercado, G., Garcia, V., Monasterio, O., 2009. Antibacterial and Antitumorigenic Properties of Microcin E492, a Pore- Forming Bacteriocin. CPB 10, 74–85. https://doi.org/10.2174/138920109787048643

Lamichhane, J.R., Varvaro, L., Parisi, L., Audergon, J.-M., Morris, C.E., 2014. Disease and Frost Damage of Woody Plants Caused by Pseudomonas syringae, in: Advances in Agronomy. Elsevier, pp. 235–295. https://doi.org/10.1016/B978-0-12-800132-5.00004-3

Lancaster, L.E., Wintermeyer, W., Rodnina, M.V., 2007. Colicins and their potential in cancer treatment. Blood Cells, Molecules, and Diseases 38, 15–18. https://doi.org/10.1016/j.bcmd.2006.10.006

Larkin, R.P., Honeycutt, C.W., Griffin, T.S., Olanya, O.M., Halloran, J.M., He, Z., 2011. Effects of Different Potato Cropping System Approaches and Water Management on Soilborne Diseases and Soil Microbial Communities. Phytopathology® 101, 58–67. https://doi.org/10.1094/PHYTO-04-10-0100

Lebot, V., 2009. Cassava: postharvest quality and marketing, in: Tropical Root and Tuber Crops Cassava, Sweet Potato, Yams and Aroids. CABI, Wallingford, England, p. 413.
Lee, K.D., Gray, E.J., Mabood, F., Jung, W.-J., Charles, T., Clark, S.R.D., Ly, A., Souleimanov, A., Zhou, X., Smith, D.L., 2009. The class IId bacteriocin thuricin-17 increases plant growth. Planta 229, 747–755. https://doi.org/10.1007/s00425-008-0870-6
Li, Y., Ducasse, R., Zirah, S., Blond, A., Goulard, C., Lescop, E., Giraud, C., Hartke, A., Guittet, E., Pernodet, J.-L., Rebuffat, S., 2015. Characterization of Sviceucin from *Streptomyces* Provides Insight into Enzyme Exchangeability and Disulfide Bond Formation in Lasso Peptides. ACS Chem. Biol. 10, 2641–2649. https://doi.org/10.1021/acschembio.5b00584
Liu, X., Vederas, J.C., Whittal, R.M., Zheng, J., Stiles, M.E., Carlson, D., Franz, C.M.A.P., McMullen, L.M., van Belkum, M.J., 2011. Identification of an N-terminal formylated, two-peptide bacteriocin from Enterococcus faecalis 710C. J Agric Food Chem 59, 5602–5608. https://doi.org/10.1021/jf104751v
Liu, Y., Lai, Q., Göker, M., Meier-Kolthoff, J.P., Wang, M., Sun, Y., Wang, L., Shao, Z., 2015. Genomic insights into the taxonomic status of the Bacillus cereus group. Sci Rep 5, 14082. https://doi.org/10.1038/srep14082
Lohans, C.T., Towle, K.M., Miskolzie, M., McKay, R.T., van Belkum, M.J., McMullen, L.M., Vederas, J.C., 2013. Solution structures of the linear leaderless bacteriocins enterocin 7A and 7B resemble carnocyclin A, a circular antimicrobial peptide. Biochemistry 52, 3987–3994. https://doi.org/10.1021/bi400359z
Lozano, J.C., 1974. Bacterial Blight of Cassava in Colombia: Epidemiology and Control. Phytopathology 64, 83. https://doi.org/10.1094/Phyto-64-83
Ma, B., Hibbing, M.E., Kim, H.-S., Reedy, R.M., Yedidia, I., Breuer, Jane, Breuer, Jeffrey, Glasner, J.D., Perna, N.T., Kelman, A., Charkowski, A.O., 2007. Host Range and Molecular Phylogenies of the Soft Rot Enterobacterial Genera *Pectobacterium* and *Dickeya*. Phytopathology® 97, 1150–1163. https://doi.org/10.1094/PHYTO-97-9-1150
Mabood, F., Zhou, X., Smith, D.L., 2014. Microbial signaling and plant growth promotion. Can. J. Plant Sci. 94, 1051–1063. https://doi.org/10.4141/cjps2013-148
Mahmud, K., Makaju, S., Ibrahim, R., Missaoui, A., 2020. Current Progress in Nitrogen Fixing Plants and Microbiome Research. Plants 9, 97. https://doi.org/10.3390/plants9010097
Makarova, K.S., Wolf, Y.I., Karamycheva, S., Zhang, D., Aravind, L., Koonin, E.V., 2019. Antimicrobial Peptides, Polymorphic Toxins, and Self-Nonself Recognition Systems in Archaea: an Untapped Armory for Intermicrobial Conflicts. mBio 10, e00715-19. https://doi.org/10.1128/mBio.00715-19
Maksimov, M.O., Pan, S.J., James Link, A., 2012. Lasso peptides: structure, function, biosynthesis, and engineering. Nat Prod Rep 29, 996–1006. https://doi.org/10.1039/c2np20070h
Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G., Foster, G.D., 2012. Top 10 plant pathogenic bacteria in molecular plant pathology: Top 10 plant pathogenic bacteria. Molecular Plant Pathology 13, 614–629. https://doi.org/10.1111/j.1364-3703.2012.00804.x
Masuda, Y., Zendo, T., Sonomoto, K., 2012. New type non-lantibiotic bacteriocins: circular and leaderless bacteriocins. Benef Microbes 3, 3–12. https://doi.org/10.3920/BM2011.0047
Mathur, H., Field, D., Rea, M.C., Cotter, P.D., Hill, C., Ross, R.P., 2018. Fighting biofilms with lantibiotics and other groups of bacteriocins. npj Biofilms Microbiomes 4, 9. https://doi.org/10.1038/s41522-018-0053-6
Mathur, H., Field, D., Rea, M.C., Cotter, P.D., Hill, C., Ross, R.P., 2017. Bacteriocin-Antimicrobial Synergy: A Medical and Food Perspective. Front. Microbiol. 8, 1205. https://doi.org/10.3389/fmicb.2017.01205
Mathur, H., Rea, M., Cotter, P., Hill, C., Ross, R., 2015. The Sactibiotic Subclass of Bacteriocins: An Update. CPPS 16, 549–558. https://doi.org/10.2174/1389203716666150515124831
McCann, H.C., Rikkerink, E.H.A., Bertels, F., Fiers, M., Lu, A., Rees-George, J., Andersen, M.T., Gleave, A.P., Haubold, B., Wohlers, M.W., Guttman, D.S., Wang, P.W., Straub, C., Vanneste,

J., Rainey, P.B., Templeton, M.D., 2013. Genomic Analysis of the Kiwifruit Pathogen Pseudomonas syringae pv. actinidiae Provides Insight into the Origins of an Emergent Plant Disease. PLoS Pathog 9, e1003503. https://doi.org/10.1371/journal.ppat.1003503

Melby, J.O., Nard, N.J., Mitchell, D.A., 2011. Thiazole/oxazole-modified microcins: complex natural products from ribosomal templates. Curr Opin Chem Biol 15, 369–378. https://doi.org/10.1016/j.cbpa.2011.02.027

Meng, Q.X., Jiang, H.H., Hanson, L.E., Hao, J.J., 2012. Characterizing a novel strain of *Bacillus amyloliquefaciens* BAC03 for potential biological control application. J Appl Microbiol 113, 1165–1175. https://doi.org/10.1111/j.1365-2672.2012.05420.x

Metelev, M., Tietz, J.I., Melby, J.O., Blair, P.M., Zhu, L., Livnat, I., Severinov, K., Mitchell, D.A., 2015. Structure, Bioactivity, and Resistance Mechanism of Streptomonomicin, an Unusual Lasso Peptide from an Understudied Halophilic Actinomycete. Chemistry & Biology 22, 241–250. https://doi.org/10.1016/j.chembiol.2014.11.017

Mew, T.W., 1987. Current Status and Future Prospects of Research on Bacterial Blight of Rice. Annu. Rev. Phytopathol. 25, 359–382. https://doi.org/10.1146/annurev.py.25.090187.002043

Michel-Briand, Y., Baysse, C., 2002. The pyocins of Pseudomonas aeruginosa. Biochimie 84, 499–510. https://doi.org/10.1016/s0300-9084(02)01422-0

Michielse, C.B., J Hooykaas, P.J., J J van den Hondel, C.A.M., J Ram, A.F., 2008. Agrobacterium-mediated transformation of the filamentous fungus Aspergillus awamori. Nat Protoc 3, 1671–1678. https://doi.org/10.1038/nprot.2008.154

Mojgani, N., 2017. Bacteriocin-Producing Rhizosphere Bacteria and Their Potential as a Biocontrol Agent, in: Mehnaz, S. (Ed.), Rhizotrophs: Plant Growth Promotion to Bioremediation. Springer Singapore, Singapore, pp. 165–181. https://doi.org/10.1007/978-981-10-4862-3_8

Moll, G., Hildeng-Hauge, H., Nissen-Meyer, J., Nes, I.F., Konings, W.N., Driessen, A.J.M., 1998. Mechanistic Properties of the Two-Component Bacteriocin Lactococcin G. J Bacteriol 180, 96–99. https://doi.org/10.1128/JB.180.1.96-99.1998

Montalbán-López, M., Sánchez-Hidalgo, M., Cebrián, R., Maqueda, M., 2012. Discovering the Bacterial Circular Proteins: Bacteriocins, Cyanobactins, and Pilins. Journal of Biological Chemistry 287, 27007–27013. https://doi.org/10.1074/jbc.R112.354688

Mouloud, G., Daoud, H., Bassem, J., Laribi Atef, I., Hani, B., 2013. New Bacteriocin from Bacillus clausii StrainGM17: Purification, Characterization, and Biological Activity. Appl Biochem Biotechnol 171, 2186–2200. https://doi.org/10.1007/s12010-013-0489-3

Mulet, M., Lalucat, J., García-Valdés, E., 2010. DNA sequence-based analysis of the *Pseudomonas* species. Environmental Microbiology. https://doi.org/10.1111/j.1462-2920.2010.02181.x

Munita, J.M., Arias, C.A., 2016. Mechanisms of Antibiotic Resistance. Microbiol Spectr 4, 4.2.15. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Nakamura, S., Chikaike, T., Yonehara, H., Umezawa, H., 1965. STRUCTURES OF BOTTROMYCINS A AND B. J Antibiot (Tokyo) 18, 60–61.

Nakayama, K., Takashima, K., Ishihara, H., Shinomiya, T., Kageyama, M., Kanaya, S., Ohnishi, M., Murata, T., Mori, H., Hayashi, T., 2000. The R-type pyocin of Pseudomonas aeruginosa is related to P2 phage, and the F-type is related to lambda phage. Mol Microbiol 38, 213–231. https://doi.org/10.1046/j.1365-2958.2000.02135.x

Nazarov, P.A., Baleev, D.N., Ivanova, M.I., Sokolova, L.M., Karakozova, M.V., 2020. Infectious plant diseases: etiology, current status, problems and prospects in plant protection. Acta Naturae 12, 46–59. https://doi.org/10.32607/actanaturae.11026

Nelson, S., 2009. Bacterial Leaf Blight of Aglaonema. University of Hawai‘i at Manoa 1–3.

Ng, K.L., Haddad, P.R., 2000. ACIDS | Liquid Chromatography, in: Encyclopedia of Separation Science. Elsevier, pp. 1854–1862. https://doi.org/10.1016/B0-12-226770-2/01401-0

Nilsen, T., Nes, I.F., Holo, H., 2003. Enterolysin A, a cell wall-degrading bacteriocin from Enterococcus faecalis LMG 2333. Appl Environ Microbiol 69, 2975–2984. https://doi.org/10.1128/aem.69.5.2975-2984.2003

Nissen-Meyer, J., Nes, I.F., 1997. Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. Arch Microbiol 167, 67–77.
Nissen-Meyer, J., Oppegård, C., Rogne, P., Haugen, H.S., Kristiansen, P.E., 2010. Structure and Mode-of-Action of the Two-Peptide (Class-IIb) Bacteriocins. Probiotics Antimicrob Proteins 2, 52–60. https://doi.org/10.1007/s12602-009-9021-z
Noda, T., Kaku, H., 1999. Growth of Xanthomonas oryzae pv. oryzae In Planta and in Guttation Fluid of Rice. Jpn. J. Phytopathol. 65, 9–14. https://doi.org/10.3186/jjphytopath.65.9
Norelli, J.L., Jones, A.L., Aldwinckle, H.S., 2003. Fire Blight Management in the Twenty-first Century: Using New Technologies that Enhance Host Resistance in Apple. Plant Disease 87, 756–765. https://doi.org/10.1094/PDIS.2003.87.7.756
Norris, G.E., Patchett, M.L., 2016. The glycocins: in a class of their own. Current Opinion in Structural Biology 40, 112–119. https://doi.org/10.1016/j.sbi.2016.09.003
Nowak, R.S., Caldwell, M.M., 1984. A test of compensatory photosynthesis in the field: Implications for herbivory tolerance. Oecologia 61, 311–318. https://doi.org/10.1007/BF00379627
Nozue, K., Park, C.-J., Ronald, P.C., 2011. Quantitative Measurements of Xanthomonas Oryzae pv. Oryzae Distribution in Rice Using Fluorescent-Labeling. J. Plant Biol. 54, 269–274. https://doi.org/10.1007/s12374-011-9164-9
Oman, T.J., Boettcher, J.M., Wang, H., Okalibe, X.N., van der Donk, W.A., 2011. Sublancin is not a lantibiotic but an S-linked glycopeptide. Nat Chem Biol 7, 78–80. https://doi.org/10.1038/nchembio.509
Ongey, E.L., Neubauer, P., 2016. Lanthipeptides: chemical synthesis versus in vivo biosynthesis as tools for pharmaceutical production. Microb Cell Fact 15, 97. https://doi.org/10.1186/s12934-016-0502-y
Oscáriz, J.C., Lasa, I., Pisabarro, A.G., 1999. Detection and characterization of cerein 7, a new bacteriocin produced by *Bacillus cereus* with a broad spectrum of activity. FEMS Microbiology Letters 178, 337–341. https://doi.org/10.1111/j.1574-6968.1999.tb08696.x
Oscáriz, J.C., Pisabarro, A.G., 2001. Classification and mode of action of membrane-active bacteriocins produced by gram-positive bacteria. Int Microbiol 4, 13–19. https://doi.org/10.1007/s101230100003
Ovchinnikov, K.V., Chi, H., Mehmeti, I., Holo, H., Nes, I.F., Diep, D.B., 2016. Novel Group of Leaderless Multipeptide Bacteriocins from Gram-Positive Bacteria. Appl Environ Microbiol 82, 5216–5224. https://doi.org/10.1128/AEM.01094-16
Padda, K.P., Puri, A., Chanway, C., 2019. Endophytic nitrogen fixation – a possible 'hidden' source of nitrogen for lodgepole pine trees growing at unreclaimed gravel mining sites. FEMS Microbiology Ecology 95, fiz172. https://doi.org/10.1093/femsec/fiz172
Pag, U., Sahl, H.-G., 2002. Multiple activities in lantibiotics--models for the design of novel antibiotics? Curr Pharm Des 8, 815–833. https://doi.org/10.2174/1381612023395439
Papo, N., Shai, Y., 2005. Host defense peptides as new weapons in cancer treatment. CMLS, Cell. Mol. Life Sci. 62, 784–790. https://doi.org/10.1007/s00018-005-4560-2
Parret, A.H.A., Schoofs, G., Proost, P., De Mot, R., 2003. Plant Lectin-Like Bacteriocin from a Rhizosphere-Colonizing *Pseudomonas* Isolate. J Bacteriol 185, 897–908. https://doi.org/10.1128/JB.185.3.897-908.2003
Parret, A.H.A., Temmerman, K., De Mot, R., 2005. Novel Lectin-Like Bacteriocins of Biocontrol Strain *Pseudomonas fluorescens* Pf-5. Appl Environ Microbiol 71, 5197–5207. https://doi.org/10.1128/AEM.71.9.5197-5207.2005
Paulin, J.P., 2000. *Erwinia amylovora* : general characteristics, biochemistry and serology., in: Vanneste, J.L. (Ed.), Fire Blight: The Disease and Its Causative Agent, Erwinia Amylovora. CABI Publishing, UK, pp. 87–115. https://doi.org/10.1079/9780851992945.0087
Perez, R.H., Zendo, T., Sonomoto, K., 2014. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microb Cell Fact 13, 1–13. https://doi.org/10.1186/1475-2859-13-S1-S3

Pieterse, R., Todorov, S.D., 2010. Bacteriocins: exploring alternatives to antibiotics in mastitis treatment. Braz. J. Microbiol. 41, 542–562. https://doi.org/10.1590/S1517-83822010000300003

Podile, A.R., Kishore, G.K., 2006. Plant growth-promoting rhizobacteria, in: Gnanamanickam, S.S. (Ed.), Plant-Associated Bacteria. Springer Netherlands, Dordrecht, pp. 195–230. https://doi.org/10.1007/978-1-4020-4538-7_6

Preciado, G.M., Michel, M.M., Villarreal-Morales, S.L., Flores-Gallegos, A.C., Aguirre-Joya, J., Morlett-Chávez, J., Aguilar, C.N., Rodríguez-Herrera, R., 2016. Bacteriocins and Its Use for Multidrug-Resistant Bacteria Control, in: Antibiotic Resistance. Elsevier, pp. 329–349. https://doi.org/10.1016/B978-0-12-803642-6.00016-2

Prudent, M., Salon, C., Souleimanov, A., Emery, R.J.N., Smith, D.L., 2015. Soybean is less impacted by water stress using Bradyrhizobium japonicum and thuricin-17 from Bacillus thuringiensis. Agron. Sustain. Dev. 35, 749–757. https://doi.org/10.1007/s13593-014-0256-z

Rammelsberg, M., M  ller, E., Radler, F., 1990. Caseicin 80: purification and characterization of a new bacteriocin from Lactobacillus casei. Arch. Microbiol. 154, 249–252. https://doi.org/10.1007/BF00248963

Rather, I.A., Galope, R., Bajpai, V.K., Lim, J., Paek, W.K., Park, Y.-H., 2017. Diversity of Marine Bacteria and Their Bacteriocins: Applications in Aquaculture. Reviews in Fisheries Science & Aquaculture 25, 257–269. https://doi.org/10.1080/23308249.2017.1282417

Redak, R.A., Purcell, A.H., Lopes, J.R.S., Blua, M.J., Mizell III, R.F., Andersen, P.C., 2004. T HE B IOLOGY OF X YLEM F LUID –F EEDING I NSECT V ECTORS OF *X YLELLA FASTIDIOSA* AND T HEIR R ELATION TO D ISEASE E PIDEMIOLOGY. Annu. Rev. Entomol. 49, 243–270. https://doi.org/10.1146/annurev.ento.49.061802.123403

Ren, H., Biswas, S., Ho, S., van der Donk, W.A., Zhao, H., 2018. Rapid Discovery of Glycocins through Pathway Refactoring in *Escherichia coli*. ACS Chem. Biol. 13, 2966–2972. https://doi.org/10.1021/acschembio.8b00599

Riley, M.A., Wertz, J.E., 2002. Bacteriocins: evolution, ecology, and application. Annu Rev Microbiol 56, 117–137. https://doi.org/10.1146/annurev.micro.56.012302.161024

Rodríguez, J.M., Martínez, M.I., Kok, J., 2002. Pediocin PA-1, a Wide-Spectrum Bacteriocin from Lactic Acid Bacteria. Critical Reviews in Food Science and Nutrition 42, 91–121. https://doi.org/10.1080/10408690290825475

Rosenberg, I.M., 1996. Protein Analysis and Purification. Birkhäuser Boston, Boston, MA. https://doi.org/10.1007/978-1-4612-2056-5

Ryan, R.P., Vorhölter, F.-J., Potnis, N., Jones, J.B., Van Sluys, M.-A., Bogdanove, A.J., Dow, J.M., 2011. Pathogenomics of Xanthomonas: understanding bacterium–plant interactions. Nat Rev Microbiol 9, 344–355. https://doi.org/10.1038/nrmicro2558

Samson, R., Legendre, J.B., Christen, R., Saux, M.F.-L., Achouak, W., Gardan, L., 2005. Transfer of Pectobacterium chrysanthemi (Burkholder et al. 1953) Brenner et al. 1973 and Brenneria paradisiaca to the genus Dickeya gen. nov. as Dickeya chrysanthemi comb. nov. and Dickeya paradisiaca comb. nov. and delineation of four novel species, Dickeya dadantii sp. nov., Dickeya dianthicola sp. nov., Dickeya dieffenbachiae sp. nov. and Dickeya zeae sp. nov. International Journal of Systematic and Evolutionary Microbiology 55, 1415–1427. https://doi.org/10.1099/ijs.0.02791-0

Sánchez-Barrena, M.J., Martínez-Ripoll, M., Gálvez, A., Valdivia, E., Maqueda, M., Cruz, V., Albert, A., 2003. Structure of Bacteriocin AS-48: From Soluble State to Membrane Bound State. Journal of Molecular Biology 334, 541–549. https://doi.org/10.1016/j.jmb.2003.09.060

Santoyo, G., Moreno-Hagelsieb, G., Del Carmen Orozco-Mosqueda, Ma., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiological Research 183, 92–99. https://doi.org/10.1016/j.micres.2015.11.008

Sarwar, A., Latif, Z., Cabaleiro, C., Amin, A., Saleem, M.A., 2019. First Report of *Streptomyces europaeiscabiei* Causing Potato Common Scab in Galicia, Spain. Plant Disease 103, 1407–1407. https://doi.org/10.1094/PDIS-08-18-1397-PDN

Schägger, H., 2006. Tricine–SDS-PAGE. Nat Protoc 1, 16–22. https://doi.org/10.1038/nprot.2006.4
Schillinger, U., L�cke, F.K., 1989. Antibacterial activity of Lactobacillus sake isolated from meat. Appl Environ Microbiol 55, 1901–1906. https://doi.org/10.1128/aem.55.8.1901-1906.1989
Schindler, C.A., Schuhardt, V.T., 1964. LYSOSTAPHIN: A NEW BACTERIOLYTIC AGENT FOR THE STAPHYLOCOCCUS. Proc Natl Acad Sci U S A 51, 414–421. https://doi.org/10.1073/pnas.51.3.414
Scholl, D., 2017. Phage Tail-Like Bacteriocins. Annu Rev Virol 4, 453–467. https://doi.org/10.1146/annurev-virology-101416-041632
Scholz, R., Vater, J., Budiharjo, A., Wang, Z., He, Y., Dietel, K., Schwecke, T., Herfort, S., Lasch, P., Borriss, R., 2014. Amylocyclicin, a Novel Circular Bacteriocin Produced by Bacillus amyloliquefaciens FZB42. J Bacteriol 196, 1842–1852. https://doi.org/10.1128/JB.01474-14
Silkin, L., Hamza, S., Kaufman, S., Cobb, S.L., Vederas, J.C., 2008. Spermicidal bacteriocins: lacticin 3147 and subtilosin A. Bioorg Med Chem Lett 18, 3103–3106. https://doi.org/10.1016/j.bmcl.2007.11.024
Simmonds, R.S., Pearson, L., Kennedy, R.C., Tagg, J.R., 1996. Mode of action of a lysostaphin-like bacteriolytic agent produced by Streptococcus zooepidemicus 4881. Appl Environ Microbiol 62, 4536–4541. https://doi.org/10.1128/AEM.62.12.4536-4541.1996
Smith, E.F., Townsend, C.O., 1907. A Plant-Tumor of Bacterial Origin. Science 25, 671–673. https://doi.org/10.1126/science.25.643.671
Sobrino-López, A., Martín-Belloso, O., 2008. Use of nisin and other bacteriocins for preservation of dairy products. International Dairy Journal 18, 329–343. https://doi.org/10.1016/j.idairyj.2007.11.009
Soltani, S., Hammami, R., Cotter, P.D., Rebuffat, S., Said, L.B., Gaudreau, H., Bédard, F., Biron, E., Drider, D., Fliss, I., 2021. Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations. FEMS Microbiology Reviews 45, fuaa039. https://doi.org/10.1093/femsre/fuaa039
Spence, C.A., Raman, V., Donofrio, N.M., Bais, H.P., 2014. Global gene expression in rice blast pathogen Magnaporthe oryzae treated with a natural rice soil isolate. Planta 239, 171–185. https://doi.org/10.1007/s00425-013-1974-1
St-Onge, R., Gadkar, V.J., Arseneault, T., Goyer, C., Filion, M., 2011. The ability of Pseudomonas sp. LBUM 223 to produce phenazine-1-carboxylic acid affects the growth of Streptomyces scabies, the expression of thaxtomin biosynthesis genes and the biological control potential against common scab of potato: Effect of PCA production ability on S. scabies. FEMS Microbiology Ecology 75, 173–183. https://doi.org/10.1111/j.1574-6941.2010.00992.x
Subramanian, S., Ricci, E., Souleimanov, A., Smith, D.L., 2016. A Proteomic Approach to Lipo-Chitooligosaccharide and Thuricin 17 Effects on Soybean GerminationUnstressed and Salt Stress. PLoS ONE 11, e0160660. https://doi.org/10.1371/journal.pone.0160660
Subramanian, S., Smith, D.L., 2015. Bacteriocins from the rhizosphere microbiome – from an agriculture perspective. Front. Plant Sci. 6. https://doi.org/10.3389/fpls.2015.00909
Suda, S., Cotter, P.D., Hill, C., Ross, R.P., 2012. Lacticin 3147--biosynthesis, molecular analysis, immunity, bioengineering and applications. Curr Protein Pept Sci 13, 193–204. https://doi.org/10.2174/138920312800785021
Sun, Z., Wang, X., Zhang, X., Wu, H., Zou, Y., Li, P., Sun, C., Xu, W., Liu, F., Wang, D., 2018. Class III bacteriocin Helveticin-M causes sublethal damage on target cells through impairment of cell wall and membrane. J Ind Microbiol Biotechnol 45, 213–227. https://doi.org/10.1007/s10295-018-2008-6
Swetwiwathana, A., Visessanguan, W., 2015. Potential of bacteriocin-producing lactic acid bacteria for safety improvements of traditional Thai fermented meat and human health. Meat Sci 109, 101–105. https://doi.org/10.1016/j.meatsci.2015.05.030
Swings, J., Van Den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T.W., Kersters, K., 1990. Reclassification of the Causal Agents of Bacterial Blight (Xanthomonas campestris pv. oryzae) and Bacterial Leaf Streak (Xanthomonas campestris pv. oryzicola) of Rice as Pathovars of

Xanthomonas oryzae (ex Ishiyama 1922) sp. nov., nom. rev. International Journal of Systematic Bacteriology 40, 309–311. https://doi.org/10.1099/00207713-40-3-309
Tabata, A., Yamada, T., Ohtani, H., Ohkura, K., Tomoyasu, T., Nagamune, H., 2019. β-Hemolytic Streptococcus anginosus subsp. anginosus causes streptolysin S-dependent cytotoxicity to human cell culture lines in vitro. J Oral Microbiol 11, 1609839. https://doi.org/10.1080/20002297.2019.1609839
Tan, S., Moore, G., Nodwell, J., 2019. Put a Bow on It: Knotted Antibiotics Take Center Stage. Antibiotics 8, 117. https://doi.org/10.3390/antibiotics8030117
Thokchom, S., Joshi, S., 2012. Probiotic and Bacteriocin Efficacy of lactic Acid Bacteria from Traditionally Fermented Foods: A Review. Assam University Journal of Science and Technology 10, 142–155.
Thomson, S.V., 2000. Epidemiology of fire blight., in: Vanneste, J.L. (Ed.), Fire Blight: The Disease and Its Causative Agent, Erwinia Amylovora. CABI Publishing, UK, pp. 9–36. https://doi.org/10.1079/9780851992945.0009
Tong, Z., Zhang, Y., Ling, J., Ma, J., Huang, L., Zhang, L., 2014. An In Vitro Study on the Effects of Nisin on the Antibacterial Activities of 18 Antibiotics against Enterococcus faecalis. PLoS ONE 9, e89209. https://doi.org/10.1371/journal.pone.0089209
Torreblanca, M., Meseguer, I., Ventosa, A., 1994. Production of halocin is a practically universal feature of archaeal halophilic rods. Lett Appl Microbiol 19, 201–205. https://doi.org/10.1111/j.1472-765X.1994.tb00943.x
Toth, I.K., van der Wolf, J.M., Saddler, G., Lojkowska, E., Hélias, V., Pirhonen, M., Tsror Lahkim, L., Elphinstone, J.G., 2011. Dickeya species: an emerging problem for potato production in Europe: Dickeya spp. on potato in Europe. Plant Pathology 60, 385–399. https://doi.org/10.1111/j.1365-3059.2011.02427.x
Tracanna, V., de Jong, A., Medema, M.H., Kuipers, O.P., 2017. Mining prokaryotes for antimicrobial compounds: from diversity to function. FEMS Microbiol Rev 41, 417–429. https://doi.org/10.1093/femsre/fux014
Tymoszewska, A., Diep, D.B., Wirtek, P., Aleksandrzak-Piekarczyk, T., 2017. The Non-Lantibiotic Bacteriocin Garvicin Q Targets Man-PTS in a Broad Spectrum of Sensitive Bacterial Genera. Sci Rep 7, 8359. https://doi.org/10.1038/s41598-017-09102-7
Varijakshapanicker, P., Mckune, S., Miller, L., Hendrickx, S., Balehegn, M., Dahl, G.E., Adesogan, A.T., 2019. Corrigendum to: Sustainable livestock systems to improve human health, nutrition, and economic status. Animal Frontiers vfz043. https://doi.org/10.1093/af/vfz043
Verdier, V., Restrepo, S., Mosquera, G., Jorge, V., Lopez, C., 2004. Recent progress in the characterization of molecular determinants in the Xanthomonas axonopodis pv. manihotis–cassava interaction. Plant Mol Biol 56, 573–584. https://doi.org/10.1007/s11103-004-5044-8
Verdier, V., Vera Cruz, C., Leach, J.E., 2012. Controlling rice bacterial blight in Africa: Needs and prospects. Journal of Biotechnology 159, 320–328. https://doi.org/10.1016/j.jbiotec.2011.09.020
Vijay Simha, B., Sood, S.K., Kumariya, R., Garsa, A.K., 2012. Simple and rapid purification of pediocin PA-1 from Pediococcus pentosaceous NCDC 273 suitable for industrial application. Microbiological Research 167, 544–549. https://doi.org/10.1016/j.micres.2012.01.001
Villalobos-Delgado, L.H., Nevárez-Moorillon, G.V., Caro, I., Quinto, E.J., Mateo, J., 2019. Natural antimicrobial agents to improve foods shelf life, in: Food Quality and Shelf Life. Elsevier, pp. 125–157. https://doi.org/10.1016/B978-0-12-817190-5.00004-5
Waisvisz, J.M., van der Hoeven, M.G., van Peppen, J., Zwennis, W.C.M., 1957. Bottromycin. I. A New Sulfur-containing Antibiotic. J. Am. Chem. Soc. 79, 4520–4521. https://doi.org/10.1021/ja01573a072
Wang, S., Huang, S., Ye, Q., Zeng, X., Yu, H., Qi, D., Qiao, S., 2018. Prevention of Cyclophosphamide-Induced Immunosuppression in Mice with the Antimicrobial Peptide Sublancin. Journal of Immunology Research 2018, 1–11. https://doi.org/10.1155/2018/4353580

Wanner, L.A., Kirk, W.W., Qu, X.S., 2014. Field efficacy of nonpathogenic *Streptomyces* species against potato common scab. J Appl Microbiol 116, 123–133. https://doi.org/10.1111/jam.12336

Wessel, D., Flügge, U.I., 1984. A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. Analytical Biochemistry 138, 141–143. https://doi.org/10.1016/0003-2697(84)90782-6

Wiebach, V., Mainz, A., Siegert, M.-A.J., Jungmann, N.A., Lesquame, G., Tirat, S., Dreux-Zigha, A., Aszodi, J., Le Beller, D., Süssmuth, R.D., 2018. The anti-staphylococcal lipolanthines are ribosomally synthesized lipopeptides. Nat Chem Biol 14, 652–654. https://doi.org/10.1038/s41589-018-0068-6

Wilson, C.R., Ransom, L.M., Pemberton, B.M., 1999. The Relative Importance of Seed-borne Inoculum to Common Scab Disease of Potato and the Efficacy of Seed Tuber and Soil Treatments for Disease Control. Journal of Phytopathology 147, 13–18. https://doi.org/10.1046/j.1439-0434.1999.147001013.x

Woraprayote, W., Malila, Y., Sorapukdee, S., Swetwiwathana, A., Benjakul, S., Visessanguan, W., 2016. Bacteriocins from lactic acid bacteria and their applications in meat and meat products. Meat Science 120, 118–132. https://doi.org/10.1016/j.meatsci.2016.04.004

Wulff, E.G., van Vuurde, J.W.L., Hockenhull, J., 2003. The ability of the biological control agent Bacillus subtilis, strain BB, to colonise vegetable brassicas endophytically following seed inoculation. Plant and Soil 255, 463–474. https://doi.org/10.1023/A:1026026917355

Yang, S.-C., Lin, C.-H., Sung, C.T., Fang, J.-Y., 2014. Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Front Microbiol 5, 241. https://doi.org/10.3389/fmicb.2014.00241

Yasmin, S., Zaka, A., Imran, A., Zahid, M.A., Yousaf, S., Rasul, G., Arif, M., Mirza, M.S., 2016. Plant Growth Promotion and Suppression of Bacterial Leaf Blight in Rice by Inoculated Bacteria. PLoS ONE 11, e0160688. https://doi.org/10.1371/journal.pone.0160688

Zacharof, M.P., Lovitt, R.W., 2012. Bacteriocins Produced by Lactic Acid Bacteria a Review Article. APCBEE Procedia 2, 50–56. https://doi.org/10.1016/j.apcbee.2012.06.010

Zadoks, R.N., Middleton, J.R., McDougall, S., Katholm, J., Schukken, Y.H., 2011. Molecular Epidemiology of Mastitis Pathogens of Dairy Cattle and Comparative Relevance to Humans. J Mammary Gland Biol Neoplasia 16, 357–372. https://doi.org/10.1007/s10911-011-9236-y

Zanetti, M., Carniel, T.K., Dalcanton, F., dos Anjos, R.S., Gracher Riella, H., de Araújo, P.H.H., de Oliveira, D., Antônio Fiori, M., 2018. Use of encapsulated natural compounds as antimicrobial additives in food packaging: A brief review. Trends in Food Science & Technology 81, 51–60. https://doi.org/10.1016/j.tifs.2018.09.003

Zasloff, M., 1987. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc. Natl. Acad. Sci. U.S.A. 84, 5449–5453. https://doi.org/10.1073/pnas.84.15.5449

Zhang, D., Xu, H., Gao, J., Portieles, R., Du, L., Gao, X., Borroto Nordelo, C., Borrás-Hidalgo, O., 2021. Endophytic Bacillus altitudinis Strain Uses Different Novelty Molecular Pathways to Enhance Plant Growth. Front. Microbiol. 12, 692313. https://doi.org/10.3389/fmicb.2021.692313

Zhang, J., Liu, G., Shang, N., Cheng, W., Chen, S., Li, P., 2009. Purification and Partial Amino Acid Sequence of Pentocin 31-1, an Anti-Listeria Bacteriocin Produced by Lactobacillus pentosus 31-1. Journal of Food Protection 72, 2524–2529. https://doi.org/10.4315/0362-028X-72.12.2524

Zhao, Yu-qiang, Tian, Y., Wang, L., Geng, G., Zhao, W., Hu, B., Zhao, You-fu, 2019. Fire blight disease, a fast-approaching threat to apple and pear production in China. Journal of Integrative Agriculture 18, 815–820. https://doi.org/10.1016/S2095-3119(18)62033-7

SUPPLEMENTS

Supplement 1

Table 1 Bacteria isolated from the soil.

Sample gathering place	Strain name	Growth temperature	Growth medium (time)
Potato field	BL1	30 °C	10×NB (48 h), LB (24 h)
	BL2	30 °C	10×NB (48 h), LB (24 h)
	BL3	30 °C	10×NB (48 h), LB (24 h)
	BL4	30 °C	BHI (48 h), LB (24 h)
	BL5	30 °C	BHI (48 h), LB (24 h)
	BL6	30 °C	BHI (48 h), LB (24 h)
	BL7	30 °C	BHI (48 h), LB (24 h)
	BL8	30 °C	BHI (48 h), LB (24 h)
	BL9	30 °C	BHI (48 h), LB (24 h)
	BL10	30 °C	BHI (48 h), LB (24 h)
	BL11	30 °C	BHI (48 h), LB (24 h)
	BL12	30 °C	BHI (48 h), LB (24 h)
	BL13	30 °C	BHI (48 h), LB (24 h)
	BL14	30 °C	BHI (48 h), LB (24 h)
	BL15	30 °C	NB (48 h), LB (24 h)
	BL16	30 °C	NB (48 h), LB (24 h)
	BL17	30 °C	NB (48 h), LB (24 h)
	BL18	30 °C	NB (48 h), LB (24 h)
	BL19	30 °C	NB (48 h), LB (24 h)
	BL20	30 °C	NB (48 h), LB (24 h)
	BL21	30 °C	NB (48 h), LB (24 h)
	BL22	30 °C	NB (48 h), LB (24 h)
	BL23	30 °C	NB (48 h), LB (24 h)
	BL24	30 °C	NB (48 h), LB (24 h)
	BL25	30 °C	BHI (48 h), LB (24 h)
	BL26	30 °C	BHI (48 h), LB (24 h)
	BL27	30 °C	BHI (48 h), LB (24 h)
	BL28	30 °C	BHI (48 h), LB (24 h)
	BL29	30 °C	BHI (48 h), LB (24 h)
Rye field	RL1	30 °C	BHI (48 h), LB (24 h)
	RL2	30 °C	BHI (48 h), LB (24 h)
	RL3	30 °C	BHI (48 h), LB (24 h)
	RL4	30 °C	BHI (48 h), LB (24 h)
	RL5	30 °C	NB (48 h), LB (24 h)
	RL6	30 °C	10×NB (48 h), LB (24 h)
	RL7	30 °C	10×NB (48 h), LB (24 h)
	RL8	30 °C	10×NB (48 h), LB (24 h)
	RL9	30 °C	NB (48 h), LB (24 h)
	RL10	30 °C	NB (48 h), LB (24 h)
	RL11	30 °C	NB (48 h), LB (24 h)
Strawberry field	BR1	30 °C	10×NB (48 h), LB (24 h)
	BR2	30 °C	10×NB (48 h), LB (24 h)
	BR3	30 °C	BHI (48 h), LB (24 h)
	BR4	30 °C	BHI (48 h), LB (24 h)
	BR5	30 °C	BHI (48 h), LB (24 h)
	BR6	30 °C	NB (48 h), LB (24 h)
	BR7	30 °C	NB (48 h), LB (24 h)
	BR8	30 °C	NB (48 h), LB (24 h)
	BR9	30 °C	BHI (48 h), LB (24 h)
	BR10	30 °C	BHI (48 h), LB (24 h)
	BR11	30 °C	BHI (48 h), LB (24 h)

Supplement 2

Table 2 List of hits for BL1 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Bacillus toyonensis*	BCT-7112	CP006863	99.30362117	10/1436	100
2	*Bacillus mobilis*	0711P9-1	MACF01000036	99.30362117	10/1436	100
3	*Bacillus pacificus*	EB422	KJ812450	99.23398329	11/1436	100
4	*Bacillus wiedmannii*	FSL W8-0169	LOBC01000053	99.09470752	13/1436	100
5	*Bacillus albus*	N35-10-2	MAOE01000087	99.09470752	13/1436	100
6	*Bacillus luti*	TD41	MACI01000041	99.09470752	13/1436	100
7	*Bacillus cereus*	ATCC 14579	AE016877	99.02506964	14/1436	100
8	*Bacillus proteolyticus*	TD42	MACH01000033	99.02506964	14/1436	100
9	*Bacillus tropicus*	N24	MACG01000025	99.02506964	14/1436	100
10	*Bacillus fungorum*	17-SMS-01	MG601116	99.02506964	14/1436	100
11	*Bacillus paramycoides*	NH24A2	MAOI01000012	98.95543175	15/1436	100
12	*Bacillus paranthracis*	Mn5	MACE01000012	98.95543175	15/1436	100
13	*Bacillus nitratireducens*	4049	KJ812430	98.95543175	15/1436	100
14	*Bacillus anthracis*	Ames	AE016879	98.88579387	16/1436	100
15	*Bacillus mycoides*	DSM 2048	ACMU01000002	98.67688022	19/1436	100
16	*Bacillus pseudomycoides*	DSM 12442	ACMX01000133	98.60724234	20/1436	100
17	*Bacillus cytotoxicus*	NVH 391-98	CP000764	97.1448468	41/1436	100
18	*Rossellomorea oryzaecorticis*	R1	KF548480	95.92760181	45/1105	75.62797013
19	*Rossellomorea marisflavi*	JCM 11544	LGUE01000011	94.90934449	73/1434	100
20	*Bacillus coahuilensis*	m4-4	ABFU01000135	94.85915493	73/1420	100
21	*Gottfriedia luciferensis*	LMG 18422	AJ419629	94.83240223	74/1432	100
22	*Bacillus seohaeanensis*	BH724	AY667495	94.77825465	73/1398	95.11864407
23	*Priestia taiwanensis*	FJAT-14571	KF040588	94.74421864	75/1427	97.899729
24	*Bacillus acidicola*	105-2	AF547209	94.70383275	76/1435	100
25	*Sutcliffiella catenulatus*	18C	LT617055	94.70383275	76/1435	98.7109905
26	*Bacillus tianshenii*	YIM M13235	KF811034	94.69644103	76/1433	100
27	*Cytobacillus purgationiresistens*	DS22	FR666703	94.64411557	76/1419	97.48811948
28	*Metabacillus herbersteinensis*	D-1-5a	AJ781029	94.62290503	77/1432	99.4568907
29	*Bacillus salacetis*	SKP7-4	LC367333	94.58689459	76/1404	97.01492537
30	*Sutcliffiella halmapala*	DSM 8723	KV917375	94.55687369	78/1433	100

Table 3 List of hits for BL5 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Bacillus velezensis*	CR-502	AY603658	99.7111913	4/1385	95.3804348
2	*Bacillus tequilensis*	KCTC 13622	AYTO01000043	99.5804196	6/1430	100
3	*Bacillus cabrialesii*	TE3	MK462260	99.5804196	6/1430	100
4	*Bacillus inaquosorum*	KCTC 13429	AMXN01000021	99.5804196	6/1430	100
5	*Bacillus subtilis*	NCIB 3610	ABQL01000001	99.5104895	7/1430	100
6	*Bacillus halotolerans*	ATCC 25096	LPVF01000003	99.4405594	8/1430	100
7	*Bacillus stercoris*	JCM 30051	MN536904	99.4405594	8/1430	100
8	*Bacillus spizizenii*	NRRL B-23049	CP002905	99.4405594	8/1430	100
9	*Bacillus mojavensis*	RO-H-1	JH600280	99.3706294	9/1430	100
10	*Bacillus nakamurai*	NRRL B-41091	LSAZ01000028	99.3706294	9/1430	100
11	*Bacillus vallismortis*	DV1-F-3	JH600273	99.2307692	11/1430	100
12	*Bacillus siamensis*	KCTC 13613	AJVF01000043	99.2307692	11/1430	100
13	*Bacillus atrophaeus*	JCM 9070	AB021181	99.020979	14/1430	100
14	*Bacillus amyloliquefaciens*	DSM 7	FN597644	98.951049	15/1430	100
15	*Bacillus glycinifermentans*	GO-13	LECW01000063	98.3916084	23/1430	100
16	*Bacillus paralicheniformis*	KJ-16	KY694465	98.3216783	24/1430	100
17	*Bacillus haynesii*	NRRL B-41327	MRBL01000076	98.041958	28/1430	100
18	*Bacillus licheniformis*	ATCC 14580	AE017333	97.9020979	30/1430	100
19	*Bacillus sonorensis*	NBRC 101234	AYTN01000016	97.7622378	32/1430	100
20	*Bacillus swezeyi*	NRRL B-41294	MRBK01000096	97.6923077	33/1430	100
21	*Bacillus aerius*	24K	AJ831843	97.4089636	37/1428	100
22	*Bacillus altitudinis*	41KF2b	ASJC01000029	96.9209237	44/1429	100
23	*Bacillus xiamenensis*	HYC-10	AMSH01000114	96.8509447	45/1429	100
24	*Bacillus safensis subsp. safensis*	FO-36b	ASJD01000027	96.7109867	47/1429	100
25	*Bacillus safensis subsp. osmophilus*	BC09	KY990920	96.7109867	47/1429	99.932019
26	*Bacillus pumilus*	ATCC 7061	ABRX01000007	96.6410077	48/1429	100
27	*Bacillus zhangzhouensis*	DW5-4	JOTP01000061	96.6410077	48/1429	100
28	*Bacillus australimaris*	NH7I_1	JX680098	96.5710287	49/1429	100
29	*Bacillus salacetis*	SKP7-4	LC367333	96.2910128	52/1402	97.0149254
30	*Rossellomorea vietnamensis*	15-1	AB099708	96.0294118	54/1360	94.165536

Table 4 List of hits for BL11 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Bacillus toyonensis*	BCT-7112	CP006863	99.5070423	7/1420	100
2	*Bacillus mobilis*	0711P9-1	MACF01000036	99.5070423	7/1420	100
3	*Bacillus wiedmannii*	FSL W8-0169	LOBC01000053	99.4366197	8/1420	100
4	*Bacillus albus*	N35-10-2	MAOE01000087	99.4366197	8/1420	100
5	*Bacillus luti*	TD41	MACI01000041	99.4366197	8/1420	100
6	*Bacillus pacificus*	EB422	KJ812450	99.4366197	8/1420	100
7	*Bacillus cereus*	ATCC 14579	AE016877	99.3661972	9/1420	100
8	*Bacillus proteolyticus*	TD42	MACH01000033	99.3661972	9/1420	100
9	*Bacillus tropicus*	N24	MACG01000025	99.3661972	9/1420	100
10	*Bacillus fungorum*	17-SMS-01	MG601116	99.3661972	9/1420	100
11	*Bacillus paramycoides*	NH24A2	MAOI01000012	99.2957746	10/1420	100
12	*Bacillus paranthracis*	Mn5	MACE01000012	99.2957746	10/1420	100
13	*Bacillus nitratireducens*	4049	KJ812430	99.2957746	10/1420	100
14	*Bacillus anthracis*	Ames	AE016879	99.2253521	11/1420	100
15	*Bacillus mycoides*	DSM 2048	ACMU01000002	99.0140845	14/1420	100
16	*Bacillus pseudomycoides*	DSM 12442	ACMX01000133	98.943662	15/1420	100
17	*Bacillus cytotoxicus*	NVH 391-98	CP000764	97.3239437	38/1420	100
18	*Rossellomorea oryzaecorticis*	R1	KF548480	95.8333333	46/1104	75.6279701
19	*Rossellomorea marisflavi*	JCM 11544	LGUE01000011	95.2045134	68/1418	100
20	*Bacillus salacetis*	SKP7-4	LC367333	95.0323974	69/1389	97.0149254
21	*Bacillus acidicola*	105-2	AF547209	94.9964764	71/1419	100
22	*Bacillus tianshenii*	YIM M13235	KF811034	94.9894143	71/1417	100
23	*Gottfriedia luciferensis*	LMG 18422	AJ419629	94.9858757	71/1416	100
24	*Rossellomorea vietnamensis*	15-1	AB099708	94.9554896	68/1348	94.165536
25	*Sutcliffiella halmapala*	DSM 8723	KV917375	94.922426	72/1418	100
26	*Metabacillus herbersteinensis*	D-1-5a	AJ781029	94.9188426	72/1417	99.4568907
27	*Bacillus spongiae*	135PIL107-10	KY451772	94.9077491	69/1355	94.5233266
28	*Bacillus coahuilensis*	m4-4	ABFU01000135	94.9044586	72/1413	100
29	*Bacillus seohaeanensis*	BH724	AY667495	94.8460988	72/1397	95.1186441
30	*Gottfriedia acidiceleris*	CBD 119	DQ374637	94.8446328	73/1416	100

Table 5 List of hits for BL17 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Bacillus toyonensis*	BCT-7112	CP006863	99.8601399	2/1430	100
2	*Bacillus wiedmannii*	FSL W8-0169	LOBC01000053	99.8601399	2/1430	100
3	*Bacillus proteolyticus*	TD42	MACH01000033	99.7902098	3/1430	100
4	*Bacillus albus*	N35-10-2	MAOE01000087	99.7902098	3/1430	100
5	*Bacillus luti*	TD41	MACI01000041	99.7902098	3/1430	100
6	*Bacillus mobilis*	0711P9-1	MACF01000036	99.7902098	3/1430	100
7	*Bacillus pacificus*	EB422	KJ812450	99.7902098	3/1430	100
8	*Bacillus fungorum*	17-SMS-01	MG601116	99.7902098	3/1430	100
9	*Bacillus cereus*	ATCC 14579	AE016877	99.7202797	4/1430	100
10	*Bacillus paramycoides*	NH24A2	MAOI01000012	99.7202797	4/1430	100
11	*Bacillus tropicus*	N24	MACG01000025	99.7202797	4/1430	100
12	*Bacillus paranthracis*	Mn5	MACE01000012	99.6503497	5/1430	100
13	*Bacillus nitratireducens*	4049	KJ812430	99.6503497	5/1430	100
14	*Bacillus anthracis*	Ames	AE016879	99.5804196	6/1430	100
15	*Bacillus mycoides*	DSM 2048	ACMU01000002	99.4405594	8/1430	100
16	*Bacillus pseudomycoides*	DSM 12442	ACMX01000133	99.3006993	10/1430	100
17	*Bacillus cytotoxicus*	NVH 391-98	CP000764	97.7606718	32/1429	100
18	*Rossellomorea oryzaecorticis*	R1	KF548480	96.0909091	43/1100	75.6279701
19	*Bacillus coahuilensis*	m4-4	ABFU01000135	95.5445545	63/1414	100
20	*Rossellomorea marisflavi*	JCM 11544	LGUE01000011	95.5182073	64/1428	100
21	*Bacillus acidicola*	105-2	AF547209	95.3781513	66/1428	100
22	*Priestia flexa*	NBRC 15715	BCVD01000224	95.2414276	68/1429	100
23	*Bacillus tianshenii*	YIM M13235	KF811034	95.2347582	68/1427	100
24	*Gottfriedia luciferensis*	LMG 18422	AJ419629	95.1646811	69/1427	100
25	*Sutcliffiella halmapala*	DSM 8723	KV917375	95.1646811	69/1427	100
26	*Bacillus salacetis*	SKP7-4	LC367333	95.0106914	70/1403	97.0149254
27	*Ectobacillus panaciterrae*	Gsoil 1517	AB245380	94.9823322	71/1415	100
28	*Sutcliffiella cohnii*	NBRC 15565	BCUW01000190	94.9579832	72/1428	100
29	*Bacillus spongiae*	135PIL107-10	KY451772	94.9561404	69/1368	94.5233266
30	*Gottfriedia acidiceleris*	CBD 119	DQ374637	94.9509116	72/1426	100

Table 6 List of hits for BL25 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Bacillus toyonensis*	BCT-7112	CP006863	99.789916	3/1428	100
2	*Bacillus mobilis*	0711P9-1	MACF01000036	99.789916	3/1428	100
3	*Bacillus pacificus*	EB422	KJ812450	99.719888	4/1428	100
4	*Bacillus wiedmannii*	FSL W8-0169	LOBC01000053	99.5798319	6/1428	100
5	*Bacillus albus*	N35-10-2	MAOE01000087	99.5798319	6/1428	100
6	*Bacillus luti*	TD41	MACI01000041	99.5798319	6/1428	100
7	*Bacillus cereus*	ATCC 14579	AE016877	99.5098039	7/1428	100
8	*Bacillus proteolyticus*	TD42	MACH01000033	99.5098039	7/1428	100
9	*Bacillus tropicus*	N24	MACG01000025	99.5098039	7/1428	100
10	*Bacillus fungorum*	17-SMS-01	MG601116	99.5098039	7/1428	100
11	*Bacillus paramycoides*	NH24A2	MAOI01000012	99.4397759	8/1428	100
12	*Bacillus paranthracis*	Mn5	MACE01000012	99.4397759	8/1428	100
13	*Bacillus nitratireducens*	4049	KJ812430	99.4397759	8/1428	100
14	*Bacillus anthracis*	Ames	AE016879	99.3697479	9/1428	100
15	*Bacillus mycoides*	DSM 2048	ACMU01000002	99.1596639	12/1428	100
16	*Bacillus pseudomycoides*	DSM 12442	ACMX01000133	99.0896359	13/1428	100
17	*Bacillus cytotoxicus*	NVH 391-98	CP000764	97.6190476	34/1428	100
18	*Rossellomorea oryzaecorticis*	R1	KF548480	96.1783439	42/1099	75.6279701
19	*Bacillus coahuilensis*	m4-4	ABFU01000135	95.3966006	65/1412	100
20	*Rossellomorea marisflavi*	JCM 11544	LGUE01000011	95.371669	66/1426	100
21	*Gottfriedia luciferensis*	LMG 18422	AJ419629	95.2949438	67/1424	100
22	*Bacillus acidicola*	105-2	AF547209	95.1646811	69/1427	100
23	*Bacillus tianshenii*	YIM M13235	KF811034	95.1578947	69/1425	100
24	*Sutcliffiella halmapala*	DSM 8723	KV917375	95.0175439	71/1425	100
25	*Gottfriedia acidiceleris*	CBD 119	DQ374637	95.0105411	71/1423	100
26	*Sutcliffiella catenulatus*	18C	LT617055	94.9509116	72/1426	98.7109905
27	*Metabacillus herbersteinensis*	D-1-5a	AJ781029	94.9438202	72/1424	99.4568907
28	*Bacillus salacetis*	SKP7-4	LC367333	94.935806	71/1402	97.0149254
29	*Rossellomorea vietnamensis*	15-1	AB099708	94.8529412	70/1360	94.165536
30	*Bacillus seohaeanensis*	BH724	AY667495	94.8497854	72/1398	95.1186441

Table 7 List of hits for BL26 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Bacillus altitudinis*	41KF2b	ASJC01000029	100	0/1427	100
2	*Bacillus xiamenensis*	HYC-10	AMSH01000114	99.9299229	1/1427	100
3	*Bacillus safensis subsp. safensis*	FO-36b	ASJD01000027	99.5795375	6/1427	100
4	*Bacillus safensis subsp. osmophilus*	BC09	KY990920	99.5795375	6/1427	99.932019
5	*Bacillus pumilus*	ATCC 7061	ABRX01000007	99.5094604	7/1427	100
6	*Bacillus zhangzhouensis*	DW5-4	JOTP01000061	99.5094604	7/1427	100
7	*Bacillus australimaris*	NH7I_1	JX680098	99.4393833	8/1427	100
8	*Bacillus atrophaeus*	JCM 9070	AB021181	97.547302	35/1427	100
9	*Bacillus siamensis*	KCTC 13613	AJVF01000043	97.3370708	38/1427	100
10	*Bacillus subtilis*	NCIB 3610	ABQL01000001	97.2669937	39/1427	100
11	*Bacillus nakamurai*	NRRL B-41091	LSAZ01000028	97.2669937	39/1427	100
12	*Bacillus velezensis*	CR-502	AY603658	97.2563177	38/1385	95.3804348
13	*Bacillus amyloliquefaciens*	DSM 7	FN597644	97.0567624	42/1427	100
14	*Bacillus tequilensis*	KCTC 13622	AYTO01000043	97.0567624	42/1427	100
15	*Bacillus halotolerans*	ATCC 25096	LPVF01000003	97.0567624	42/1427	100
16	*Bacillus cabrialesii*	TE3	MK462260	97.0567624	42/1427	100
17	*Bacillus inaquosorum*	KCTC 13429	AMXN01000021	97.0567624	42/1427	100
18	*Bacillus stercoris*	JCM 30051	MN536904	97.0567624	42/1427	100
19	*Bacillus spizizenii*	NRRL B-23049	CP002905	97.0567624	42/1427	100
20	*Bacillus mojavensis*	RO-H-1	JH600280	96.9866854	43/1427	100
21	*Bacillus vallismortis*	DV1-F-3	JH600273	96.9866854	43/1427	100
22	*Bacillus paralicheniformis*	KJ-16	KY694465	96.6339411	48/1426	100
23	*Bacillus glycinifermentans*	GO-13	LECW01000063	96.5638149	49/1426	100
24	*Bacillus swezeyi*	NRRL B-41294	MRBK01000096	96.4936886	50/1426	100
25	*Bacillus licheniformis*	ATCC 14580	AE017333	96.4235624	51/1426	100
26	*Bacillus sonorensis*	NBRC 101234	AYTN01000016	96.4235624	51/1426	100
27	*Bacillus haynesii*	NRRL B-41327	MRBL01000076	96.4235624	51/1426	100
28	*Metabacillus idriensis*	SMC 4352-2	AY904033	96.2623413	53/1418	96.8113976
29	*Bacillus aerius*	24K	AJ831843	95.8567416	59/1424	100
30	*Bacillus gobiensis*	FJAT-4402	CP012600	95.7223001	61/1426	100

Table 8 List of hits for BR2 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Pseudomonas silesiensis*	A3	KX276592	99.787234	3/1410	100
2	*Pseudomonas mandelii*	NBRC 103147	BDAF01000092	99.7163121	4/1410	100
3	*Pseudomonas frederiksbergensis*	JAJ28	AJ249382	99.5741661	6/1409	100
4	*Pseudomonas piscium*	P50	LR797558	99.4318182	7/1232	84.4413982
5	*Pseudomonas meliae*	CFBP 3225	JYHE01000183	99.2907801	10/1410	100
6	*Pseudomonas tremae*	CFBP 6111	AJ492826	99.2907801	10/1410	100
7	*Pseudomonas cannabina*	CFBP 2341	AJ492827	99.2907801	10/1410	100
8	*Pseudomonas caspiana*	FBF102	LOHF01000033	99.2907801	10/1410	100
9	*Pseudomonas lini*	CFBP 5737	AY035996	99.2892679	10/1407	100
10	*Pseudomonas amygdali*	CFBP 3205	JYHB01000005	99.2198582	11/1410	100
11	*Pseudomonas ficuserectae*	JCM 2400	AB021378	99.2159658	11/1403	98.6995209
12	*Pseudomonas caricapapayae*	ATCC 33615	D84010	99.1489362	12/1410	98.6310746
13	*Pseudomonas mucoides*	P154a	LR797589	99.1071429	11/1232	84.4413982
14	*Pseudomonas congelans*	DSM 14939	FNJH01000022	99.0780142	13/1410	100
15	*Pseudomonas cerasi*	58	LT222319	99.0780142	13/1410	100
16	*Pseudomonas pisciculturae*	P115	LR797573	99.0610329	12/1278	87.5942426
17	*Pseudomonas syringae*	KCTC 12500	KI657453	99.0070922	14/1410	100
18	*Pseudomonas savastanoi*	ATCC 13522	AB021402	99.0056818	14/1408	100
19	*Pseudomonas arsenicoxydans*	CECT 7543	LT629705	98.9346591	15/1408	100
20	*Pseudomonas viciae*	11K1	MN698727	98.9346591	15/1408	100
21	*Pseudomonas prosekii*	LMG 26867	LT629762	98.8636364	16/1408	100
22	*Pseudomonas marginalis*	ATCC 10844	AJ308309	98.7933635	16/1326	92.6662097
23	*Pseudomonas neuropathica*	P155	LR797591	98.7824675	15/1232	84.4413982
24	*Pseudomonas brassicacearum subsp. neoaurantiaca*	ATCC 49054	EU391388	98.7224982	18/1409	99.6577687
25	*Pseudomonas chlororaphis subsp. chlororaphis*	NBRC 3904	BCZX01000031	98.7215909	18/1408	100
26	*Pseudomonas thivervalensis*	DSM 13194	LHVE01000021	98.7197724	18/1406	100
27	*Pseudomonas avellanae*	BPIC 631	AKBS01001374	98.6524823	19/1410	100
28	*Pseudomonas kilonensis*	DSM 13647	LHVH01000037	98.6505682	19/1408	100
29	*Pseudomonas mediterranea*	CFBP 5447	AUPB01000004	98.6505682	19/1408	100
30	*Pseudomonas bijieensis*	L22-9	MT835388	98.6467236	19/1404	100

Table 9 List of hits for RL2 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Pseudomonas lini*	CFBP 5737	AY035996	99.9290277	1/1409	100
2	*Pseudomonas arsenicoxydans*	CECT 7543	LT629705	99.4326241	8/1410	100
3	*Pseudomonas piscium*	P50	LR797558	99.4318182	7/1232	84.4413982
4	*Pseudomonas prosekii*	LMG 26867	LT629762	99.3617021	9/1410	100
5	*Pseudomonas viciae*	11K1	MN698727	99.3617021	9/1410	100
6	*Pseudomonas brassicacearum subsp. neoaurantiaca*	ATCC 49054	EU391388	99.2198582	11/1410	99.6577687
7	*Pseudomonas mucoides*	P154a	LR797589	99.1883117	10/1232	84.4413982
8	*Pseudomonas chlororaphis subsp. chlororaphis*	NBRC 3904	BCZX01000031	99.1489362	12/1410	100
9	*Pseudomonas silesiensis*	A3	KX276592	99.1489362	12/1410	100
10	*Pseudomonas thivervalensis*	DSM 13194	LHVE01000021	99.1477273	12/1408	100
11	*Pseudomonas bijieensis*	L22-9	MT835388	99.1465149	12/1406	100
12	*Pseudomonas mandelii*	NBRC 103147	BDAF01000092	99.0780142	13/1410	100
13	*Pseudomonas kilonensis*	DSM 13647	LHVH01000037	99.0780142	13/1410	100
14	*Pseudomonas mediterranea*	CFBP 5447	AUPB01000004	99.0780142	13/1410	100
15	*Pseudomonas corrugata*	ATCC 29736	D84012	99.0773598	13/1409	98.6291981
16	*Pseudomonas pisciculturae*	P115	LR797573	99.0610329	12/1278	87.5942426
17	*Pseudomonas veronii*	DSM 11331	JYLL01000074	99.0070922	14/1410	100
18	*Pseudomonas kielensis*	MBT-1	MW377589	99.0070922	14/1410	100
19	*Pseudomonas migulae*	CIP 105470	AF074383	98.9361702	15/1410	100
20	*Pseudomonas chlororaphis subsp. aureofaciens*	NBRC 3521	BBQB01000031	98.9361702	15/1410	100
21	*Pseudomonas frederiksbergensis*	JAJ28	AJ249382	98.9354152	15/1409	100
22	*Pseudomonas brassicacearum subsp. brassicacearum*	DBK11	AF100321	98.9354152	15/1409	99.9314599
23	*Pseudomonas marginalis*	ATCC 10844	AJ308309	98.8704819	15/1328	92.6662097
24	*Pseudomonas extremaustralis*	14-3	AHIP01000073	98.8652482	16/1410	100
25	*Pseudomonas chlororaphis subsp. piscium*	DSM 21509	LHUZ01000017	98.8652482	16/1410	100
26	*Pseudomonas spelaei*	SJ/9/1	HQ844525	98.8652482	16/1410	99.314599
27	*Pseudomonas yamanorum*	8H1	EU557337	98.8644429	16/1409	98.4235778
28	*Pseudomonas chlororaphis subsp. aurantiaca*	DSM 19603	CP027746	98.7943262	17/1410	100
29	*Pseudomonas sivasensis*	P7	JAAOWU010000041	98.7943262	17/1410	100
30	*Pseudomonas fildesensis*	KG01	MK859934	98.7943262	17/1410	100

Table 10 List of hits for RL6 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Pseudomonas silesiensis*	A3	KX276592	99.7855611	3/1399	100
2	*Pseudomonas mandelii*	NBRC 103147	BDAF01000092	99.7140815	4/1399	100
3	*Pseudomonas frederiksbergensis*	JAJ28	AJ249382	99.5708155	6/1398	100
4	*Pseudomonas piscium*	P50	LR797558	99.4318182	7/1232	84.4413982
5	*Pseudomonas meliae*	CFBP 3225	JYHE01000183	99.2852037	10/1399	100
6	*Pseudomonas tremae*	CFBP 6111	AJ492826	99.2852037	10/1399	100
7	*Pseudomonas cannabina*	CFBP 2341	AJ492827	99.2852037	10/1399	100
8	*Pseudomonas caspiana*	FBF102	LOHF01000033	99.2852037	10/1399	100
9	*Pseudomonas lini*	CFBP 5737	AY035996	99.2836676	10/1396	100
10	*Pseudomonas amygdali*	CFBP 3205	JYHB01000005	99.2137241	11/1399	100
11	*Pseudomonas ficuserectae*	JCM 2400	AB021378	99.2097701	11/1392	98.6995209
12	*Pseudomonas caricapapayae*	ATCC 33615	D84010	99.1422445	12/1399	98.6310746
13	*Pseudomonas mucoides*	P154a	LR797589	99.1071429	11/1232	84.4413982
14	*Pseudomonas congelans*	DSM 14939	FNJH01000022	99.0707648	13/1399	100
15	*Pseudomonas cerasi*	58	LT222319	99.0707648	13/1399	100
16	*Pseudomonas pisciculturae*	P115	LR797573	99.0610329	12/1278	87.5942426
17	*Pseudomonas syringae*	KCTC 12500	KI657453	98.9992852	14/1399	100
18	*Pseudomonas savastanoi*	ATCC 13522	AB021402	98.9978525	14/1397	100
19	*Pseudomonas arsenicoxydans*	CECT 7543	LT629705	98.9262706	15/1397	100
20	*Pseudomonas viciae*	11K1	MN698727	98.9262706	15/1397	100
21	*Pseudomonas prosekii*	LMG 26867	LT629762	98.8546886	16/1397	100
22	*Pseudomonas marginalis*	ATCC 10844	AJ308309	98.7897126	16/1322	92.6662097
23	*Pseudomonas neuropathica*	P155	LR797591	98.7824675	15/1232	84.4413982
24	*Pseudomonas brassicacearum subsp. neoaurantiaca*	ATCC 49054	EU391388	98.7124464	18/1398	99.6577687
25	*Pseudomonas chlororaphis subsp. chlororaphis*	NBRC 3904	BCZX01000031	98.7115247	18/1397	100
26	*Pseudomonas thivervalensis*	DSM 13194	LHVE01000021	98.7096774	18/1395	100
27	*Pseudomonas avellanae*	BPIC 631	AKBS01001374	98.6418871	19/1399	100
28	*Pseudomonas kilonensis*	DSM 13647	LHVH01000037	98.6399427	19/1397	100
29	*Pseudomonas mediterranea*	CFBP 5447	AUPB01000004	98.6399427	19/1397	100
30	*Pseudomonas bijieensis*	L22-9	MT835388	98.6360373	19/1393	100

Table 11 List of hits for RL7 isolate from EzBioCloud 16S database.

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Mismatch/Total nt	Completeness (%)
1	*Bacillus wiedmannii*	FSL W8-0169	LOBC01000053	99.7902098	3/1430	100
2	*Bacillus albus*	N35-10-2	MAOE01000087	99.7902098	3/1430	100
3	*Bacillus luti*	TD41	MACI01000041	99.7902098	3/1430	100
4	*Bacillus cereus*	ATCC 14579	AE016877	99.7202797	4/1430	100
5	*Bacillus proteolyticus*	TD42	MACH01000033	99.7202797	4/1430	100
6	*Bacillus tropicus*	N24	MACG01000025	99.7202797	4/1430	100
7	*Bacillus fungorum*	17-SMS-01	MG601116	99.7202797	4/1430	100
8	*Bacillus paramycoides*	NH24A2	MAOI01000012	99.6503497	5/1430	100
9	*Bacillus paranthracis*	Mn5	MACE01000012	99.6503497	5/1430	100
10	*Bacillus nitratireducens*	4049	KJ812430	99.6503497	5/1430	100
11	*Bacillus toyonensis*	BCT-7112	CP006863	99.5804196	6/1430	100
12	*Bacillus mobilis*	0711P9-1	MACF01000036	99.5804196	6/1430	100
13	*Bacillus anthracis*	Ames	AE016879	99.5804196	6/1430	100
14	*Bacillus pacificus*	EB422	KJ812450	99.5104895	7/1430	100
15	*Bacillus mycoides*	DSM 2048	ACMU01000002	99.3706294	9/1430	100
16	*Bacillus pseudomycoides*	DSM 12442	ACMX01000133	99.3006993	10/1430	100
17	*Bacillus cytotoxicus*	NVH 391-98	CP000764	97.6923077	33/1430	100
18	*Rossellomorea oryzaecorticis*	R1	KF548480	96	44/1100	75.6279701
19	*Bacillus tianshenii*	YIM M13235	KF811034	95.3749124	66/1427	100
20	*Bacillus coahuilensis*	m4-4	ABFU01000135	95.3323904	66/1414	100
21	*Rossellomorea marisflavi*	JCM 11544	LGUE01000011	95.3081232	67/1428	100
22	*Sutcliffiella halmapala*	DSM 8723	KV917375	95.2347582	68/1427	100
23	*Gottfriedia luciferensis*	LMG 18422	AJ419629	95.1612903	69/1426	100
24	*Bacillus acidicola*	105-2	AF547209	95.1014696	70/1429	100
25	*Priestia flexa*	NBRC 15715	BCVD01000224	95.0314906	71/1429	100
26	*Gottfriedia acidiceleris*	CBD 119	DQ374637	94.9473684	72/1425	100
27	*Bacillus salacetis*	SKP7-4	LC367333	94.9394155	71/1403	97.0149254
28	*Ectobacillus panaciterrae*	Gsoil 1517	AB245380	94.9116608	72/1415	100
29	*Margalitia shackletonii*	LMG 18435	LJJC01000006	94.8879552	73/1428	100
30	*Rossellomorea vietnamensis*	15-1	AB099708	94.856723	70/1361	94.165536

Supplement 3

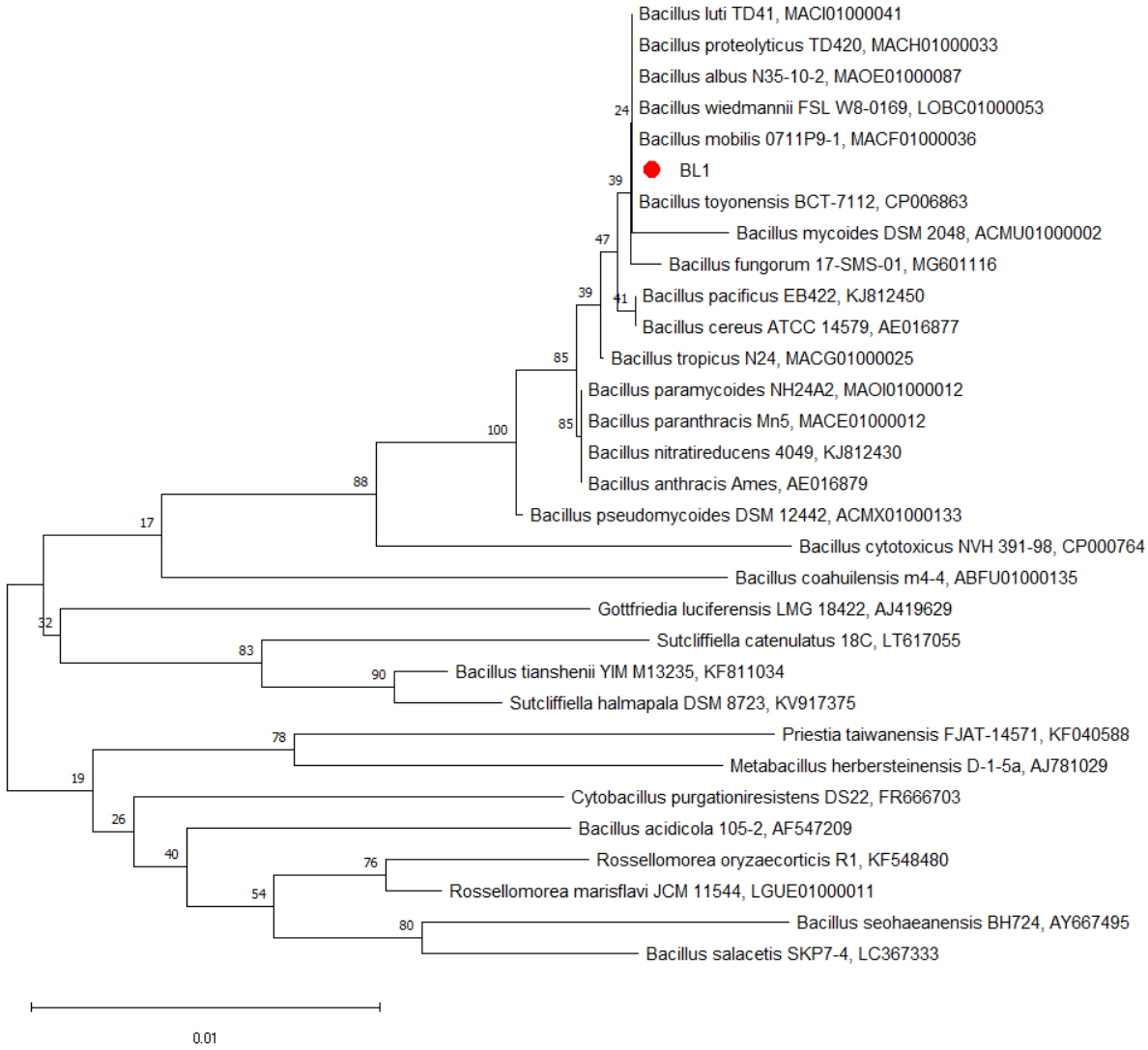

Figure 1 Phylogenetic tree depicting the relationship between strain BL1 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.01 nucleotide substitutions per site.

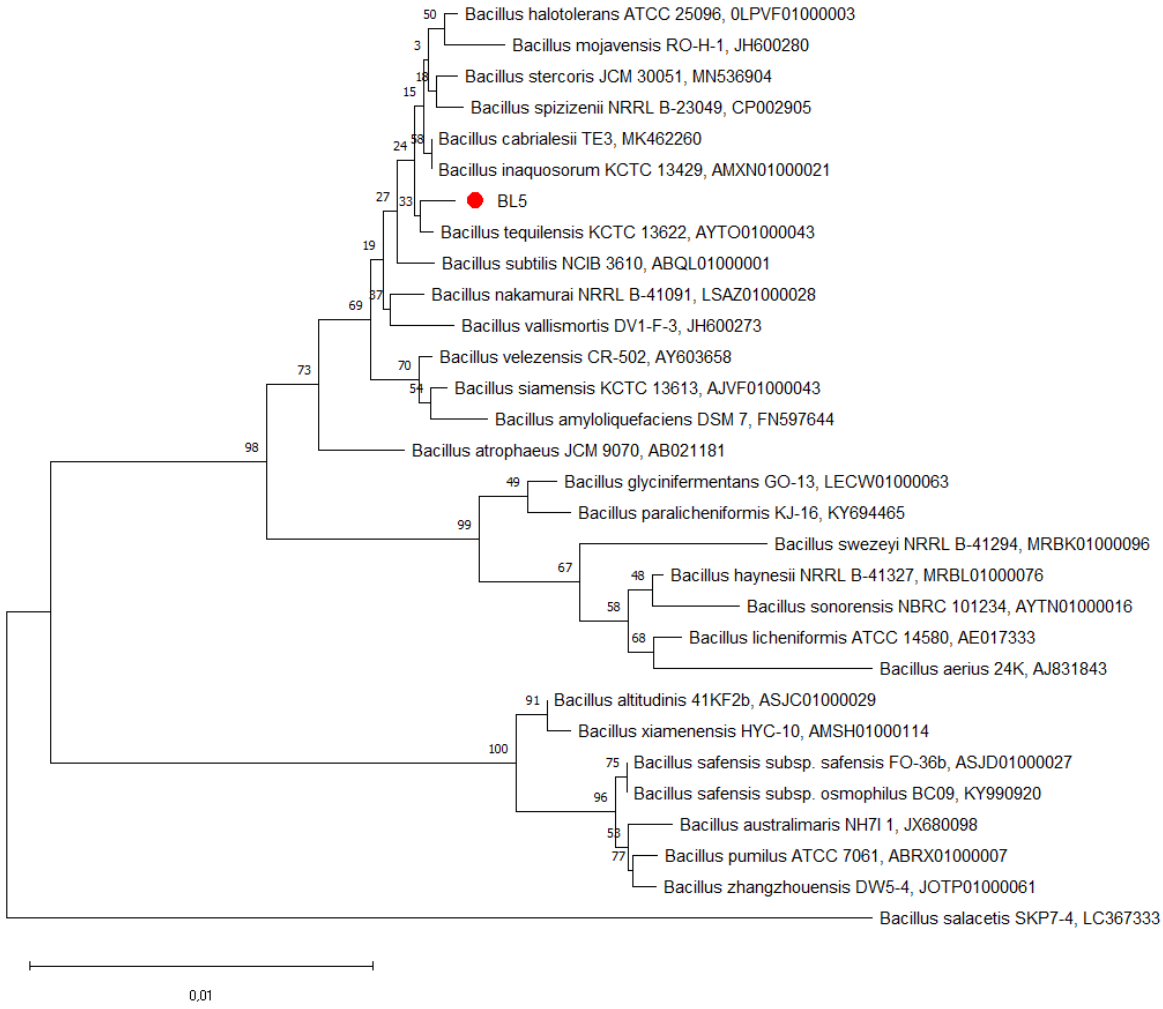

Figure 2 Phylogenetic tree depicting the relationship between strain BL5 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.01 nucleotide substitutions per site.

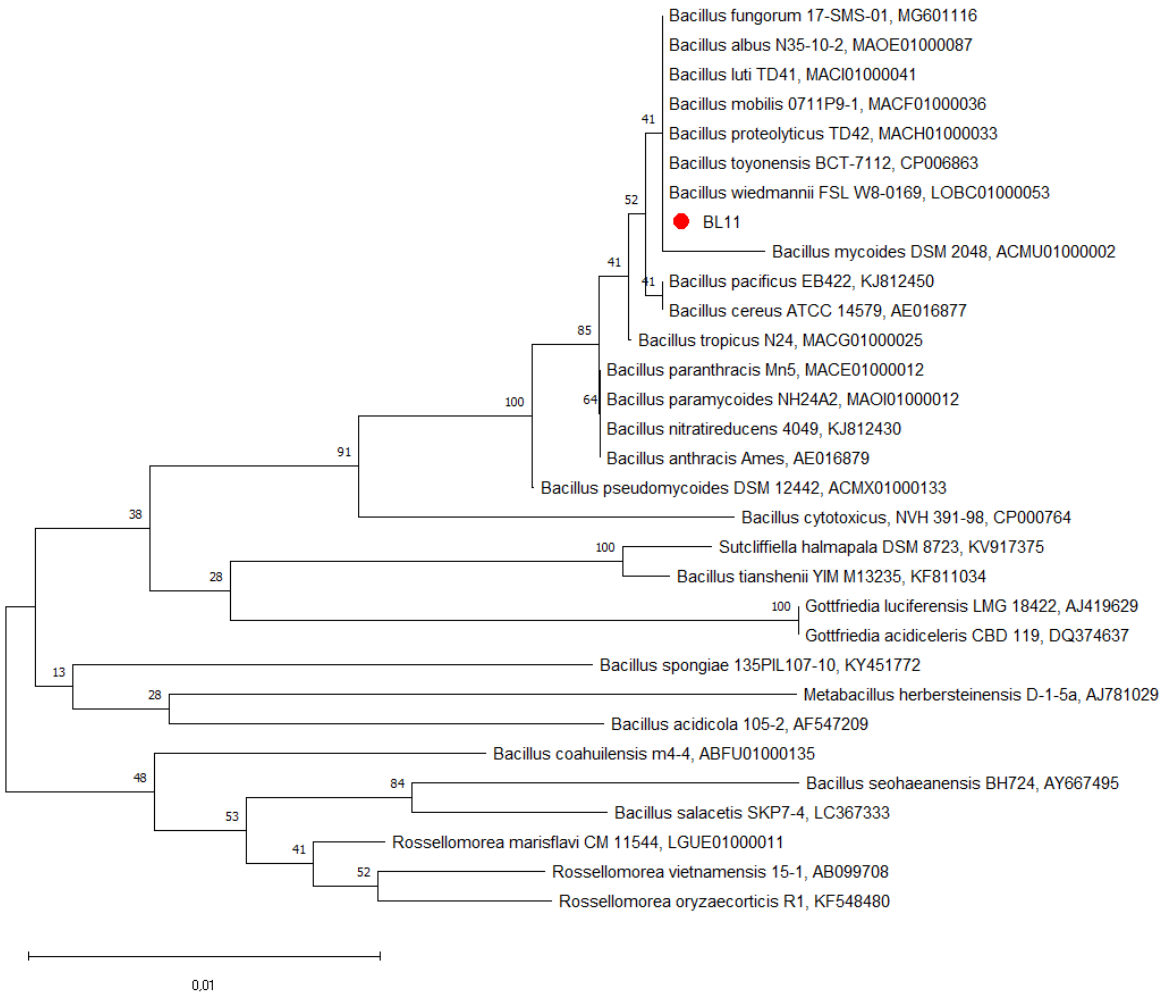

Figure 3 Phylogenetic tree depicting the relationship between strain BL11 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.01 nucleotide substitutions per site.

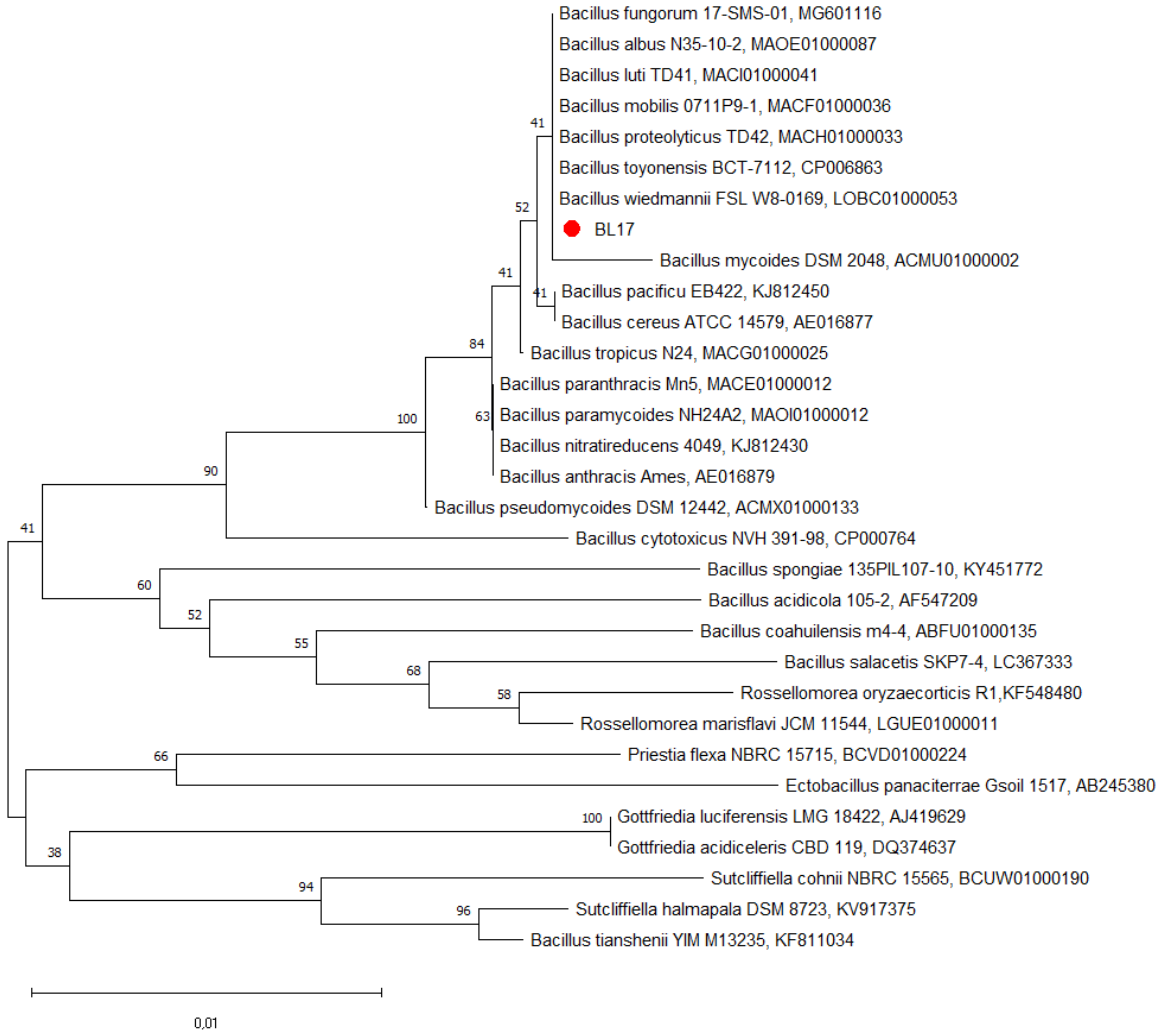

Figure 4 Phylogenetic tree depicting the relationship between strain BL17 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.01 nucleotide substitutions per site.

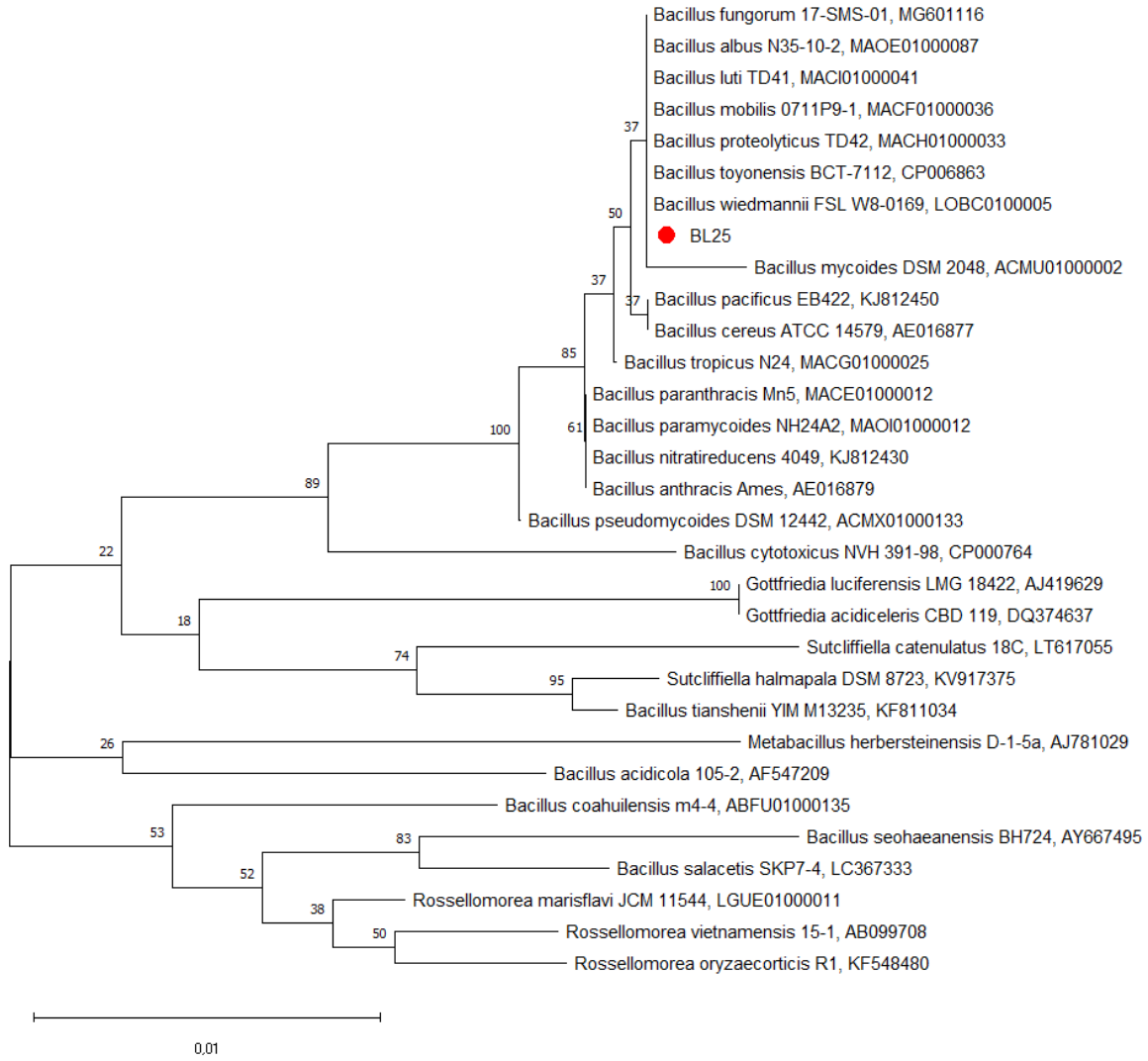

Figure 5 Phylogenetic tree depicting the relationship between strain BL25 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.01 nucleotide substitutions per site.

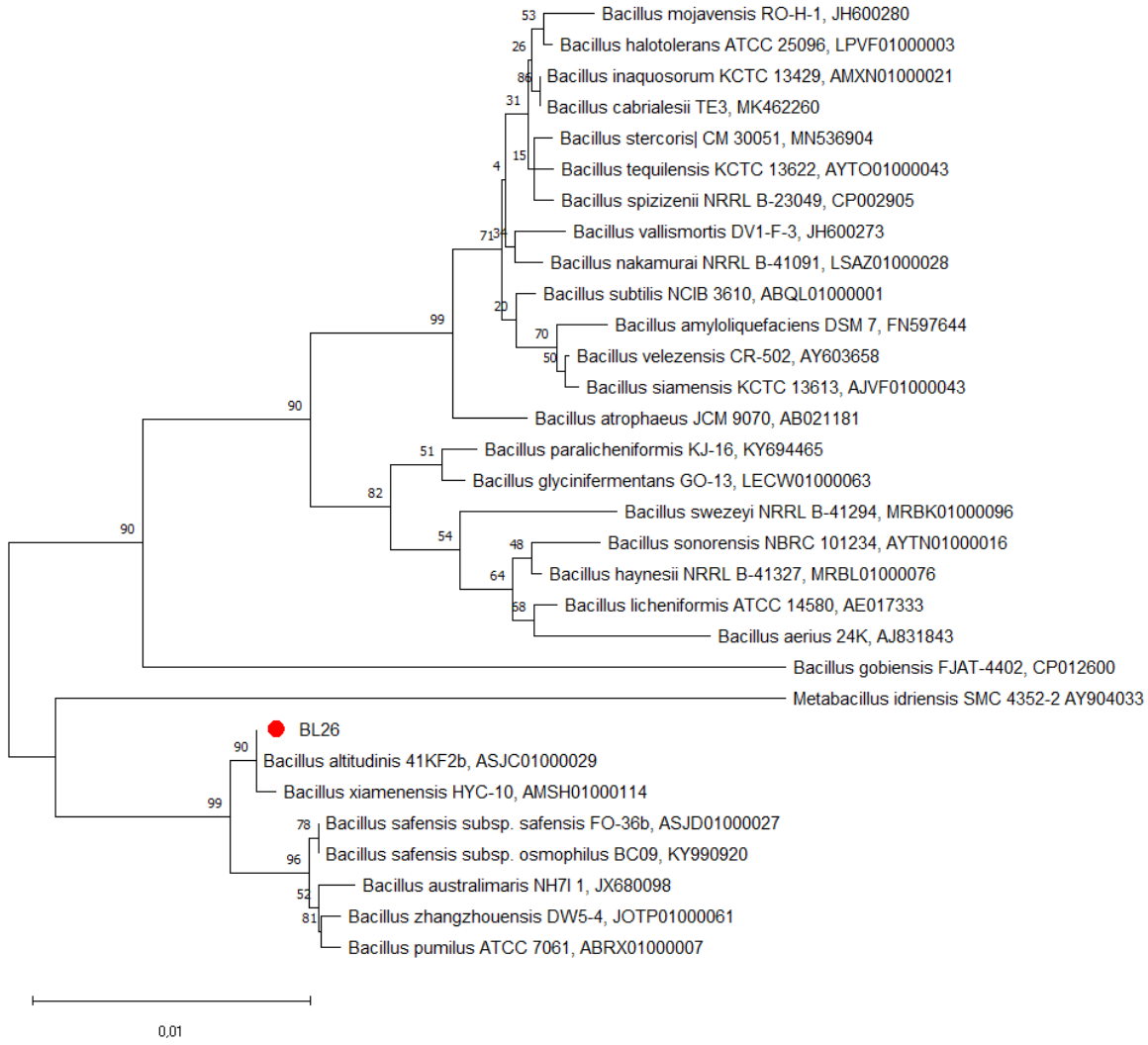

Figure 6 Phylogenetic tree depicting the relationship between strain BL26 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.01 nucleotide substitutions per site.

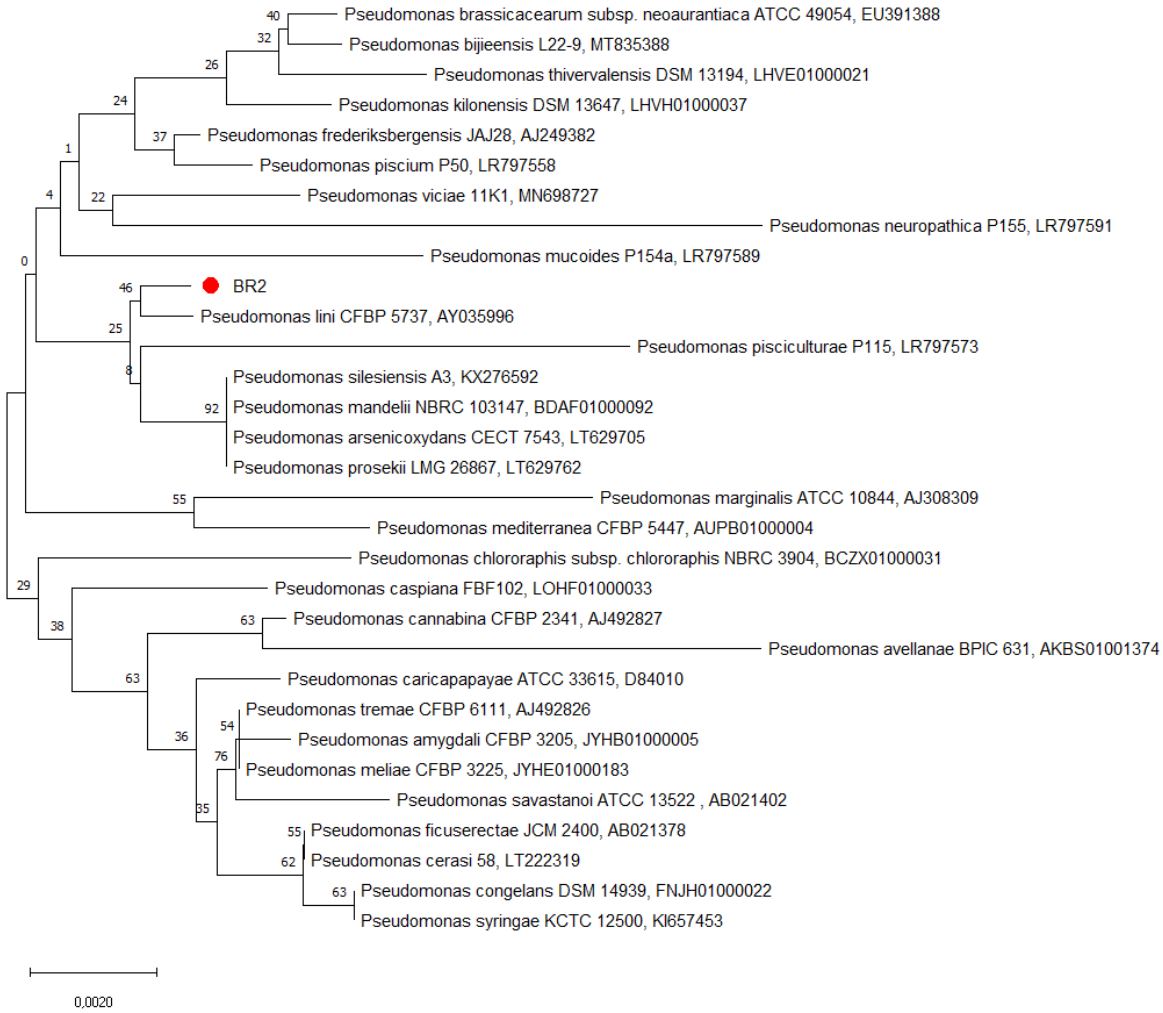

Figure 7 Phylogenetic tree depicting the relationship between strain BR2 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.002 nucleotide substitutions per site.

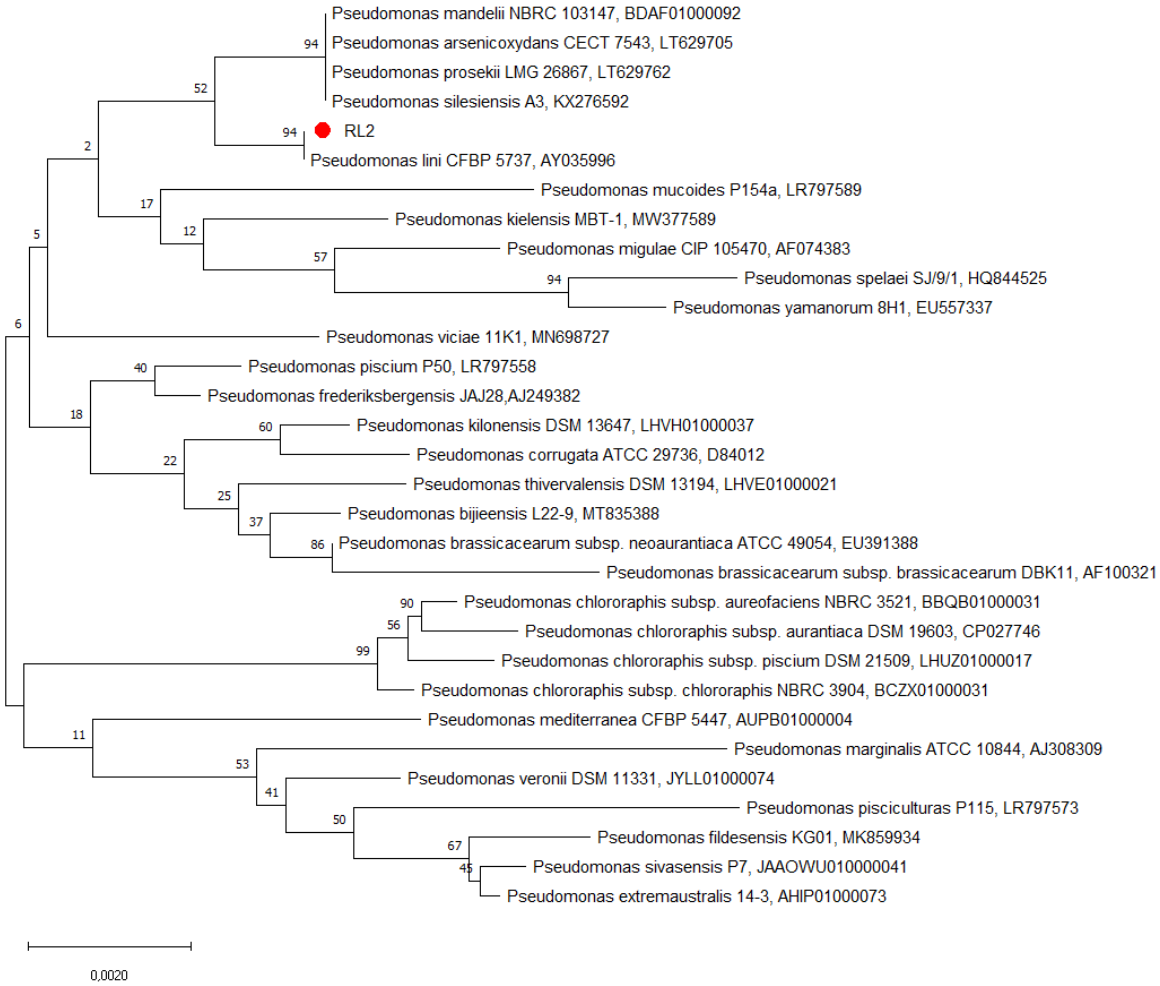

Figure 8 Phylogenetic tree depicting the relationship between strain RL2 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.002 nucleotide substitutions per site.

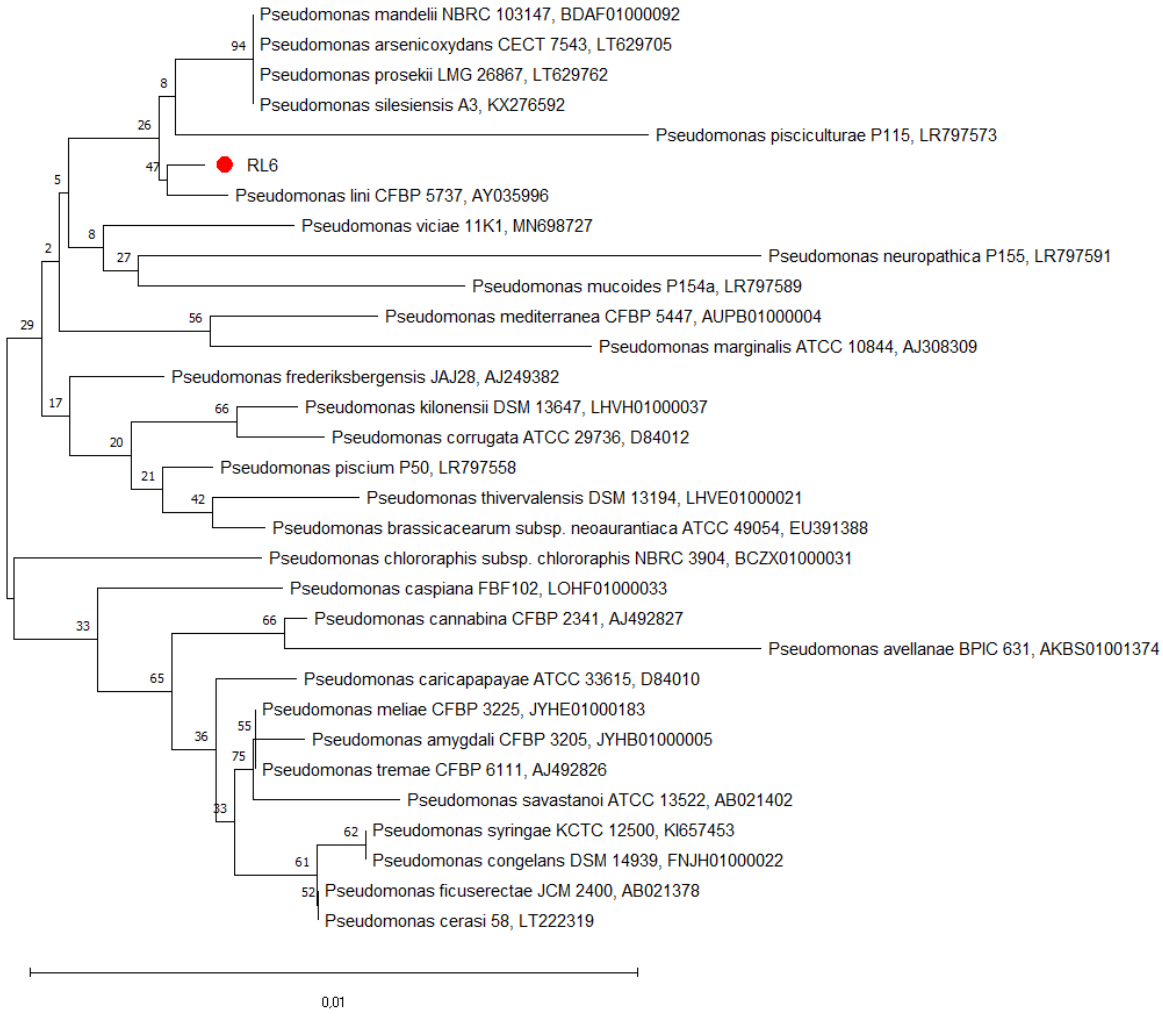

Figure 9 Phylogenetic tree depicting the relationship between strain RL6 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.01 nucleotide substitutions per site.

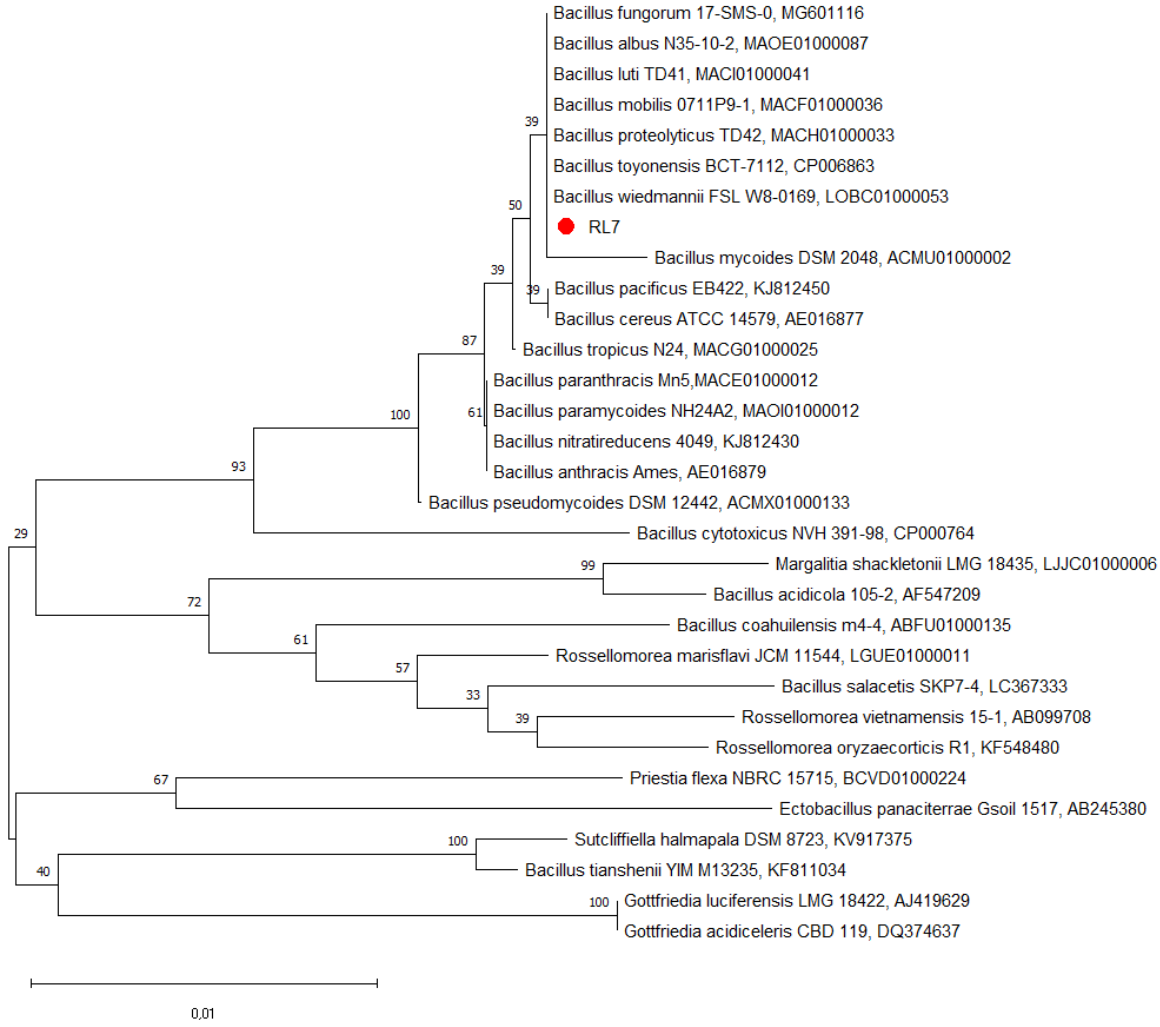

Figure 10 Phylogenetic tree depicting the relationship between strain RL7 and its closest relatives as deduced from 16S rRNA sequence analysis. This analysis involved 31 nucleotide sequences. The tree was constructed by using the neighbour-joining method. Bar, 0.01 nucleotide substitutions per site.

Supplement 4

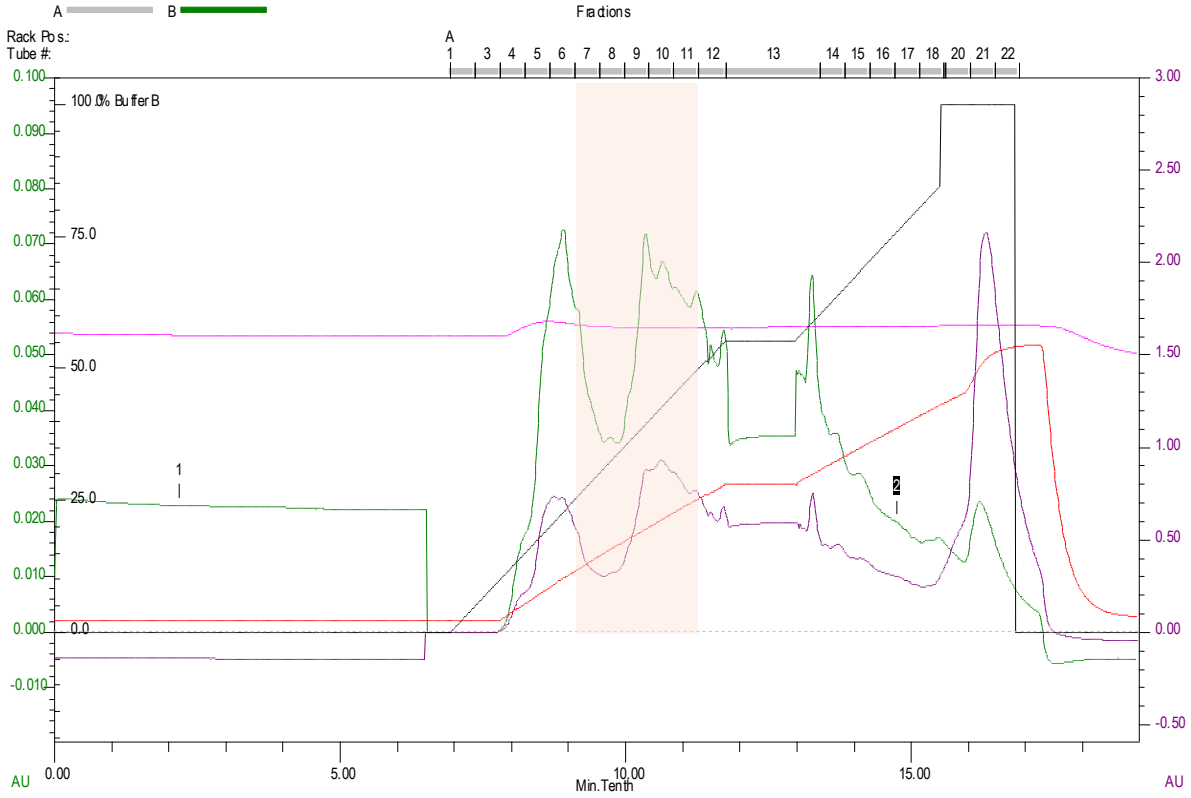

Figure 11 Anion-exchange chromatography. The most active factions are marked with a light orange background. Purple curve - 214 nm, green - 280 nm absorbance, black - NaCl gradient.

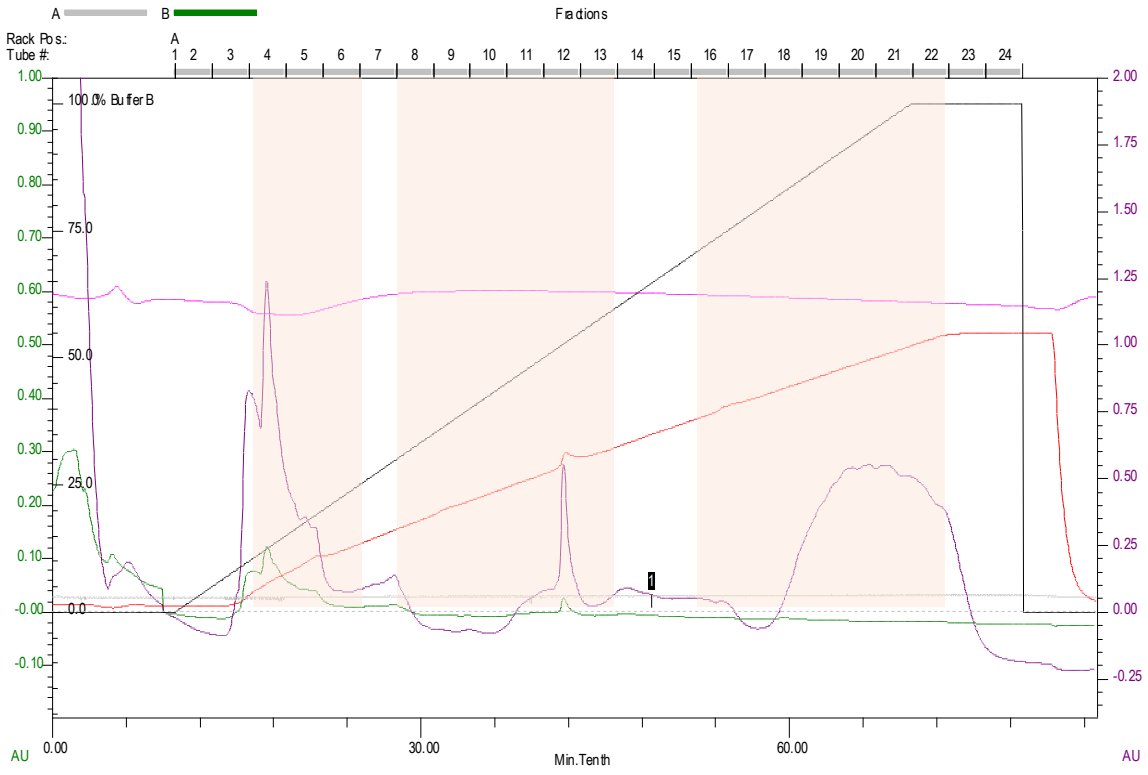

Figure 12 Anion-exchange chromatography. The most active factions are marked with a light orange background. Purple curve - 214 nm, green - 280 nm absorbance, black - NaCl gradient.

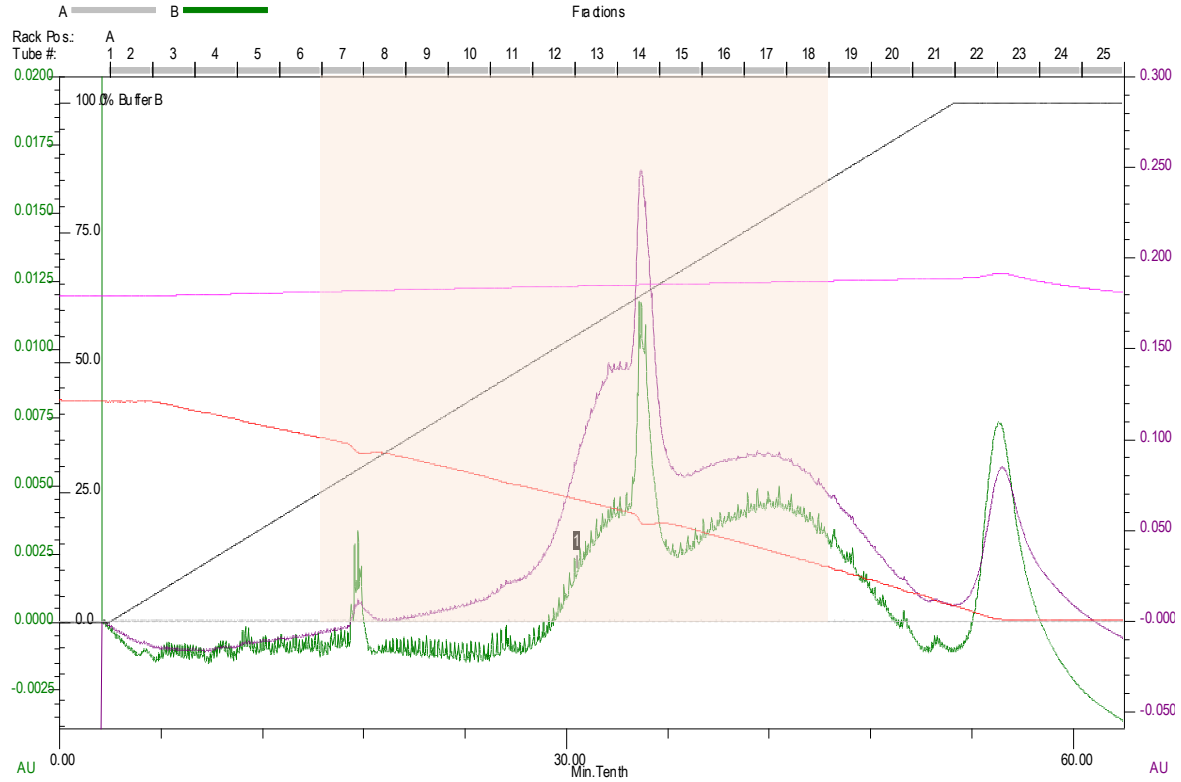

Figure 13 Hydrophobic interaction chromatography. The most active factions are marked with a light orange background. Purple curve - 214 nm, green - 280 nm absorbance, black – buffer B4 gradient.