IDENTIFICATION OF CANDIDATE GENES FOR BIOSURFACTANT PRODUCTION IN *LACTOBACILLUS* SPECIES

By

PRYSCILA VELASCO VAZQUEZ

Bachelor of Engineering in Environmental Systems

Universidad Politecnica de Durango

Durango, Mexico

2013

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
August, 2017

IDENTIFICATION OF CANDIDATE GENES FOR

BIOSURFACTANT PRODUCTION IN

LACTOBACILLUS SPECIES

Thesis Approved:

Dr. Patricia Rayas-Duarte

Thesis Adviser

Dr. Patricia Canaan

Dr. Li (Maria) Ma

ACKNOWLEDGEMENTS

I owe my deepest appreciation to John Moore, CFO and Siobhan Reilly, CEO of Log10, LLC for granting this project. They have made available their support in a number of ways that only family would. I am heartily thankful for the opportunity from the very beginning.

It is a pleasure to thank those who made this thesis possible. My dear committee members, Patricia Rayas-Duarte, Patricia Canaan, and Maria Ma Li, whose expertise, encouragement, guidance and enthusiasm had lasting effect.

I offer my regards and blessings to all my family and truly friends who supported me in any respect during the completion of the project, regardless of the long distance. And also, the closest ones, especially thanks to my tia Miriam.

I dedicate this work to my parents, Veronica and Marco, and grandparents, Roberto, Esther and Leo. I am thankful for their constant love and support. Last, but not least I would like to thank my sister, the one that I've missed the most, Diana for her unconditional support all along.

Name: PRYSCILA VELASCO-VAZQUEZ

Date of Degree: MAY, 2017

Title of Study: IDENTIFICATION OF CANDIDATE GENES FOR BIOSURFACTANT PRODUCTION IN *LACTOBACILLUS* SPECIES

Major Field: FOOD SCIENCE

Abstract: Certain bacteria develop biofilm in the environments they colonize which provides shelter from antimicrobials and other sanitation procedures. Undesired biofilm formation is a serious problem in the food and healthcare industries. Probiotic biosurfactant-producing Lactobacilli with demonstrated antimicrobial activity can be used as a deterrent of pathogenic biofilms. Currently there is no biosurfactant PCR detection method. Genes responsible for biosurfactant production in Lactobacilli are currently undiscovered.

The purpose of this study is to identify candidate genes within Lactobacilli that share similarity and/or identity with known genes required for the production of biosurfactants in other bacterial species.

An in-silico study was performed through the use of the NCBI BLAST algorithms, and compared gene sequences of *L. casei*, *L. rhamnosus*, *L. fermentum*, *L. acidophilus*, *L. pentosus*, *L. paracasei*, *L. helveticus, L. plantarum, L. delbrueckii, L. jensenii, L. reuteri, L. brevis* (each of which has been shown to produce surlactin) with previously identified biosurfactant-producing genes of various bacteria. A set of primer sequences for a qPCR screening methodology was design to screen *Lactobacillus* strains.

The best candidate found was selected based upon its conserved domain features and nearby ORFs; Nonribosomal peptide synthetase (NRPS) and polyketide synthetase (PKS), respectively. Similarity was shown between NRPS SrfA (that regulates production of surfactin by *Bacillus subtilis),* and NRPS NpsA from *L. plantarum* WCFS1. NRPS NpsA is 5289 amino acid long and it is consensus within seven *L. plantarum* strains. It is formed by five modules that are highly similar but not identical. Each module includes adenylation, condensation and thiolation domains.

Therefore, further analyses of NRPS NpsA putative surlactin producer need to be performed. NRPSs can produce a variety of secondary metabolites, not only biosurfactants. The confirmation of the regulatory protein would help to design a rapid PCR screening method, using the designed primers, that may be helpful in the clinical field and food industry.

Keywords: *probiotic, Lactobacilli, biofilm, biosurfactant, surlactin, genes*

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

ABBREVIATIONS

Table 1. Abbreviations list

Acronym	Definition
ACP	Acyl carrier protein
ASM	American Society for Microbiology
BATH	Bacterial adhesion to hydrocarbons assay
BHL	Butyryl homoserine lactone
BLAST	Basic local alignment search tool
BS	Biosurfactant
CD	Conserved domains
CGH	Comparative genome hybridization
CMC	Critical micelle concentration
CUP	Chaperone usher pathway
CoA	Coenzyme A
DDBJ	DNA DataBank of Japan
EHEC	Entero hemorrhagic *E. coli*
ENA	European Nucleotide Archive
EPS	Extracellular polymeric substances
ERS	Economic Research Service
ESTs	Expressed sequence tags
FAO	Food and Agriculture Organization
GIT	Gastrointestinal tract
HIC	Hydrophobic interaction chromatography
HSL	Homoserine lactone
INSDC	International Nucleotide Sequence Database Collaboration
KEGG	Kyoto Encyclopedia of Genes and Genomes
KR	Keto reductase
LAB	Lactic acid bacteria
LTAs	Lipoteichoic acids
MELs	Mannosylerythritol lipids
MEOR	Microbial enhanced oil recovery
MRSA	Methicillin-resistant Staphylococcus aureus

Acronym	Definition
NMR	Nuclear magnetic resonance
NRPSs	Nonribosomal peptide synthetases
ORFs	Open reading frames
PCP	Peptidyl carrier protein
PGA	Polyglucosamine
PKS	Polyketide synthetase
PTS	Phosphotransferases
QSS	Quorum sensing system
SDPs	Sortase-dependent proteins
UPEC	Uropathogenic *E. coli*
USDA	United States Department of Agriculture
WHO	World Health Organization
WTAs	Wall teichoic acids

CHAPTER I

INTRODUCTION

1. Statement of problem

Abiotic surfaces in food processing and medical environments offer a niche to bacteria for biofilm formation. Therefore, the prevalence of pathogenic biofilms in these areas represent a harm to safety and health. Probiotic biosurfactant-producing Lactobacilli can be used to replace pathogenic biofilms. However, current methods based on the surface tension and cell hydrophobicity, for the detection of biosurfactant requires its extraction and purification, which are time consuming. There is no Polymerase Chain Reaction (PCR) screening method for probiotic biosurfactant-producing bacteria, and their regulatory genes are undiscovered.

2. Purpose of the study

a) To identify similarities between the known genes encoding the production of biosurfactants in Lactobacilli.

b) To identify and compare genes responsible for the production of biosurfactant in Lactobacilli.

c) To determine which species among Lactobacilli have the identified genes in b).

3. Hypotheses

1. The gene responsible for the surfactant production in *Lactobacillus* spp. is similar to the ones found in other bacterial species, *Bacillus*, *Pseudomonas, Acinobacter,* or *Serratia.*

A set of primers can be designed to identify this gene in the genomic sequence of *Lactobacillus* spp

CHAPTER II

REVIEW OF THE LITERATURE

1. Biofilms

Bacteria represent the most prosperous form of life on Earth, in terms of total biomass, diversity and extent of habitat colonized. It is known that it is the ability of their genotype to respond phenotypically to environmental factors, rather than the power of its genetic repertoire, that has produced the singular success of bacteria. Biofilm fossils are included in the oldest records of life on Earth with 3.5 billion years (Schopf et al., 1983).

Morphological and physiological properties of microorganisms have been described as they grow in vitro, where optimal growth conditions are provided. However, in natural environments, nearly every species of microorganisms, bacteria (with 90% of all of them), fungi, algae and protozoa are commonly found to be attached to surfaces as biofilms. Therefore, there are differences between their planktonic and sessile states such as, genotype, phenotype, and mechanisms of adaptation. Henrici first reported in 1933 observation about the existence of biofilms, concluding that "water bacteria are not free floating organisms, but grow upon submerged surfaces" (Henrici, 1933).

1.1. Biofilm definition

Biofilms are sessile communities of microorganisms that grow attached to a surface or interphase and embedded in a self-produced extracellular matrix of extracellular polymeric biomolecules, such as DNA (Costerton, Lewandowski, Caldwell, Korber, & Lappinscott, 1995). Bacteria coordinate efforts with neighbors to accomplish biofilm development to protect themselves from environmental stresses (e.g., attack by immune system, desiccation, antimicrobials, protozoa ingestion). Sessile bacteria inside a biofilm can stand 1000 times higher biocide concentration than their planktonic state with respect to growth rate and gene transcription (Van Houdt & Michiels, 2010).

1.2. Types of Biofilms

Biofilms are omnipresent. They are, within living organisms symbiotically or pathogenically, attached to what is known as biotic surfaces such as, epithelial tissue, teeth, plants, etc. They can also colonize inert (i.e., abiotic) surfaces such as metal, wood, plastic, rocks, and glass where water and nutrients are available. Most of the times biofilms constitute a community formed by multispecies, whereas single species biofilm exists in a variety of infections on medical implants (Costerton et al., 1995). Multispecies biofilms are regulated by community factors, different atmospheric conditions (i.e., aerobic or anaerobic), pH values and redox potential (Satpathy, Sen, Pattanaik, & Raut, 2016). Regardless their ecosystem and type of surface in which microorganisms are attached to form a biofilm, they can be present as part of the environment, or purposely put there; thus, they are either desired or undesired. Therefore, they can be divided into two categories, beneficial or harmful.

1.1 Beneficial biofilms

Beneficial biofilms are those whose presence is considered to be convenient for the host, leading to symbiotic relationships in case attached to a biotic surface. In natural environments, symbiotic biofilms have been found to induce plant growth and to protect plants from phytopathogens, soil bacteria, whereas others are involved in pathogenesis (Bogino, Oliva Mde, Sorroche, & Giordano, 2013). Bioremediation is a technology for remediating groundwater and soil at many sites contaminated with hazardous wastes. This is facilitated by enhanced gene transfer among biofilm organisms and by the increased bioavailability of pollutants (i.e., fuel oil, gasoline, chlorinated solvents, nitro aromatics, heavy metals) for degradation (Singh, Paul, & Jain, 2006). Wastewater treatment technologies have been developing filtration systems that adapted a biofilm on the filter media, in which microorganisms use the organic matter found in that water (Sehar & Naz, 2016).

The use of lactic acid bacteria (LAB) biofilms in food and food processing plants hasn't been only for the control of food-borne pathogens, but also for the extension of shelf life and sensory quality improvement (Mozzi, Raya, & Vignolo, 2016). Most LAB present in food agricultural products are considered safe due their GRAS (Generally Recognized As Safe) status. For example, in cheese processing plants using traditional wooden vats for milk fermentation (Licitra et al., 2007). These cheeses are made with raw milk that develops biofilms on the vat wood, becoming a resident biofilm that acts as a reservoir of strains that allows milk fermentation and cheese ripening without adding exogenous starters (Didienne et al., 2012).

Human and animal gastrointestinal tracts (GIT) are a niche for their autochthonous microbiota. Natural endothelial surfaces are covered by biofilms of native bacteria that condition the tissue to protect it against the adhesion of extraneous organisms. It is known that virtually all body fluids provide sufficient organic nutrients for optimal bacterial growth. For example, studies have found that the human vagina is protected by *Lactobacillus* spp. due its physiological activity against *Candida albicans* involved in vaginitis studies (Malik, Petrova, & Claes, 2013; Terraf, Juarez Tomas, Nader-Macias, & Silva, 2012).

1.1 Harmful biofilms

Biofilm development also contributes to the virulence of phytopathogenic bacteria through various mechanisms, including blockage of xylem vessels (transportation system for water and nutrients in vascular plants), increased resistance to plant antimicrobial compounds, and/or enhanced colonization of specific habitats (Bogino et al., 2013). In the United States, according to the American Society for Microbiology (ASM), physician visits that are attributable to infectious diseases have costs estimated at more than $120 billion (htpp://www.asm.org/index.php/position-statements-and-testimony?id=2288). Biofilms are implicated in various diseases such as otitis media (the most common acute ear infection in children), bacterial endocarditis (infection of the inner surface of the heart and its valves), cystic fibrosis (a chronic disorder resulting in increased susceptibility to serious lung infection). Sources of hospital-acquired infections may include the presence of biofilms on the surfaces of catheters, medical implants, valves, stents and shunts (Satpathy et al., 2016). Biofilms that form on medical implants are difficult to remove where surgical intervention would be the only way to overcome such infections

(Neethirajan, Clond, & Vogt, 2014).

Biofilms are also found in the household environment. Poor disinfection practices and ineffective cleaning products may contribute to the occurrence of illnesses linked with pathogenic microorganisms attached to toilets, sinks, drains, countertops, and cutting boards in the kitchen and bath. In 2014, the United States Department of Agriculture (USDA) and the Economic Research Service (ERS) have conducted research into the economic cost of foodborne illnesses in the US, in which 8.9 million Americans suffer foodborne disease, and the resulting economic cost is $15.6 billion per year (https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx).

Additionally, in food industry, biofilms can also persist the cleaning processes and be the cause of contamination of equipment, as it was found with 59% of foodborne disease out breaks investigated in France (Gomez, Ramiro, Quecan, & de Melo Franco, 2016).

L. monocytogenes is commonly found in food-processing environments, and it has been isolated from both meat and dairy processing plants (Winkelströter, Tulini, & De Martinis, 2015; Wong, 1998), also *E. coli* O157:H7 has the potential to form biofilm on different surfaces used in food industry. Common sites for the presence of *Salmonella* spp. in food-processing plants are filling or packaging equipment, floor, drains, walls, cooling pipes, conveyors, collators for assembling product for packaging, racks for transporting products, hand tools, gloves, freezers, etc., which are usually made of plastics (Pompermayer & Gaylarde, 2000). Generally, more biofilm was produced in low nutrient conditions, as can be found in specific food-processing environments, compared to high nutrient conditions (Stepanovic, 2004).

1.3. Biofilm formation

Bacteria form biofilms regulated by dynamic processes, such as a defense mechanism against environmental conditions or as colonization mechanism to remain in a favorable niche. The external conditions trigger adaptation changes in the phenotype, regulated by the genotypic alteration of its own inhabitants which then leads to further maturation of the biofilm (Jefferson, 2004). Five sequential steps are followed to form a biofilm: 1) planktonic cells adhesion to the surface, 2) bacterial division and adsorption, 3) early development, production of cell-cell signaling molecule, 4) firmly mature biofilm architecture with extracellular polymeric substances (EPS), and 5) disassembly of the matrix and dispersion of single cell (Fig. 1) (O'Toole & Wong, 2016; Satpathy et al., 2016). The factors controlling biofilm formation include recognition of attachment sites on a surface, nutritional cues, changes of pH and temperature, exposure to antibiotics and biocides, and host defense mechanisms (Basak, N, O, & Kumar Mallick, 2013).

The initial bacterial attachment is driven by Brownian motion and gravitational forces, and depends upon surrounding hydrodynamic forces. These attractive or repulsive forces include electrostatic and hydrophobic interactions, as well as van der Waals forces among others. Velocity and direction toward or away from the contact surface are affected by medium properties, along with bacterial cell-surface composition. The importance of flagellar motility, as an advantage to overcome hydrodynamic and repulsive forces, for initial attachment has been documented for several pathogens, including *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Escherichia coli* ((Lemon, Higgins, & Kolter, 2007; O'Toole & Kolter, 1998).

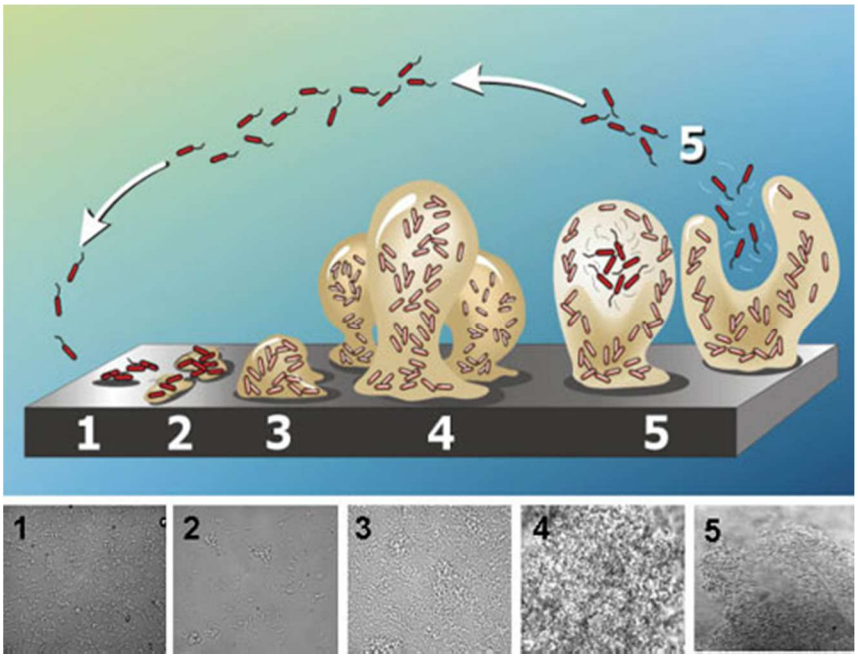

Figure 1. Biofilm formation stages 1) Cells adhesion to the surface, 2) Bacterial division and adsorption, 3) Early development with production of cell-cell signaling molecules, 4) Maturation of biofilm architecture with extracellular polymeric substances, and 5) Disassembly of the matrix and dispersion of cells.

This initial attachment is not definitive yet, is rather reversible and dynamic, when bacteria can go back to their planktonic population if they are diminished by nutrient availability, and hydrodynamic forces or repulsive forces (Dunne, 2002).

Irreversible attachment is accomplished by bacteria that can weather cut off forces and maintain a steadfast grip on the surface. For example, uropathogenic *E. coli* (UPEC) and other *E. coli* pathogenic serotypes rely heavily on type 1 pili ((Kostakioti, Hadjifrangiskou, & Hultgren, 2013; Martinez, Mulvey, Schilling, Pinkner, & Hultgren, 2000; Pratt & Kolter, 1998), which are composed by multiple subunits of adhesive organelles compiled by the chaperone usher pathway (CUP) (Waksman & Hultgren, 2009). CUP pili systems are differentially expressed and are believed to facilitate attachment in a niche-specific manner (Welch et al., 2002). It has been demonstrated that

the adherence is mediated by the FimH adhesin at the tip of type 1 pili, which recognizes mannosylated moieties (Thumbikat et al., 2009). FimH presumes to have a key role in UPEC pathogenesis; it mediates binding and invasion to human bladder epithelial cells, binds to human uroplakin (a plasma membrane protein complex on urinary bladder epithelial cells), and is significant in a murine preclinical model of cystitis (Martinez et al., 2000). In addition to type 1 pili, curli fibers and Antigen 43 have been shown to mediate attachment and interbacterial interactions on abiotic surfaces (Henderson et al., 1997; Kjaergaard et al., 2000; Cegelski et al., 2009). Additionally, curli enables binding to the eukaryotic extracellular matrix components laminin, fibronectin, and plasminogen (Vidal et al., 1998; Cookson et al., 2002; Uhlich et al., 2006).

Furthermore, *P. aeruginosa*, an extensively studied pathogen and biofilm former, also uses a number of attachment organelles to irreversibly adhere to a surface. Besides flagella, *P. aeruginosa* uses type IV pili-mediated twitching motility to wade through the liquid interface and contact the surface, maintain adherence, and move across the attachment surface (O'Toole & Kolter, 1998). Similar to UPEC, *P. aeruginosa* express several CUP fimbriae, of which CupA is involved in surface adherence and auto aggregation (Vallet et al., 2001; Klebensberger et al., 2009).

On the other hand, the gram-positive *Enterococci* are nonmotile and, up until recently, were thought to possess no adhesive pili. Enterococcal adhesins that mediate adherence to eukaryotic extracellular matrix components, have been identified; such as SagA, Acm (*E. faecium*), and Ace (*E. faecalis*), which bind collagen (Mohamed et al., 2006), and the surface protein Esp, which has been shown to promote biofilm formation on abiotic surfaces in *esp*-expressing *E. faecalis* strains (Toledo-Arana et al., 2001). Additionally,

reports showed the presence of Enterococcal biofilm pili (Ebp) in *E. faecalis* and demonstrated their contribution to biofilm formation, endocarditis, and urinary tract infection (Guiton et al., 2009; Kline, Dodson, Caparon, & Hultgren, 2010).

Surface contact causes responses that precede to gene expression changes, up-regulating factors favoring sessility, such as those implicated in the extracellular matrix formation (Bhomkar, Materi, Semenchenko, & Wishart, 2010; Morici et al., 2007). Further studies reported that polyglucosamine (PGA) and colanic acid contribute to biofilm architecture with PGA being prevalent among clinical isolates, including UPEC (Agladze, Wang, & Romeo, 2005; Prigent-Combaret & Lejeune, 1999; X. Wang, Preston, & Romeo, 2004), (Cerca et al., 2007).

Extracellular matrix composition of *P. aeruginosa* has been more investigated, and has been shown to vary depending on environmental conditions (Harmsen, Yang, Pamp, & Tolker-Nielsen, 2010). Pel and Psl are the primary EPS components, Psl increases *Pseudomonas* attachment to airway epithelial cells, whereas augmented expression of *pel* in colony variants isolated from cystic fibrosis patients was associated with *P. aeruginosa* persistence in lung airways (Jackson, Starkey, Kremer, Parsek, & Wozniak, 2004; Ma, Jackson, Landry, Parsek, & Wozniak, 2006; Vasseur, Vallet-Gely, Soscia, Genin, & Filloux, 2005). (Garcia-Medina, Dunne, Singh, & Brody, 2005). Furthermore, Borlee et al. identified CdrA, a large secreted adhesin, which is expressed in the biofilm in response to high levels of the universal signal 3,5-cyclic diguanylic acid (c-di-GMP) and binds Psl, stabilizing biofilm structures (Borlee et al., 2010). Besides EPS, several studies have demonstrated that eDNA is critical for cell-to-cell connections of *Pseudomonas* biofilms (Whitchurch et al., 2002; Yang et al., 2007). While the biofilm

matures, lysis of bacterial subpopulation increases eDNA, this in response to the *P. aeruginosa* quinolone signal (Pqs) QSS (Allesen-Holm et al., 2006).

The mature biofilm has an active community trading and sharing products for its maintenance, and when it is ready, dispersal step becomes an option. Bacteria have evolved maneuvers to recognize environmental variations and assess whether it is still beneficial to reside within the biofilm or whether it is time to resume a planktonic lifestyle. There are different tactics to accomplish biofilm dispersion: degrading the matrix, ending the synthesis of the biofilm matrix compounds, or disrupting noncovalent interaction between matrix components. This last step is accomplished with the production of biosurfactants (Hong, Lee, & Wood, 2010; Karatan & Watnick, 2009; Rowe, Withers, & Swift, 2010; Sauer et al., 2004). For instance, EPS-degrading enzymes, such as alginate lyase in *P. aeruginosa,* contribute to bacterial detachment from the matrix. And also, in *E. coli*, the CsrA protein was shown to repress PGA synthesis, also assisting in dispersion (X. Wang et al., 2004). In addition to EPS, surfactant molecules are produced, reducing the interactions between surface and bacteria. Cell death creates voids within the biofilm, serving as an additional dispersal mechanism that frees resident live bacteria, as shown by studies in *P. aeruginosa* (Webb et al., 2003). Dispersing bacteria have the ability to reinitiate the process of biofilm formation, on encountering an appropriate environment.

Kolodkin-Gal and colleagues reported that *Bacillus subtilis* biofilm disassembly is facilitated by a mixture of D-amino acids (D-leucine, D-methionine, D-tyrosine, and D-tryptophan) that are produced during the stationary phase of growth and get incorporated into the peptide side chains of peptidoglycan in place of the terminal D-alanine

(Kolodkin-Gal et al., 2010). This D-amino acid incorporation interferes with the anchoring of adhesive fibers on the cell surface, leading to fiber dissociation and loss of bacterial adherence, without influencing bacterial growth or expression of matrix components. Exogenous addition of the D-amino acid mixture or the individual D-amino acids disrupted preformed biofilms of *B. subtilis* and other bacterial species. Further studies revealed that D-amino acids work together with norsperimidine, another factor produced by *B. subtilus*, to cause biofilm disassembly (Kolodkin-Gal et al., 2010). Thus, D-amino acid/norsperimidine treatment may hold promising potential in preventing or eradicating biofilms (Kostakioti et al., 2013).

Coordination occurs through a series of mechanisms of cell-to-cell communication called quorum sensing system (QSS) that is discussed in detail below (Davies et al., 1998).

Quorum Sensing System

The capacity to recognize the population density by measuring the accumulation of a specific signaling molecule that members of the community secret, is conferred by a QSS. In order to activate the response, the population density must be high enough to accumulate the signal in the extracellular environment. Because QSS regulatory networks are usually very intricate and may include several genes whose products affect biofilm development at different stages, it is not always easy to understand how the activation of QSS finally triggers biofilm dispersion (Solano, Echeverz, & Lasa, 2014). Some pathways have been studied to find a genetic and enzymatic regulatory mechanisms in the biofilm formation. Winkelströter (2015) identified the bacteriocin plantaricin NC8 produced by *L. paraplantarum* FT259 and detected its gene, which they suggested may

be responsible for the antimicrobial activity observed in biofilm formed by co-culture against *L. monocytogenes* (Winkelströter et al., 2015).

1.4. Detection of biofilms

Diverse procedures and devices, have been applied for investigating microbial adhesion on surfaces. They differ widely in experimental conditions, detection equipment, and the variables that can be measured (Wagner et al., 2014). Examples of methods to detect biofilm production in laboratory are, tissue culture plate, tube method, Congo Red Agar method, bioluminescent assay, piezoelectric sensors, and fluorescent microscopic examination(Hassan et al., 2011). Microbes cannot be measured in situ, experimental studies use medium and growth optimal conditions to mimic biotic environments. Thus, discontinuous systems have major drawbacks for fundamental studies of microbial adhesion on abiotic surfaces (Wagner et al., 2014). In contrast to in vitro studies on biofilm formation, a different transition from initial attachment to colony formation and exponential growth could not be observed in the marine environment by Fischer and colleagues (2014). Initial attachment was followed by an adaptation phase of low growth and homogeneously distributed solitary bacterial cells. Moreover, the biofilm formation process was suggested to be modeled by three consecutive development stages endorsed to initial bacterial attachment, bacterial growth, and attachment and growth of unicellular eukaryotic microorganisms (Fischer, Friedrichs, & Lachnit, 2014).

2. **Biosurfactants**

Biosurfactants (BSs) have been defined by Cooper (1986) and Banat (1995) as "structurally diverse and heterogeneous groups of surface-active amphipathic molecules synthesized by microorganisms". Moreover, most recent reports have discovered that also some plants and animals are surfactant producers. Biosurfactants are capable of interaction with the phase boundary between two phases in a heterogeneous system, defined as the interface, leading to the reduction of its tension (Fig. 2). BSs are produced on the third stage of biofilm formation to disrupt interaction between its matrix components. Hence, freeing resident live bacteria. Therefore, BSs offer a widespread applicability and advantage due to their specific modes of action, low toxicity, and relative ease of preparation (L. Rodrigues, Banat, Teixeira, & Oliveira, 2006).

It has been known that hydrocarbon-degrading microorganisms produce biosurfactants to increase the apparent aqueous solubility of the hydrocarbon by forming micelles (stable aggregates of 10 to 200 molecules) or to change cell surface properties to bring the organism to such hydrocarbon. BSs can be intracellular (remain attached to the cell wall) or can be excreted out of the cell to the media (extracellular). Intracellular BS structure

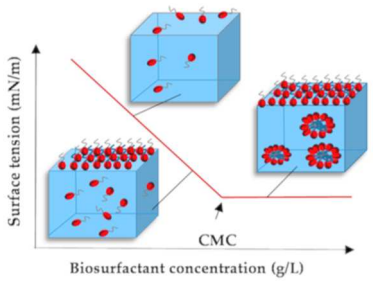

Figure 2. Illustration of regions in which micelle formation occurs (critical micelle concentration CMC). Surfactant molecule: red polar head soluble in water, hydrocarbon tail soluble in oil (Santos, Rufino, Luna, Santos, & Sarubbo, 2016)

include membrane lipids and promote the transport of insoluble substrates through the membrane. Whereas extracellular (or cell-free) BS help on the substrate solubilization and are usually a complex structure of lipids, proteins and carbohydrates (Desai & Banat, 1997). Understanding the different roles and significance of biosurfactants to microbes is a key step towards the study of these compounds. Such roles could be unique to the physiology and ecology of the producing microorganisms; therefore, it is difficult to identify general functions that are common among all biosurfactants. Regarding such roles, alterations on the surface or interfacial tension are needed for the erection of fruiting bodies, gliding motility, swarming of cells, and biofilm formation and development (McInerney, 2010).

Adhesion is a physiological mechanism for growth and survival of cells in natural environments. The primary process affecting bacteria transport, which determines its fate in the subsurface, is the growth of bacteria on water insoluble hydrocarbons. Thus, the bacterial growth on some surfaces is influenced by the BS, that forms a conditioning film on an interface, thereby stimulating certain microorganisms to attach to the interface, while inhibiting the attachment of others. These results indicate that the attachment or detachment from surfaces occurs as needed (Rosenberg, 1999). In fact, mechanisms for detachment appear to be indispensable for all attached microorganisms to facilitate dispersal and colonization of new surfaces.

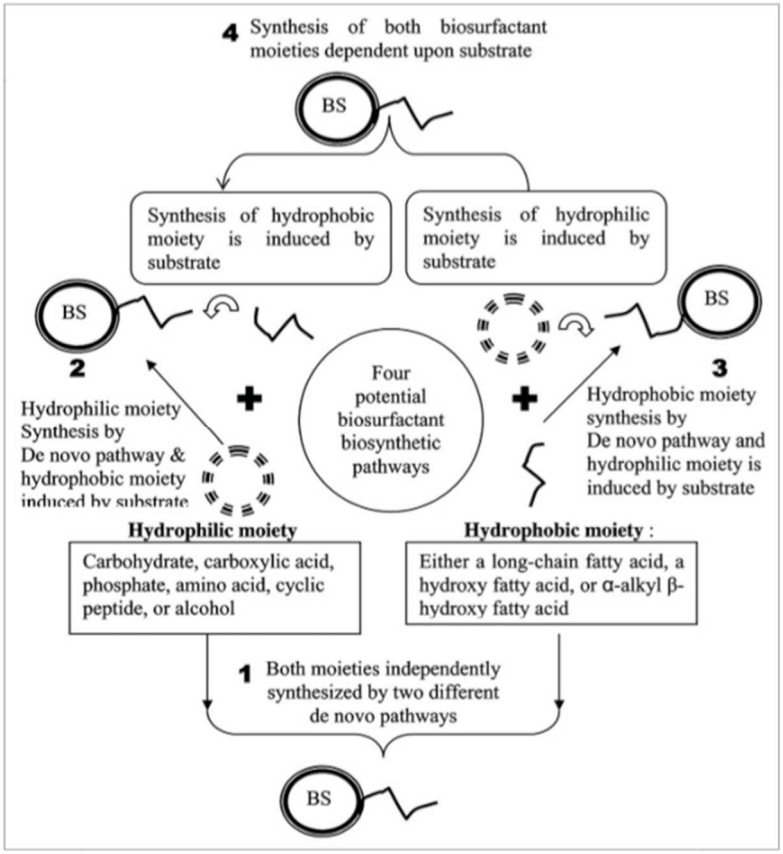

Figure 3. Syldatk and Wagner proposed assumptions in biosynthetic pathways of biosurfactants (Ramkrishna, 2010b)

In addition to the two main natural roles of biosurfactants, increasing availability of hydrophobic substrates and regulating attachment/detachment to and from surfaces, BS seems to be an evolutionary defense strategy as evidenced by antimicrobial activity.

Syldatk and Wagner (1987) proposed four possible biosynthetic pathways, that are shown in Figure 3, both moieties are synthesized independently of the growth substrate (de novo), with a hydrophobic carbon source such as fatty acids and triglycerides, the lipid moieties are directly derived from the carbon source, but the sugar is synthesized de novo, or the sugar moiety is directly derived from the carbon source, but the lipid component is synthesized de novo.

The release and composition of BS from each bacterial genus has been shown to be growth-dependent and to vary with each specie and strain. It has been suggested that in order to evaluate the properties of a BS, conservation methods are needed for over 120 days, to estimate the commercial validity of the product. Some of these methods are used separately or in combination (such as heating, acid precipitation, solvent extraction, ultrafiltration) to purify the supernatant and prevent growth of mold (Vijayakumar & Saravanan, 2015). To select a specific recovery method three major criteria should be considered, the cost of the extraction method, the purpose of the final product due to its influence in the purity level required, and the particular fermentation process in which the method may be adapted (Heyd et al., 2008). Because of these criteria and the diversity of biosurfactants, the recovery methods for each type of BS are discussed under each category.

The United States, Canada, China and Western Europe surfactant industry was valued at $20 billion in 2006, with these countries representing 70% of the worldwide market (Janshekar et al., 2007). The biosurfactant market is growing rapidly, and according to a report, global biosurfactant market was worth USD 1.7 billion in 2011, which is expected to reach USD 2.2 billion in 2018, growing at an average annual growth rate of 3.5% from 2011 to 2018 (http://www.prweb.com/releases/2012/11/prweb10158903.htm accessed on 12th February, 2017). The high cost of biosurfactant production is one of the major restraint of BS commercializing. In fact, only surfactin and rhamnolipids are commercially available and the later are the only biosurfactant thus far that has been approved by US Environmental Protection Agency for its use in food products, pharmaceuticals, and cosmetics (Nitschke & Costa, 2007).

2.1. Physicochemical properties of biosurfactants

Biosurfactants are characterized by physicochemical properties, surface and interfacial activity, emulsification, specificity, toxicity and biodegradability, temperature and pH; which are common to most surfactants produced by microorganisms.

1.1 Surface and interfacial activity

Efficiency of a surfactant is measured by its critical micelle concentration (CMC), that is a remarkable feature of biosurfactants, much lower than chemically made surfactants (Fig. 2). Having a low CMC means that BS are effective at low concentrations; thus, small amounts are needed to reduce interfacial tension. These ranges from 1 to 2000 mg/L. On the other hand, effectiveness is evaluated with the interfacial (oil/water) and surface tensions are respectively approximately 1 and 30 mN/m. A reduction of the water surface tension from 72 to 35 mN/m and the interfacial tension of n-hexadecane from 40 to 1 mN/m, is considered as a good surfactant (Santos et al., 2016). When surfactants monomers are added into a solution, the surface or interfacial tension will be reduced until the surfactant concentration reaches the CMC. At the CMC, surfactant monomers begin to spontaneously associate into structured aggregates such as vesicles, micelles, and lamellae (continuous bilayers). These aggregates are formed due to various weak chemical interaction between the polar head groups and the nonpolar tail groups such as hydrophobic, Van der Waals, and hydrogen bonding (Soberon-Chavez, 2011).

The affecting factors to the CMC for any surfactant include its structure, pH, temperature and ionic strength of the solution; thus, the aggregate structure is dictated by the polarity of the solvent in which the surfactant is dissolved. For instance, in oil, the polar head

groups will aggregate in the center of the micelle whereas the hydrophobic tails will be turned toward the outside of the micelle (Soberon-Chavez, 2011).

1.1 Emulsification

An emulsifying or dispersing mediator not only affects a reduction in the average particle size but also modifies the surface properties of the particle in a fundamental manner. Only a small amount can noticeably alter the surface charge, hydrophobicity and most remarkably pattern identification based on the three-dimensional structure of the adherent polymer (Velikonja & Kosaric, 1993). Depending upon its composition and the ecology, a biosurfactant, can be either emulsifier or de-emulsifier, because of the two basic types of emulsion, oil-in-water and water-in-oil (Santos et al., 2016). The addition of biosurfactants to an emulsion, may lead to a higher stability of the heterogeneous system. Whereas emulsification activity is a natural process of many hydrocarbon-degrading microorganisms, due the interfacial surface is between water and oil, can be a limiting factor. This evidence is indirect, emulsification may provide an evolutionary advantage for the microorganism that produces the emulsifier (Ramkrishna, 2010b).

1.1 Specificity

As a result of their complex molecules with specific functional groups, biosurfactants often have more specific action than chemically synthesized materials. For example, specificity of emulsan towards a mixture of aliphatic and aromatic hydrocarbons and that of solubilizing factor of Pseudomonas PG1 towards pristane (a saturated terpenoid alkane, used as a lubricant, a transformer oil, and an anti-corrosion agent, biological marker, plasmocytomas inducer and in production of

monoclonal antibodies) (Source: *PubChem Compound*. USA: National Center for Biotechnology Information (NCBI). 26 March 2005. Identification and Related Records).

1.1 Toxicity and Biodegradability

Biosurfactants have shown less toxic or non-toxic effects when compared to synthetic surfactants. Since up to 18% of detergent and household cleaning products components are surfactants, their environmental impact is a concern. Hence, BS offer an advantage due its lower toxicity and biodegradability features, which also make them adequate for bioremediation and waste treatment (Soberon-Chavez, 2011).

BS also exhibit a natural promotion of high biodegradability (Banat, 2000). They can be used as emulsifiers, de-emulsifiers, wetting and foaming agents, which are desirable qualities in different industries. Some are suitable alternatives to synthetic medicines and antimicrobial agents, and as detergents in petroleum, petrochemicals, environmental management, agrochemicals, cosmetics, pharmaceuticals, commercial laundry detergents, functional food ingredients, and in the mining industry (Ramkrishna, 2010a). A review (A.M. Shete, 2006) reports more than 250 patents obtained on BS. They also have potential use in modern day agricultural practices as a hydrophilizing and wetting agent for achieving the equal distribution of fertilizers and pesticides in the soils (Cameotra et al., 2010).

2.2. Factors affecting biosurfactant production

The physicochemical factors affecting the production of surfactant are indeed dependent on the producing microorganism, its growth conditions and the composition of the BS that is expected as well. Because of that, it is difficult to standardize production methods

for a class of biosurfactants. Instead, the optimization efforts are quite specific and some examples are discussed below.

2.2.1. Carbon source

Several studies have demonstrated that the carbon source affects the production yield and composition of biosurfactants. For example, *Candida bombicola* showed high yields of sophorolipids, 120 g/L, after eight days using oil and sugar as carbon sources. In contrast, when glucose and canola oil were used as carbon sources, the highest yield of sophorolipids was 8 g/L obtained by *C. lipolytica (*Santos et al., 2016*)*. Moreover, a yield of the protein-lipid-carbohydrate biosurfactant was 4.5 g/L, produced by *C. lipolytica*, when industrial waste was used as carbon source. Surfactin like biosurfactant was reported from *Bacillus subtilis* MTCC 2423 in which sucrose was the best carbon source (Cameotra & Makkar, 2004)

2.2.2. Nitrogen source

The second most important supplement for biosurfactant production in fermentative processes is the nitrogen source, and more so the C/N ratio. High C/N ratios limit bacterial growth and increases the production of metabolites. On the other hand, high levels of nitrogen lead to the synthesis of cellular material and limits the buildup of products. *P. aeruginosa* uses amino acids, nitrates and ammonium as nitrogen sources. Urea and ammonium nitrate have been also used as nitrogen sources for *Candida* species (Brint & Ohman, 1995).

2.3. Classification of biosurfactants

Whereas synthetic surfactants are commonly classified per the nature of their polar groups, BS are generally classified based on their biochemical composition and microbial origin. Most of biosurfactants are either anionic or neutral, whereas those that contain amine groups are cationic. The hydrophilic moiety can be an amino acid, alcohol, carbohydrate, cyclic peptide or phosphate carboxyl, and the hydrophobic moiety has long-chain fatty acids. The microbial surfactants are complex molecules in the range of peptides, fatty acids, glycolipids such as trehalose lipids, rhamnolipids, sophorolipids, diglycosyl diglycerides and mannosylerythritol lipids; lipopeptides like surfactin, iturin, fengycin and lichenysin. Table 2 is a display and summary of the principal BS type reviewed in this paper as classified by their composition. Biosurfactants can be also classified according to their low or high molecular weight, the low molecular weight type is the most studied group that includes two important subgroups: glycolipids and lipopeptides (Gudina, Rangarajan, Sen, & Rodrigues, 2013).

2.3.1. Glycolipids

Glycolipid biosurfactants are composed of a hydrophobic moiety that consists in a long-chain fatty acid (that can be aliphatic, hydroxylated, or unsaturated) combined with a hydrophilic carbohydrate-based component (such as glucose, trehalose, mannose, galactose, sophorose, or rhamnose). The best-known families of glycolipid biosurfactants are rhamnolipids, sophorolipids, trehalose lipids, and mannosylerythritol lipids (MELs) (Paulino et al., 2011).

Table 2. Major types of biosurfactants and their producing organisms (Modified from (Sekhon, Khanna, & Cameotra, 2011)

Biosurfactant type	**Producing microorganisms**
GLYCOLIPIDS	
Diglycosyl diglycerides	*Lactobacillus fermenti*
Rhamnolipids	*Pseudomonas* spp.
Sophorolipids	*Candida* spp. *Torulopsis bombicola*
Trehalose lipids	*Mycobacterium* spp.
PHOSPHOLIPID AND FATTY ACIDS	
Phospholipids	*Thiobacillus thiooxidans*
Fatty acids	*Corynebacterium* spp.
Neutral lipids	*Nocardia erythropolis*
LIPOPEPTIDES AND LIPOPROTEINS	
Polymyxin E1	*Bacillus polymyxa*
Surfactin	*Bacillus subtilis*
Polymyxin	*Bacillus polymyxia*
Gramicidin	*Bacillus brevis*
Serrawettin	*Serratia marcescens*
Viscosin	*Pseudomonas fluorescens*
POLYMERIC SURFACTANTS	
Emulsan (lipoheteropolysaccharide)	*Acinetobacter calcoaceticus* RAG-1
Liposan	*Candida lipolytica*
Protein PA	*Pseudomonas aeruginosa*
Carbohydrate-lipid-protein	*Pseudomonas fluorescens*
PARTICULATE SURFACTANT	
Vesicles	*Acinetobacter calcoaceticus*
Cells	Various bacteria

2.3.1.1.Rhamnolipids

Rhamnolipids are glycolipids composed by a hydrophilic group, one or two rhamnose molecules in *L*-form, linked to a hydrophobic group that can be a saturated or unsaturated β-hydroxy fatty acids (Figure 3). This type of surfactants can be produced using different low-cost substrates, alkanes, molasses, fructose, glycerol, pyruvate, citrates, glucose and olive oil, among other agro-industrial wastes, which is an advantage approach in environment (Benicasa et al., 2010; Lawniczak et al., 2013). In fermentative processes a variety of isoforms can be obtained, therefore their physicochemical properties may vary (Desai & Banat, 1997; Gudina, Fernandes, Teixeira, & Rodrigues, 2015). For instance, the reduction of water surface tension is between from 72 to 29-31 mN/m and the CMC values can vary from 20 to 225 mg/L in water (Syldatk et al., 1985; Dubeau et al., 2009), due to these characteristics, rhamnolipids are one of the most potent biosurfactants.

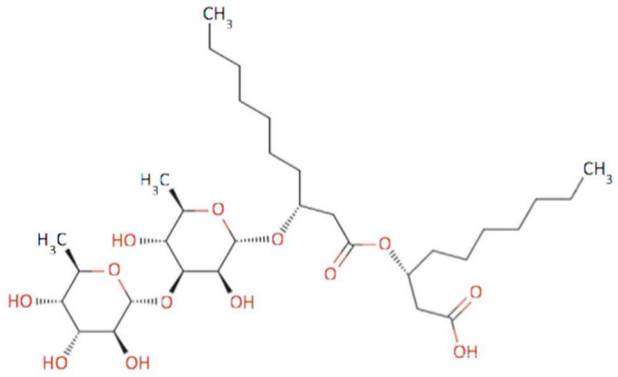

Figure 4. Structure of rhamnolipid that is predominantly produced by Pseudomonas species (Ramkrishna, 2010b).

Additionally, they present low toxicity and biodegradability, and have been focused on the biodegradation of petroleum hydrocarbons. They are incorporated in washing powder due to the 10% enhanced oil removal in combination with lipase (Bafghi & Fazaelipoor,

2012). However, rhamnolipids have been described as potentially toxic to some types of vegetation (Marecik et al., 2012).

P. aeruginosa is an opportunistic human pathogen found in long-term infections occurring in immunocompromised patients. Rhamnolipids are among the predominant virulence factors of *P. aeruginosa* (Burch, Shimada, Browne, & Lindow, 2010). The synthesis of this biosurfactant is influenced by numerous factors at both genetic control and environmental level; hence, it is regulated by QSS, a mechanism for cell density-dependent gene regulation and limitation of specific nutrients (van Ditmarsch & Xavier, 2011). Genes involved in rhamnolipid biosynthesis were found to be plasmid-encoded. The foundations of these understandings were deeply studied since 1963 and 1995 and are discussed below.

Per a proposed biosynthetic pathway (Fig. 5) (Burger et al., 1963), rhamnolipid synthesis is initiated with a reaction involving dimerization of two β-hydroxydecanoic acid chains, and proceeds by two sequential glycosyl transfer reactions, each catalyzed by a different rhamnosyltransferase. It was until 1994-1995 that Ochsner and Reiser used random transposon mutagenesis and genetic complementation (is a biological process that allows genes to be transferred to a host organism's chromosome, modifying the function of an extant gene on the chromosome and causing mutation) to identify the primary biosynthetic and regulatory genes. They found that the expression of rhlAB is positively controlled by QSS. The genes responsible to produce rhamnolipids were found to be grouped in an *rhl* gene cluster (Fig. 5). RhlA and RhlB are encoded by genes organized in an operon, that is flanked by the regulatory genes *rhlR* and rhlI (Ochsner, Koch, Fiechter, & Reiser, 1994). An operon is a set of genes transcribed under the control of an operator

gene that share related functions. Archeal and bacterial genomes normally contain a small number of highly conserved operons and a much larger number of unique ones. The members of these eukaryotic gene clusters contribute to a common function but do not usually share sequence similarity. These gene clusters therefore represent functional gene organizations with operon-like features (physical clustering and co-regulation) (Osbourn & Field, 2009).

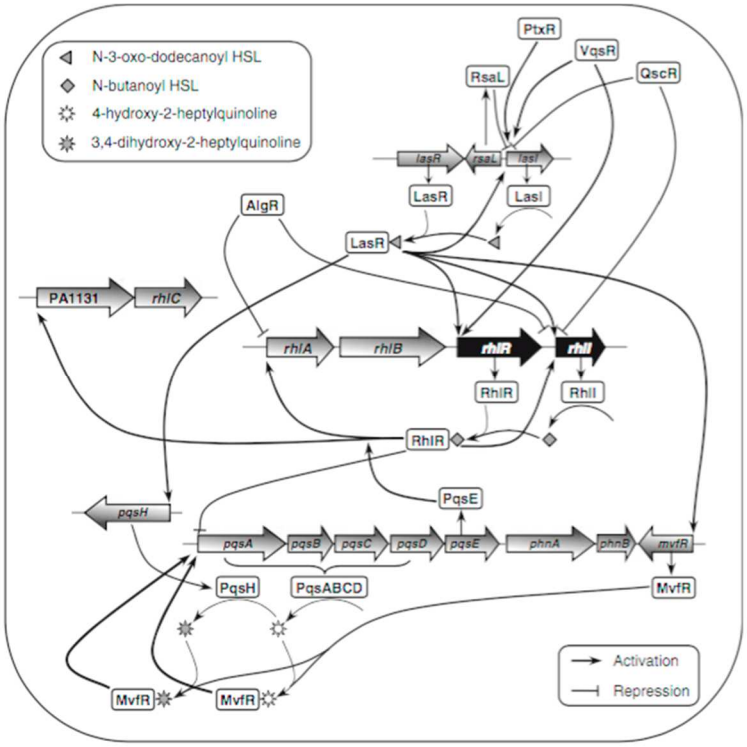

Figure 5. Genetic regulation of rhamnolipid biosynthesis in P. aeruginosa (Soberon-Chavez, 2011)

Moreover, RhlA is involved in the synthesis of rhamnosyltransferase precursor substrates or in the stabilization of the RhlB protein (Ochsner et al., 1994). *rhl*C encodes the second rhamnosyltransferase, and its expression had been shown to be coordinately regulated with rhlAB by the same QSS pathway (Rahim et al., 2001). The *rhl*R and *rhl*I act as regulators of the rhlAB gene expression. RhlI protein forms N-acylhomoserine lactones,

that act as autoinducers and influence RhlR regulator protein. Induction of *rhl*AB depends on QSS transcription activator RhlR complexed with the autoinducer N-butyryl-homoserine lactone (C4-HSL) (Medina, 2003; Rahim et al., 2001).

Another QSS that has an influence on the biosynthesis of rhamnolipid is encoded by *las*R and *las*I. The *las* system is both a positive and a negative regulator of the *rhl* system. The *lasI* and *rhlI* products are *N*-oxododecanoyl homoserine lactone (OdDHL, 3OC12HSL or PAI-1) (Pearson et al., 1994) and *N*-butyryl homoserine lactone (BHL, C4-HSL or PAI-2) respectively (Winson et al., 1995). The *las* system regulates the *rhl* system, which in turn regulates rhamnolipid synthesis (Soberon-Chavez, 2011).

Rhamnolipid production is promoted by enhanced C/N ratio (Winson et al., 1995) and inhibited by higher iron concentration (Guerra-Santos et al., 1986). Additionally, it was demonstrated that if ammonium is used instead of nitrate, as a nitrogen source, along with excessive iron, it decreases *rhl*A expression and swarming motility (Deziel et al., 2003).

Studies have demonstrated anti-tumor and anti-proliferative properties using gamma irradiation-enhancement of rhamnolipid biosurfactant against growth of a group of human cancer cell lines. The inhibitory effect of the BS is correlated with its cytotoxicity, possibly caused by surface tension reduction of the culture medium; thus, further studies in vivo might be needed to confirm such activities (Lotfabad et al., 2010).

Rhamnolipids have been also applied in food industry as antimicrobial and emulsifying agents and to disrupt (or prevent) biofilm formation of foodborne pathogens (Do Valle Tomes & Nitschke, 2012; Haba et al., 2014; Magalhaes & Nitschke, 2013; Banat et al., 2014; Philips, 2016). The suggested anti-adhesive property preventing the formation of

bacterial aggregates consists in the modification of surface hydrophobicity and interference into adhesive properties of microorganisms (Dusane et al., 2010). Moreover, when the biofilm has been formed, its removal by rhamnolipids could be due to interactions into micro colonies and alteration of biofilm environment and in the deletion of EPS; thus, causing the biofilm disruption (Diaz De Rienzo & Martin, 2016).

Heyd et al. (2008) reviewed the recovery methods of rhamnolipids, and the most common methods include acid precipitation, solvent extraction, adsorption, ion exchange chromatography, ultrafiltration, and foam fractionation. Acid precipitation lowers the pH to approximately 2, so the negative charges on the rhamnolipids are neutralized, which make them less soluble in the aqueous phase. And then, aluminum sulfate precipitates rhamnolipids by salting out. Finally, centrifugation is used to recover the precipitated rhamnolipid (Deziel et al., 1999; Zhang & Miller, 1992).

The secondly common used method is solvent extraction, where precipitation is achieved by acidification and then extracted with organic solvents, ether or ethyl acetate (Mata-Sandoval et al., 1999; Lepine et al., 2002; (Sekhon & Rahman, 2014)).

Other methods of rhamnolipid recovery are adsorption methods, that use hydrophobic adsorbents such as 16 polystyrene resin or amberlite XAD2 which retain amphiphilic substances through hydrophobic interactions. After rhamnolipid has been adsorbed, methanol releases it by elution (Dubey et al., 2005). Ultrafiltration using a membrane cutoff of 10 KDa leads to an almost complete retention of rhamnolipids at neutral pH (Haussler et al., 1998). Foam fractionation depends on the foaming capacity of rhamnolipids, the foam collapses in a separated receptacle by the action of shear forces or

acids, after directed out of the fermentation vessel. Then, the lamella (water in the film surface) is allowed to drain by gravitational force, causing a higher concentration of the surfactant in the collapsed foam (Sarachat et al., 2010).

2.3.1.2.Sophorolipids

Sophorolipids are generally produced by nonpathogenic yeast species (such as *Candida albicans, Candida bombicola, Candida floricola, Cryptococcus* spp., *Wickerhamiella domercqiae, and Pichia anomala*). This type of glycolipids structurally consists of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid through a glycosidic bond. (Hirata et al., 2009; Basak et al., 2014; Konish et al., 2015). Surface tension values of the range 40-24 mN/m (in water from 72.8 mN/m) and CMC values of 40-100 mg/L have been recorded for these compounds (Van Bogaert et al., 2011; Dengle-Pulate et al., 2013; Diaz De Rienzo et al., 2015). Sophorolipids initially have yields of 70 g/L as reported by Cooper and Paddock (1984), and extensive optimization of the culture conditions have yields of over 400 g/L (Pekin et al., 2005), which is an attractive alternative for petroleum-based surfactants.

These biosurfactants present two major forms, acidic or lactonic sophorolipids (Fig. 5). Sophorose is the hydrophilic disaccharide of the biosurfactant, with an unusual β-1,2 bond and may contain acetyl groups at the 6'- and/or 6" positions. The hydrophobic part of the amphiphilic molecule is made up by a terminal or sub terminal hydroxylated fatty acid, β-glycosidically linked to the sophorose molecule. The carboxylic end of the fatty acid is either free (acidic) or internally esterified at the 4' or, in some rare cases, at the 6'- or 6"-position (lactonic form) (Asmer et al., 1988; Van Bogaert et al., 2011). When

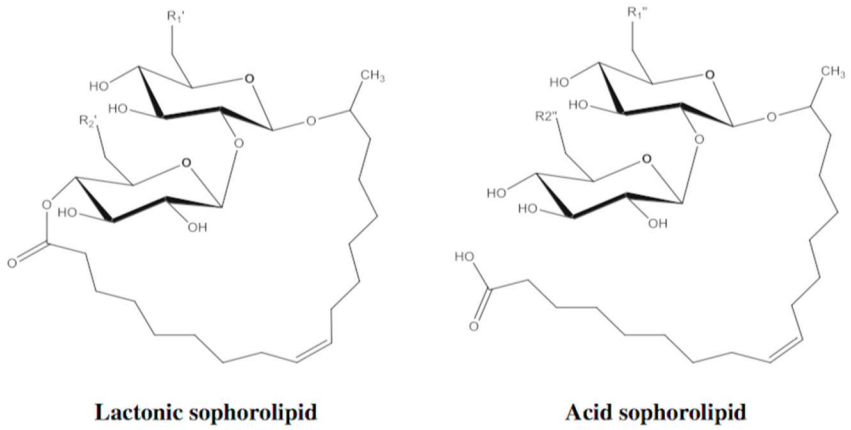

Figure 6. Lactonic and acid sophorolipid structures produced by Candida spp. (Paulino et al., 2016)

comparing the two sophorolipid types, the acidic forms have shown superior foaming capacity and solubility, whereas the lactonic forms have shown better surface and antimicrobial activities (Yang et al., 2012; Concaix 2003).

Various studies have demonstrated the influence of substrates in production and characteristics of sophorolipids. For instance, the use of alternative raw materials such as soy, molasses, animal fat, deproteinized whey and waste cooking oil, achieved good enough yields to reduce overall production cost (Shah et al., 2007; Solaiman et al., 2007; Shin et al., 2008).

Sophorolipids are one of the most common applied biosurfactant in the cosmetic and cleaner industries and with products available in the market due to their high yields and results indicating no irritation and allergic reaction on human skin (Hillion et al., 1998), translated into economic advantages (Van Bogaert & Soataert, 2011). In addition, a microbial enhanced oil recovery (MEOR) process consisting in the injection of a sophorolipid mixture in well treatment operations in the petroleum industry was patented by Baker Hughes Company (Amstrong et al., 2015).

Other potential applications for sophorolipids have been studied and patented. For example, the patents developed by researchers from the Polytechnic Institute of New York University and Synthezyme Company, claimed biopesticide and antifungal and anti-inflammatory agents in different sophorolipids compositions (Gross and Schofield, 2011). Saha et al. (2005) reported sophorolipid diacetate ethyl ester derivative has better anti-HIV and spermicidal activities than monoacetylated and nonacetylated ethyl esters. Moreover, synergistic action of sophorolipids with antibiotic cefaclor (second generation cephalosporin antibiotic used to treat bacterial infections) was reported (Joshi-Navarrete & Prabhune, 2013) against pathogenic microorganisms *E. coli* ATCC 8739 and *Staphylococcus aureus* ATCC 29737. In another study by Zhang et al. (2016), the results suggested that sophorolipids could be used as sanitizers in wash water for control of foodborne pathogens. Antimicrobial potential of diacetylated lactone form of sophorolipid derived from glucose and oleic acid was evaluated against *E. coli* O157:H7 in vitro, in which the pathogen was inactivated. It was also evaluated on spinach leaves during storage, where no significant reduction of *E. coli* O157:H7 was observed (X. Zhang et al., 2016). Furthermore, Diaz De Rienzo et al. (2015) used a mixture of acidic and lactonic sophorolipids obtained from fed-batch cultivation of *C. bombicola* ATCC 22214 with glucose and rapeseed oil as substrate, to demonstrate antimicrobial properties and biofilm disruption capacity of these BS against *Cuprivavidus necator* ATCC 17699, *B. subtilis* BBK006, and *S. aureus* ATCC 9144. Moreover, anticancer activity studies performed by Chen et al. (2006) and Shao et al. (2012) reported that the sophorolipid produced by *W. domercqiae* induced apoptosis in H7402 human liver cancer cells with inhibition of cell proliferation by blocking cell cycle at G1 (growth) and partly S phase

(DNA replicate). In addition, Rashad et al. (2014) screened anticancer activity of *C. bombicola* NRRL Y-17069 sophorolipid and the results showed inhibition of urokinase and histone deacetylase activities, being promising anticancer agent in hepatocellular carcinoma HepG2 and lung cancer A549 (Rashad et al., 2014).

The suggested sophorolipid biosynthesis pathway by (Soberon-Chavez, 2011) describes two main inputs, a hydrophilic substrate such as glucose and a lipophilic substrate. Since sophorolipid-producing yeast strains such as *C. bombicola* and *C. apicola* can grow on alkanes, they possess the enzymes for the terminal oxidation of alkanes (cytochrome P450 mono oxygenases belonging to the CYP52 family). In the subsequent steps, the derived alcohol will be converted via an aldehyde to its corresponding fatty acid, which can then be metabolized in β-oxidation or act as precursor for specific biosynthetic processes such as sophorolipid synthesis.

Additionally, fatty acids directly supplemented to the medium or derived from lipids act as feedstock for sophorolipid synthesis. If no hydrophobic substrate is present in the medium, fatty acids will be formed de novo starting from acetyl-coenzyme A (CoA) derived from the glycolysis pathway (Van Bogaert et al., 2008).

2.3.1.3.Trehalose lipids

Trehalose lipids, also known as trehalolipids, are a group of glycolipids mainly produced by gram-positive bacteria of Actinomycetales, such as *Mycobacterium* spp.*, Micrococcus* spp.*, Nocardia* spp.*, Gordonia* spp.*, Dietzia* spp.*, Tsukamurella* spp.*, Skermania* spp.*, Williamsia* spp.*, Corynebacterium* spp.*, Brevibacteria* spp.*, Arthrobacter* spp.*,* and *Rhodcooccus* spp. However, yeasts and fungi species have been reported as producers of

this type of biosurfactants (Soberon-Chavez, 2011). As a result of the structural diversity of these compounds, they have different surfactant physicochemical properties. Most of them showed a moderate to good reduction of surface tension of water, from 72 to 43-19 mN/m while the CMC values are 0.7-37 mg/L with 1-2000 mg/L the range for a BS considered as good, the lowest the value, the more effective the surfactant (Yakimov et al., 1999; Marques et al., 2009).

Trehalolipids, like other glycolipid biosurfactants, are composed of a hydrophilic moiety (i.e., trehalose) in combination with fatty acid groups and their hydrophobic moieties are more diverse than other glycolipids, aliphatic acids and mycolic acids and triesters (Gautier et al., 1992; Asselineau & Asselineau, 1978; Petrikov et al., 2013). Trehalose is a non-reducing disaccharide formed from two glucose units joined by a 1-1 α bond. This bond makes trehalose very resistant to acid hydrolysis, with high thermostability, and nonreactive to Maillard reaction (Higashima, 2002; Shao, 2011). Furthermore, trehalose contributes to the biological properties attributed to trehalose lipids due to its cryoprotection properties, growth regulation in plants, osmoregulation, protection of the cornea against oxidative damage caused by UVB rays and suppression of the proinflammatory cytokines (Duong et al., 2006; Higashima, 2002; Cejkova et al., 2011).

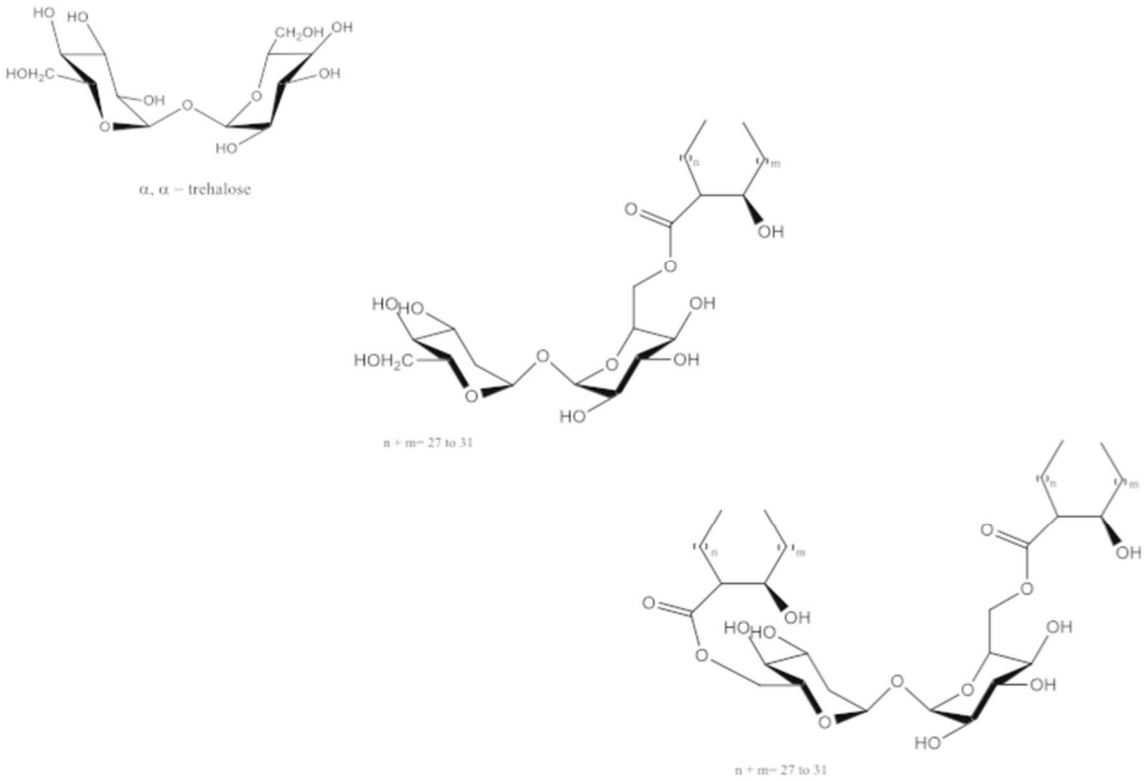

Figure 7. Structures of trehalose lipids (monomycolate and dimycolate) produced by Mycobacteria species (Paulino et al., 2016).

Trehalose lipids are found in different forms such as, trehalose monomycolates, dimycolates (Fig. 8), trimycolates, nonionic acylated trehalose derivatives, anionic trehalose tetraesters, and succinoyl trehalolipids (Kugler et al., 2014). The most known among trehalolipids is called the "cord factor", 6,6'-trehalose lipid dimycolate and it is composed by two mycolic acids of variable number of carbons esterified to the 6-hydroxyl group of each glucose (Ishikawa et al., 2009; Ryll et al., 2001; Shao, 2011). The cord factor structure varies greatly among mycobacterial species, and the mycolyl moiety has been related with toxicity and antigenicity, thereby constituting potential virulence (Ryll et al., 2001; Hunter et al., 2006; Guidry et al., 2007; Ishikawa et al., 2009).

The production of most trehalose lipids is growth-dependent, substrate and cell wall-associated, and the yield is lower than other glycolipids (Shao, 2011). Uchida et al.

(1998) reported a high trehalose lipid yield of 40 g/L using *Rhodococcus* sp. SD-74 with n-hexadecane under highly osmotic conditions. The substrates used for trehalolipids productions can be n-alkanes or nonalkanes, for instance the oil degrading bacteria *Rhodococcus* have optimal yield production using n-alkanes (Niesher et al., 2006). Whereas, in the absence of n-alkanes or other lipophilic carbon sources trehalose lipids have been produced by *Brevibacterium vitarumen* 12143 (Laneelle and Asselineau 1976), and different pathogenic *Mycobacteriaceae* (Asselineau and Asselineau, 1978).

The problem of low concentration in the production of trehalolipids has been tackled by statistical methods to increase yields and reduce process costs. For instance, Mutalik et al. (2008) achieve an increase of 3.2 to 10.9 g/L in the concentration of trehalose lipids, produced by *Rhodococcus* spp. MTCC2574 using n-hexadecane as a substrate (Mutalik et al., 2008). Additionally, genetic engineering has been used to improve production of trehalolipids in *Gordonia amarae* with the insertion and expression of the *Vitreoscilla* hemoglobin gene (*vgb*), resulting in enhancement of trehalose lipid production in a medium supplemented with hexadecane (Dogan et al., 2006).

The biological activities of trehalolipids include immune regulation, antiviral properties, inhibitory activity on calcium-dependent protein kinase C of human promyelocytic leukemia HL60 cells, inhibitory effects in growth and differentiation-induced against human leukemia cells (Lang & Philip, 1998; Sudo et al., 2000; Baeva et al., 2014).

2.3.1.4.Mannosylerythritol lipids

Mannosylerythritol lipids (MELs) are a family of nonionic glycolipid biosurfactants that contains 4-O-β-D-mannopyranosyl-*meso*-erythritol as the hydrophilic group and a fatty acid and/or an acetyl group as the hydrophobic moiety (Kitamoto, 2008). MELs are

mainly produced by *Pseudozyma* spp., *Ustilago* spp., and also by *Schizonella* spp., *Kurtzmanomyces* spp., *Candida* spp. (Kitamono et al., 2002; Haskins et al., 1955; Deml et al., 1980; Kitamoto, 1990; Kakugawa et al., 2002). Based on the degree of acetylation at C4 and C6 positions, the MELs produced by *Pseudozyma aphidis* are classified as MEL-A (diacetylated), MEL-B (monoacetylated at C6'), MEL-C (monoacetylated at C4') and MEL-D (deacetylated) (Kitamoto et al., 2001; Rau et al., 2005; Sajna et al., 2013).

MEL-A is the most common type, among all the types of MELs and it has shown a reduction of the surface tension of water from 72 mN/m to values below 30 mN/m (Morita et al., 2009; Konishi et al., 2007). *Kurtzmanomyces* sp. I-11, *Candida antarctica* KCTC 7804, *P. aphidis* and *Pseudozyma rugulosa* NBRC 10877 were reported to produce MELs with a major MEL-A composition (Kakugawa et al., 2002; Kim et al., 1999; Fan et al., 2014; Morita et al., 2006). *Pseudozyma tsukubaensis* and *Ustilago scitaminea* NBRC 32730 produce high percentages of MEL-B glycolipids and are quite different in structure, 1-O-β-(2',3'-di-O-alka(e)noyl-6'-O-acetyl-D-mannopyranosyl)-D-erythritol with the fatty acid chain length C8 and C14 and, 4-O-β-(-2',3'-di-O-alka(e)noyl-6'-O-acetyl-D-mannopyranosyl)-erythritol with the fatty acid chain length C8 and C10, respectively (Fukuoka et al., 2008; Morita et al., 2009). Among MEL-C producers, *Pseudozyma hubeiensis* KM-59, *Pseudozyma shanxiensis*, *Pseudozyma siamensis* CBS 9960 and *Pseudozyma graminicola* CBS 10092 have shown to reduce the surface tension of water to 33.8 mN/m at a CMC of 0.00036 M (Konishi, 2008; Konishi, 2007; Morita et al., 2008).

The genetic basis of the glycolipid production and regulation in fungi is largely unknown, only *Ustilago maydis* has reports of genes involved in its MEL biosynthesis (Hewald, Josephs, & Bolker, 2005). Spockener et al. (1999) found that *U. maydis* produces ustilipids (mannosylerythritol lipid and ustilagic acid (cellobiose lipid)) under nitrogen starvation conditions, and the yield and ratio of both classes of glycolipids depend on the available carbon source. The gene *emt1,* a putative glycosyltransferase, is required for the production of MELs and its expression is enhanced by nitrogen starvation. Whereas, the gene *cyp1* is essential for the production of cellobiose lipid in *U. maydis* (Hewald et al., 2005). Additionally, it has been suggested that the *U. maydis emt1* protein may use GDP-mannose for the generation of the mannosylerythritol moiety of ustilipids and this reaction has to be stereospecific, because only mannosyl-D-erythritol is generated (Hewald et al., 2005). Moreover, Hewald et al. (2006) identified the gene cluster for the biosynthesis of MEL by *U. maydis,* which contains five proteins (Fig. 9), one glycosyltransferase, three acyltransferases, and one export protein of the major facilitator family (Hewald et al., 2006).

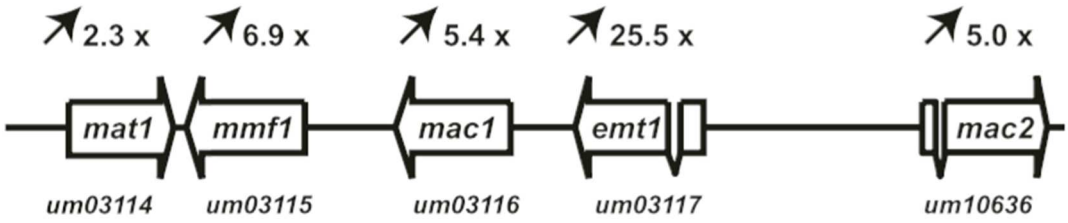

Figure 8. Biosynthetic gene cluster for the biosynthesis of MEL by Ustilago maydis (Hewald et al., 2006).

It was demonstrated by mutation analysis, that the putative acyltransferases, mac1 and mac2, are both essential for the biosynthesis of MEL. The generation of mannosylerythritol by mannosylation of erythritol, which is most probably catalyzed by

emt1, would be the first step in the biosynthetic pathway of MELs (Fig. 10) in *U. maydis* (Hewald et al., 2006). Mannosylerythritol is subsequently acylated with fatty acids by the putative acyltransferases mac1 and mac2 at positions C2 and C3, respectively. This acylation reaction appears to be essential for secretion because deletion of either mac1 or mac2 abolished MEL production completely. However, the order of activity of these two enzymes is unclear (Hewald et al., 2006).

Another study by Morita et al. (2006) identifying the genes involved in the biosynthesis of MEL by *P. antarctica* T-34 in the presence of soybean oil, indicated that mannosyltransferase and acyltransferase were involved in the biosynthetic pathway. Real-time reverse transcriptase-PCR was used to generate 398 expressed sequence tags (ESTs) assembled into 146 contiguous sequences, followed by basic local alignment search tool (BLAST) that showed that 21.4% of all contigs were orphans, while 78.6% showed similarity to sequences in the protein database. A 60.3% of all contiguous sequences share significant identities to hypothetical protein of *U. maydis* (Morita et al., 2006).

The biosynthesis of MEL is not growth-associated, and it can also be produced by using resting cells (cells in stationary phase) of yeast. These glycolipids act as energy storage material in the yeast like triacylglycerols (Kitamoto et al., 2002). Soybean oil, olive oil and safflower oil were found to be the best carbon sources using *Pseudozyma* spp., when compared to other vegetable oils such as palm oil, corn oil, rapeseed oil, and coconut oil (Morita et al., 2008). The best nitrogen source reported was sodium nitrate (0.3%, w/w) against ammonium nitrate and ammonium sulfate, while the decrease of pH by the

consumption of ammonium salts leads to a decrease in MEL yield (Kitamoto et al., 1990; Rau et al., 2005; Konishi et al., 2008). The MEL yield increased about 50% when erythritol, glucose and mannose were added by *P. rugulosa* (Morita et al., 2006).

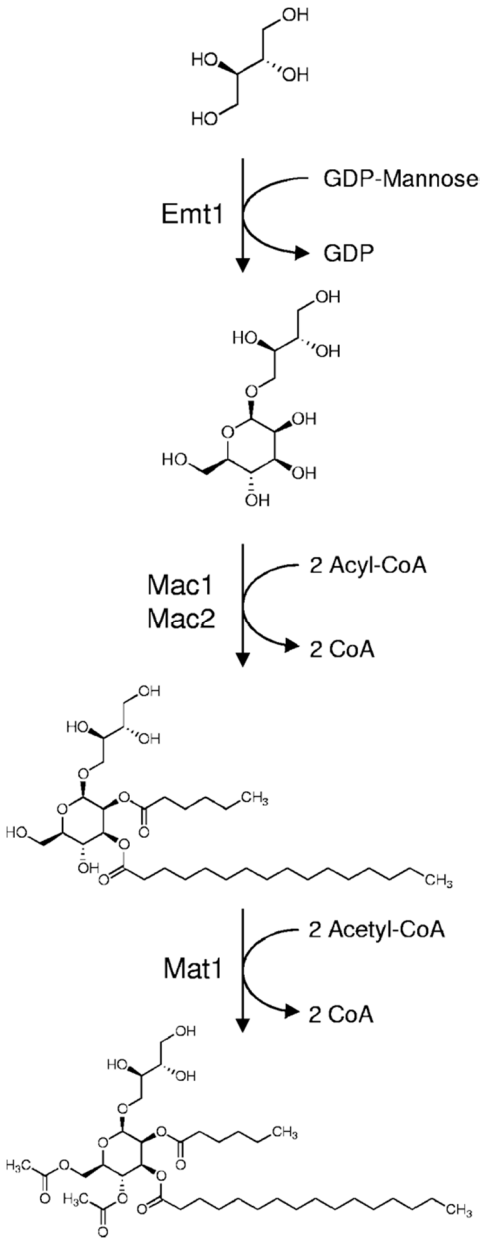

Figure 9. Proposed biosynthetic pathway of MEL under nitrogen limitation (Hewald et al., 2006)

MELs have self-assembly properties that can be defined as the spontaneous and reversible organization of molecular units into ordered structures by noncovalent interaction. Ionic and nonionic surfactants can form three-dimensional lyotropic liquid crystals such as, cubic, lamella, sponge, and hexagonal phases, which are stabilized by hydrogen bonds between the sugar moieties. MELs also self-assemble into monolayer, liposomes, large unilamellar, and multilamellar vesicles (Imura et al., 2006, 2007; Kitamono, 2008; Worakitkanchanakul et al., 2008).

The biological activities of MELs include antimicrobial, antioxidant, induction of cell differentiation and apoptosis (Arutchelvi & Doble, 2011). MEL-A and MEL-B showed strong activity against gram-positive bacteria, lower activity against gram-negative bacteria, and no activity against fungi (Deml et al., 1980). Takahashi et al. (2012) reported that MEL-B from *U. scitaminea* and MEL-C from *P. hubeiensis* showed high and moderate antioxidant activity, respectively, when compared with arbutin, a strong scavenger used as positive control. Another study using MEL-A and MEL-B produced by *P. antarctica* T-34 demonstrated the inhibition of inflammatory mediators from mast cells (a type of white blood cells) (Morita et al., 2011).

Mannosylerythritol lipids can also be used as a vehicle for gene and in drug delivery due to their ability to self-assemble into thermodynamically stable vesicles with the ability to fuse with the membrane (Inoh et al., 2004; Kitamoto 2008). Additionally, MELs are applied in cosmetics for skin care and repairing effects on the damaged hair (Yamamoto et al., 2012; Morita et al., 2010). Moreover, Sajna et al. (2013) indicated the potential use of MELs in laundry detergent formulations, being effective at different temperature and pH conditions when removing stains in fabric wash analysis.

The major challenge to the incorporation of glycolipids in products consists in the development of stable and cost-effective biotechnological methods in large scale, to increase their availability to the global market. Other less known microorganisms have shown the production of glycolipids, such as *Burkholderia* spp., *Myxococcus* spp., *Enterobacter* spp., *Pseudoxanthomonas* spp., *Acinetobacter* spp., and recently *Streptomyces* strains. On the other hand, the most studied nonpathogenic strains that produce glycolipids are *Pseudomonas putida*, *Acinetobacter calcoaceticus*, *Enterobacter asburiae*, and *Burkholderia thailandensis* (Paulino et al., 2016). There are a few studies reporting *Lactobacillus* biosurfactant as glycolipid, but also other reports state different composition. Therefore, BS produced by *Lactobacillus* spp. are discussed under its own category (2.4).

2.3.2. Lipopeptides and lipoproteins

The lipopeptide and lipoprotein types of biosurfactants consist of a high number of cyclic hydrophilic peptides attached to a hydrophobic fatty acid chain. The discovery and study of these BS has been reported since 1950s and more than hundred different compounds have been described, mainly produced by *Bacillus* spp. (Soberon-Chavez, 2011). Among their biological activities surface activity, anti-cellular and anti-enzymatic, are also swarming motility and biofilm formation. Both gram-positive and gram-negative bacterial genera, *Bacillus* and *Pseudomonas* respectively, produce a wide range of effective lipopeptides. Interestingly, this type of BS has been the most analyzed for its biosynthetic mechanisms and gene regulation systems (Roongsawang, Washio, & Morikawa, 2010).

Because of their complex and diverse structures lipopeptide biosurfactants produced by *Bacillus* spp. are classified into surfactins, iturins, fengycins or plipastatins, and the novel groups of kurstakins, reported in 2000 (Hathout et al., 2000). Furthermore, lipopeptide BS produced by *Pseudomonas* spp. have been classified into six groups: amphisin, syringomycin, viscosin, putisolvin, syringopeptin and tolaasin (Gross & Loper, 2009).

Nonribosomal peptide synthesis, a biosynthetic pathway of lipopeptide biosurfactants

Nonribosomal peptide synthetases (NRPSs) are multi-modular enzymes that recognize, activate, modify, and link the amino acid intermediates to create lipopeptides (Gevers et al., 1968; Koglin & Walsh, 2009). Biosynthesis of Nonribosomal peptides occurs via the function of the catalytic unit, referred to as a module. Usually, the modules are ordered in a co-liner sequence, and each module is composed of specific domains that are responsible for catalyzing different enzymatic activities (Strieker, Tanovic, & Marahiel, 2010). Although, the linear structure of the NRPS system is the most common, they can also be assembled in an iterative and nonlinear manner. In the linear strategy (Type A) the number and sequence of the modules in the NRPS equals the number and order of amino acids in the peptide product. On the other hand, in type B, iterative structure, the modules or domains of the synthetase are used more than once to synthesize the peptide, which consists of repeated sequences. Lastly, nonlinear (Type C) NRPSs generate peptides in which the sequence of amino acids does not correlate to the arrangement of modules on the synthetase template (Hur, Vickery, & Burkart, 2012). The adenylation (A) domain is responsible for amino acid recognition and adenylation at the expense of ATP to form an acyl-adenylate intermediate. After that, the adenylated amino acid covalently binds to a phosphopantetheine carrier of the adjacent thiolation (T) or peptidyl

carrier protein (PCP) domain. Then, the condensation (C) domain catalyzes the peptide bond formation of two consecutively bound amino acids. Epimerization (E) domain is an example of modification domains, which catalyzes the conversion of L-amino acids to D-isomers, and they are typically associated with the module that incorporates D-amino acids. The last domain, thioesterase (Te), is associated with a termination module and is responsible for cyclization and release of the product peptide (Hur et al., 2012; Strieker et al., 2010). Gene clusters encoding NRPSs for lipopeptide BS biosynthesis have been identified. These gene clusters share similarities in the modular architecture of their repetitive catalytic units (Roongsawang et al., 2010; Soberon-Chavez, 2011) and are discussed under each type of BS.

2.3.2.1.Lipopeptides produced by *Bacillus* species

Bacillus species are rod-shaped, endospore-forming aerobic or facultative anaerobic, gram-positive bacteria. The many species of the genus exhibit a wide range of physiologic abilities that allow them to live in every natural environment (Baron, 1996).

2.3.2.1.1. Surfactin family

Surfactin-like biosurfactant family is composed of approximately 20 different lipopeptides, such as surfactin, lichenysin A/D, B, C, G, surfactant BL86, pumilacidin, and bamylocin A (Roongsawang et al., 2010). Their common structure contains a heptapeptide with a chiral sequence LLDLLDL interlinked with a β-hydroxy fatty acid and a D-Leu in position 3 and 6 α L-Asp in position 4 (Fig. 11). In position 2,4 and 7 amino acid residues belong to the aliphatic group Val, Leu and Ile (Peypoux et al., 1991; Itokawa et al., 1994; Bonmatin et al., 1995). Usually, surfactin isoforms coexist in the

cell as a mixture of several peptidic variants with a different aliphatic chain length (Tang et al., 2007).

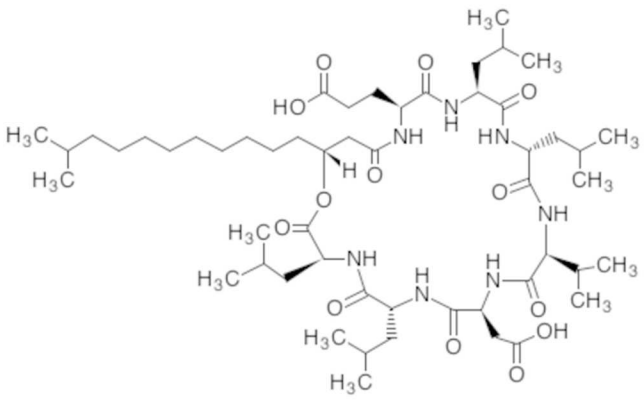

Figure 10. Detailed structure of a surfactin biosurfactant. Source: Kyoto Encyclopedia of Genes and Genomes, KEGG database (Kanehisa et al., 2000; Kanehisa et al., 2016; Kanehisa et al., 2017).

Surfactin reduces the surface tension of water from 72 to 27 mN/m at a CMC of 25-220 mg/L depending on its variants. The first application identified by Arima et al. (1968) for surfactin was as an inhibitor of fibrin (non-globular protein involved in the coagulation of blood) clot formation (Arima, Kakinuma, & Tamura, 1968). With further analysis, it was discovered its antimicrobial, anti-tumor, anti-fungal, anti-viral, hemolytic, and insecticidal activities (Eberl, Molin, & Givskov, 1999; Gudina et al., 2013; Meena & Kanwar, 2015) . Surfactins are also involved on biofilm formation and inhibition (i.e., interfering with attachment of the cells to surfaces), swarming motility, and fruiting body formation (Kearns and Losick, 2003; Julkowska, Obuchowski, Holland & Seror, 2005). The commercial potential of surfactin could not be fully realized, as a therapeutic agent, due to its hemolytic property (Ramkrishna, 2010a). These strong antimicrobial and anti-viral actions could be a consequence of its ability to form ion-conducting channels in bacterial cell membranes by exploiting its detergent-like action on cell membranes, also

called membrane active properties. This mode of action drastically reduces the chance of the development of resistance in microbes and hence, offers a promising alternative in the treatment of raging multidrug-resistant infectious diseases (Amram, 2000). Sekhon and colleagues demonstrated that BS production and release of esterases by the microbial cells have been shown to be synchronized and symbiotically beneficial in some *Bacillus* species (Sekhon et al., 2011).

Studies (Das, Mukherjee, & Sen, 2008) have shown that different biosynthetic pathways and specific enzymes are involved in surfactin, which is produced as a result of nonribosomal biosynthesis catalyzed by a large multienzyme peptide synthetase complex called the surfactin synthetase. Peypoux et al. (1999) demonstrated that surfactin is coded by four open reading frames (ORFs) named as srfA, srfB, srfC (Fig. 12 and 13) and srfD. *B. subtilis* has been found to regulate surfactin production by a cell density-responsive mechanism not based on homoserine lactone but utilizing a peptide pheromone, ComX (Das et al., 2008). An ORF is a portion of a DNA molecule that, when translated into amino acids, contains no stop codons. The genetic code reads DNA sequences in groups of three base pairs, which means that a double-stranded DNA molecule can read in any of six possible reading frames, three in the forward direction and three in the reverse. A long ORF is likely part of a gene and are often used, along with other evidence, to initially identify candidate protein-coding regions or functional RNA-coding regions in a DNA sequence (U.S. National Library of Medicine. https://ghr.nlm.nih.gov/resources#medicalterminology Retrieved on 12/13/16).

Similarly, lichensyn, a lipopeptide produced by *Bacillus licheniformis* coded by lichenysin operon (lchA) consists of three peptide synthetase genes licAA, lic AB, licAC

and licAD, and they are transcribed in the same direction (Marahiel et al., 1999). The lic operon of *B. licheniformis* is 26.6 kb long and consists of genes licA (three modules), licB (three modules) and licC (one module) (Fig. 12). The domain structures of these seven modules resemble that of surfactin synthetases SrfA-C. The modular organization of lichenysin synthetases Lic A to LicC was also found to be identical with that of surfactin synthetases. There is another gene called LicTE which codes for a thioesterase like protein (Yakimov et al., 1998). Lichenysins were first reported by Jenneman et al. (1983) for its applications in MEOR. The strain then identified as *B. licheniformis* JF-2, was re-identified as *Bacillus mojavensis* JF-2 ATCC 39307, isolated and patented at

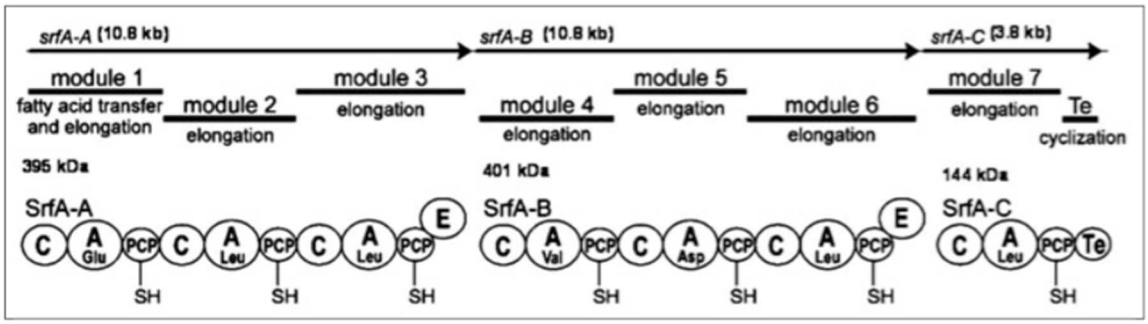

Figure 11. Surfactin synthetase reaction: three ORFs their modules, domains and amino acids (Mootz et al., 2002).

Oklahoma University for MEOR (McInerney, Jenneman & Knapp, 1985). It has been extensively studied and found to produce lichenysin BS in aerobic and anaerobic conditions, in which pH, temperature, calcium or salt concentration does not affect its surface activity (McInerney, Javaheri & Nagle, 1989).

2.3.2.1.2. Iturin family

The iturin biosurfactants family consists in cyclic lipoheptapeptides linked by a β-amino acid residue, such as iturin, bacillomycin, and mycosubtilin (Roongsawang et al., 2010).

These BSs have shown antibiotic activity, and enhanced swarming motility, for instance, iturin A is an antifungal lipopeptide BS produced by certain *B. subtilis* strains such as *B. subtilis* RB14 (Isogai, Takayama, Murakoshi & Suzuki, 1982; Leclere, Marti, Bechet, Fickers & Jacques, 2006).

The NRPS gene cluster of bacillomycin D (*bam/bmy*), mycosubtilin (*myc*), and iturin A (*itu*) is composed of four ORFs, *itu*D, *itu*A, *itu*B, and *itu*C (Fig. 13) (Saravanakumari & Nirosha, 2012). *bam* and *bmy* were found to be identical gene clusters in *B. subtilis* AU195 and *Bacillus amyloliquefaciens* FZB42, respectively. The first ORF-*bmy*D and *itu*D gene encodes a putative malonyl-CoA transacylase. The second ORF- *bmyA, mycA,*

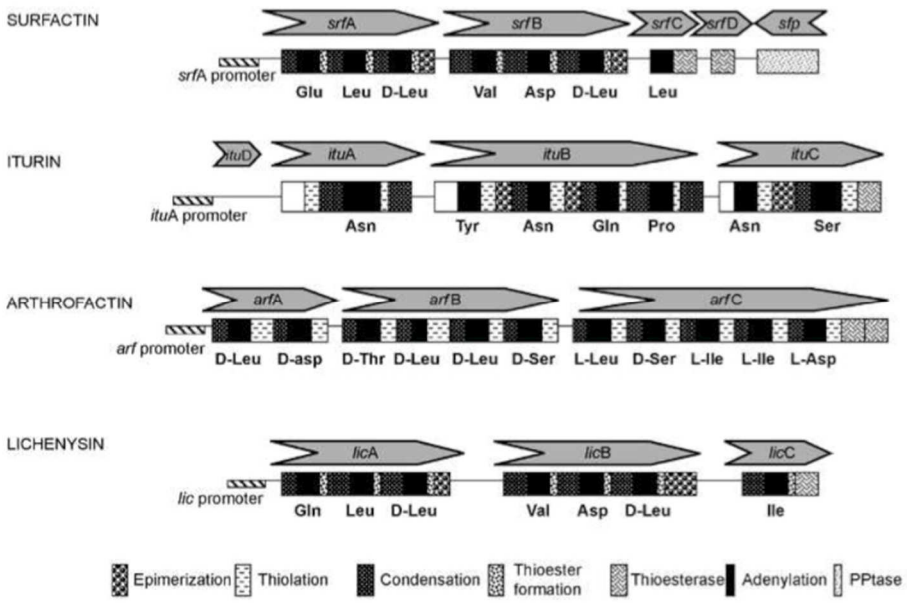

Figure 12. Structural organization of the genes encoding various lipopeptide biosurfactant synthetases. These genes show a high degree of structural similarity (Das et al., 2008)

and *itu*A encodes three functional domains homologous to β-ketoacyl synthetase, amino transferase, and amino acid. The *itu*B gene encodes a peptide synthetase consisting of four amino acid adenylation domains, two of which are flanked by an epimerization

domain. The *itu*C gene encodes another peptide synthetase that has two adenylation domains, one epimerization domain, and a thioesterase domain which probably helps in peptide cyclization (Tsuge, Akiyama & Shoda, 2001; Moyne, Cleveland & Tuzun, 2004; Hansen, Bumpus, Aron, Kelleher & Walsh, 2007). Simpson et al. (2011) developed a primer set srfA3/licA3 that could detect BS produced by *B. subtilis, B. licheniformis, B. megaterium,* and *B. sonorensis.*

2.3.2.1.3. Fengycins or plipastatins

Fengycin is an anti-fungal antibiotic effective against filamentous fungi but ineffective against yeast and bacteria, it is also referred to as plipastatins when Tyr_3 and Tyr_9 is present as the L- and D- form, respectively (Jha, Joshi, & S, 2016). These BS are produced by *Bacillus* spp. such as *B. subtilis* F29-3 which was isolated from a potato farm and its fengycin production was optimized from 1.2 g/L to 3.5 g/L (Wei, Wang, Chen, & Chen, 2010). Fengycin synthetase (Fen) contains five co-linear NRPS subunits: FenC, FenD, FenE, FenA, and FenB. Similar to SrfA and Lic, Fen is also composed of an N-acyl domain at the N-terminus of FenC, conventional E-domains, and a typical type I Te-domain.

2.3.2.2.Lipopeptides produced by Pseudomonas species

Pseudomonas is a genus of gram-negative, aerobic Gammaproteobacteria. The members of the genus demonstrate a great deal of metabolic diversity and consequently can colonize a wide range of niches. *Pseudomonas* spp. produce cyclic lipopeptide biosurfactant such as viscosin, amphisin, syringomycin, tolaasin, syringopeptin and putisolvin and their regulatory genes have been identified (Braun et al., 2010; Andersen

et al., 2003; Dubern et al., 2006). Additionally, no other cyclic lipopeptides have been discovered in *Pseudomonas* spp. Das and Mukherjee (2008) have demonstrated crude petroleum-oil biodegradation efficiency of biosurfactant producing *P. aeruginosa* M and NM strains isolated from petroleum oil contaminated soil in India (Das et al., 2008).

2.3.2.2.1. Syringomycin

Syringomycin is a phytotoxin and a key factor of *Pseudomonas syringae* B301D virulence, that reduces surface tension to 33 mN/m with a CMC of 1250 mg/L. Syringomycin is synthesized by NRPSs, SyrBl and SyrE, and three modifying protein systems (SyrB2, SyrC, SyrP). The first eight amino acids in SyrE are arranged in a line of eight modules, but the ninth module (SyrBl), which incorporates the last amino acid, is located in the upstream region. This confirms that absolute co-linearity is not essential for NRPS synthesis (N. Wang, Lu, Yang, Sze, & Gross, 2006; J. Zhang, Quigley, & Gross, 1997).

2.3.2.2.2. Syringopeptin

Syringopeptin is also produced by *P. syringae* B301D composed by large peptide moiety, with 22-25 amino acid residues. This lipopeptide BS has a CMC of 820 mg/L and reduces surface tension to 40.2 mN/m (N. Wang et al., 2006). SypA, SypB, and SypC are the NRPSs involved in the biosynthesis of syringopeptin, and represent the largest NRPSs among those reported for prokaryotes. Similar to Syr synthetase, there is no E domain present in Syp synthetase; in contrast SypC contains two unique C-terminal Te domains presumably to catalyze the release and cyclization of syringopeptin (Scholz-Schroeder, Soule, & Gross, 2003).

2.3.2.2.3. Arthrofactin

Arthrofactin it is a cyclic lipopeptide produced by *Pseudomonas* sp. MIS38 (previously misidentified as *Arthrobacter* spp.) and belongs to the amphisin group. It reduces the surface tension of water from 72 to 24mN/m with a CMC of 13.5 mg/L (Morikawa et al., 1993). Arthrofactin is one of the most effective lipopeptide BS; it has anti-fungal properties and has been reported as an enzyme inhibitor from *Pseudomonas* spp. DSS73 (Andersen et al., 2003). The arthrofactin synthetase gene cluster is formed by arfA, arfB, and arfC, which contains two, four, and five functional modules, respectively (Fig. 14).

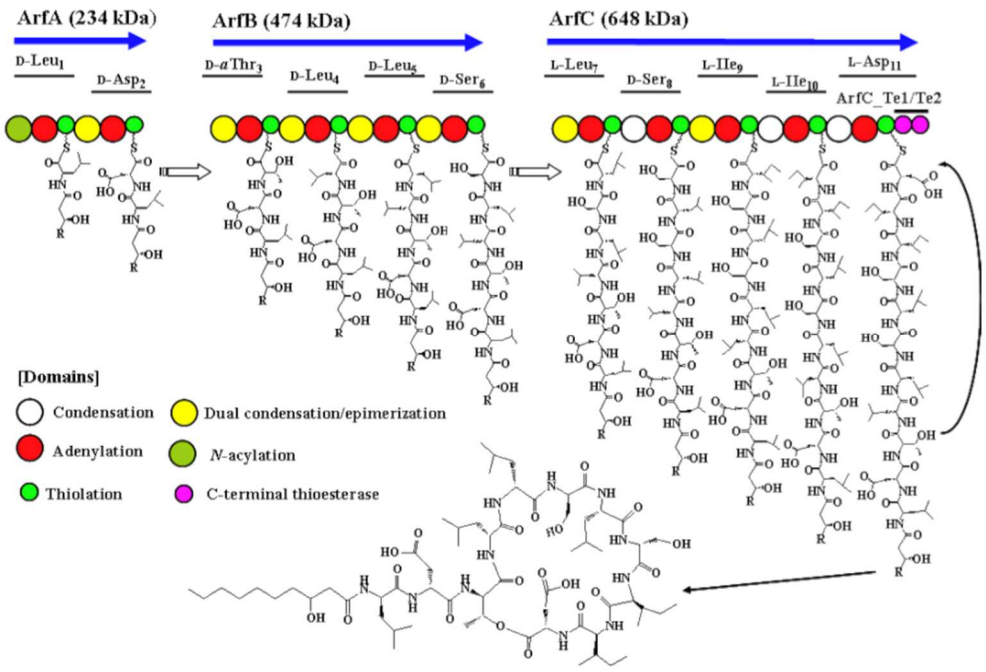

Figure 13. The arthrofactin biosynthesis assembly line (Roongsawang, Washio, & Morikawa, 2007).

ArfB was found to be the gene absolutely essential for arthrofactin production as its disruption impaired it. Moreover, Arf represents a novel NRPS architecture that features

tandem Te-domains and dual C/E domains, which suggests that those are functional in *Pseudomonas* spp. (Roongsawang et al., 2010).

2.3.2.2.4. Viscosin

Viscosin and massetolide are lipononapeptides BS produced by *P. fluorescens* SBW25 and *P. fluorescens* SS101, respectively(de Bruijn et al., 2007). Viscosin has been more studied than massetolide; viscosin is a good emulsifier that reduces surface tension to 28 mN/m with a CMC of 10-15 mg/L. Additionally, inhibition of metastasis of prostate cancer cell line and anti-fungal activity has been accredited to viscosin. Moreover, both viscosin and massetolide are involved in biofilm formation and swarming motility of *Pseudomonas* cells (Nielsen, Christophersen, Anthoni, & Sorensen, 1999). The synthesis of these BS is encoded by viscA/massA, viscB/massB, and viscC/massC ORFs that are assembled by NRPS system (de Bruijn et al., 2007). Their amino acid sequences analysis identified two modules in ViscA/MassA, four modules in ViscB/MassB, and three modules in ViscC/MassC. A, T and D domains are in each module, whereas none of the five D-amino acid-incorporation modules presents a cognate E domain, but contains a C/E domain (Roongsawang et al., 2007).

2.3.2.2.5. Putisolvin

Putisolvin is a cyclic lipododecapeptide composed of 12 amino acid peptides linked to a hexanoic lipid by an ester linkage between the ninth serine residue and the C-terminal carboxyl group. Kuiper and colleagues (2004) isolated *P. putida* PL 1445 from soil heavily contaminated with polycyclic aromatic hydrocarbons; two surfactants (putisolvin I and putisolvin II) were found to inhibit biofilm formation, have anti-fungal activity and

zoo sporicidal agent (Kruijt, Tran, & Raaijmakers, 2009). Putisolvin biosynthesis is NRPS assembled, three encoding genes have been identified, *psoA, psoB*, and *psoC* (Dubern, Coppoolse, Stiekema, & Bloemberg, 2008); and contain two, seven, and three functional modules, respectively. Amino acid sequence analysis of the C domains indicated that dual C/E domains are organized downstream of the first nine modules (Dubern et al., 2008; Roongsawang et al., 2003). Three heat shock genes *dnaK, dnaJ* and *grpE* positively regulates the biosynthesis of putisolvin (Dubern et al*.,* 2005).

2.3.2.2.6. Syringafactin

Syringafactin has shown surfactant activity and is essential for the swarming motility of the producing strains, however, its contribution to pathogenicity has not been studied. *P. syringae* B728 and *P. syringae pv. tomato* CD3000 produce syringafactin, which gene clusters have been identified, syfA and syfB to encode three and five NRPS modules, respectively (de Bruijn et al., 2007). The N-acyl domain present in the initiating module of syfA indicated that syringafactin would contain an N-terminal fatty acid chain. SyfB contains tandem Te-domains at the C-terminus (Berti, Greve, Christensen, & Thomas, 2007). It has been suggested that the syringafactin NRPS system evolved from the arthrofactin system, with three modules of arthrofactin NRPS deleted, followed by fusing the N-terminus of ArfA with a portion of ArfB (Roongsawang et al., 2010). The same authors suggest that in the deleted modules was the module involved in cyclization of arthrofactin through the threonyl residue (Roongsawang et al., 2010).

2.3.2.2.7. Serrawettin

Serratia is a genus of gram-negative, facultative anaerobic, endospore forming, rod-shaped bacteria of the Enterobacteriaceae family. The most common and pathogenic of the species in the genus, *Serratia marcescens*, usually causes nosocomial (originated in a hospital) infections. *S. marcescens* produces the antibiotic surfactant, serrawettin W1. A single gene, *pswP* is responsible for its production. This gene has a high homology with genes of the NRPSs family. Serrawettin W2 is produced by *Serratia liquefaciens* MG1, its synthesis is catalyzed by a peptide synthetase which is encoded by swrA gene (Soberon-Chavez, 2011). The reduction of the surface tension of *S. liquefaciens*, *S. marcescens* and *Serratia rubidaea* (produces rubiwettin BSs) produced biosurfactant, is in the range to 25.8-32.2 mN/m (Soberon-Chavez, 2011). Reports of the serrawettin CMC have not been found.

2.3.3. Polymeric biosurfactants

Polymeric biosurfactants are high molecular weight biopolymers, such as emulsan, alasan, liposan and biodispersan. These BSs have shown properties like high viscosity, tensile strength and resistance to shear (Ramkrishna, 2010b) .

2.3.3.1.Emulsan

Acinetobacter species are known to produce emulsan and alasan (Kok et al., 1993). *Acinetobacter calcoaceticus* RAG-1 produces a polymeric BS, emulsan; which is characterized as a polyanionic amphipathic heteropolysaccharide. The heteropolysaccharide backbone consists of repeating units of trisaccharide of N- acetyl-d-galactosamine, N-acetylgalactosamine uronic acid, and a N-acetyl amino sugar

(Ramkrishna, 2010b). *Acinetobacter lwoffii* RAG-1 also produces emulsan as a minicapsule on the cell surface which is released into the medium as a protein-polysaccharide complex. This release is triggered by an esterase that if removed, a polymer called apoemulsan is formed which cannot carry the emulsification of non-polar, hydrophobic, aliphatic materials (Zosim et al., 1986). The emulsan gene cluster termed wee encodes the genes wza, wzb, wzc, wzx, wzy required for emulsan biosynthesis (Nakar & Gutnick, 2001) of *A. lwoffii* RAG-1. Moreover, it has been demonstrated that Wzc and Wzb are a protein tyrosine kinase and protein tyrosine phosphatase, respectively and deletion in either of the two genes gave rise to an emulsan-defective phenotype (Nakar & Gutnick, 2003; Bach et al., 2003).

2.3.3.2.Alasan

Alasan is a complex of an anionic polysaccharide containing covalently bound alanine and three proteins biosurfactant produced by *Acinetobacter radioresistens* KA-53. *Acinetobacter* is a genus of gram-negative bacteria belonging to the wider class of Gammaproteobacteria. *Acinetobacter* species are a non-motile group of bacteria commonly found in soil and water. Alasan produced by *A. radioresistens* KA-53 was reported to solubilize and degrade polyaromatic hydrocarbons (Chen, Huang, Zhang, & Ding, 2012). The surface-active component of alasan is a 35.77 KDa protein called AlnA. alnA gene encodes AlnA protein which is responsible for the emulsification activity. It has been suggested that *A. radioresistens* KA53 releases AlnA, AlnB and AlnC together as a complex under stressed conditions (Das et al., 2008). Recombinant AlnB had no emulsifying activity but stabilized oil-in-water emulsion generated by AlnA. AlnB amino acid sequence has strong homology to the family of antioxidant enzymes known as

peroxiredoxins thus expression of AlnB protects *E. coli* from toxic concentrations of organic peroxide (Ramkrishna, 2010b).

2.3.3.3.Liposan

Liposan is composed of protein (17% w/v) and a carbohydrate portion, which is a heteropolysaccharide consisting of glucose, galactose, galactosamine and galacturonic acid. Liposan was isolated from *C. lipolytica,* and it was reported as a water soluble emulsifier (Cirigliano & Carman, 1984, 1985).

2.4. Lactobacilli and its biosurfactants, surlactin

LAB form a group of gram-positive, non-spore forming cocci, coccobacilli, or rods, with a nucleotide composition of less than 50 mol% GC and with lactic acid as the major carbohydrate catabolism end product (Stiles and Holzapfel, 1997). As such, LAB include the genera *Lactobacillus;* several of these species are proven to be probiotics and are GRAS (Table 3). The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), has defined probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). One of the most important mechanism of probiotic action is the capacity of probiotics to beneficially affect the host by direct effects on the microbiota. Conventionally, most attention is given to the antipathogenic properties of probiotics by competition for nutrients, the production of antimicrobials, and competitive exclusion (Wood & Warner, 2003).

Table 3. List of Lactobacillus species GRAS by FDA (Source: https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/default.htm)

GRAS notice number	Name of live microbial culture(s)
171,463	*Lactobacillus acidophilus, Lactobacillus lactis,* and *Pediococcus adicilactici*
231,429	*Lactobacillus casei*
254, 409, 410, 440	*Lactobacillus reuteri*
281, 288	*Lactobacillus rhamnosus*
357, 502	*Lactobacillus acidophilus*

Lactobacilli have been used for centuries in food preservation to prevent microbial spoilage, since then its capacity to inhibit pathogens has been well known. The health effects of lactobacilli have been proven under various conditions and the best evidence is for the treatment and prevention of enteric infections and post antibiotic syndromes. More examples of the health benefits are the effectiveness against acute infectious diarrhea and recurrence of *Clostridium difficile*-associated diarrhea, prevention of necrotizing enterocolitis in preterm neonates, and prevention and treatment of inflammatory bowel disease, prevention of colorectal cancer, and treatment of irritable bowel syndrome. Moreover, not only GIT benefits have been proven; prevention and treatment of urogenital diseases and vaginosis, prevention of atopic disease and food hypersensitivity, and the prevention of dental caries. Although the level of tolerance when lactobacilli is ingested is usually high, there are some reports regarding a not positive response of the host (Wood & Warner, 2003).

2.4.1. Physiological and metabolic characteristics of lactobacilli

The availability of probiotic factors and the adaptation to their environment (e.g., a host or abiotic surfaces) relies on specific metabolic and physiological characteristics of

lactobacilli. Cell surface structures of these microorganisms, such as peptidoglycan, teichoic acids, EPSs and cell surface proteins, play a key role in survival and probiotic mechanisms, since they are in direct contact with the environment (Lebeer, Vanderleyden, & De Keersmaecker, 2008).

The cell wall of lactobacilli is a thick 20-100 nm peptidoglycan multilayer that protects the cell against lysis and provides structural integrity. It is covalently and noncovalent, with teichoic acids, polysaccharides, and proteins (Delcour, Ferain, Deghorain, Palumbo, & Hols, 1999).

Teichoic acids are anionic cell wall polymers made of polyglycerol phosphate or polyribitol phosphate repeating units covalently anchored to either peptidoglycan (wall teichoic acids, WTAs) or attached to the cytoplasmic membrane (lipoteichoic acids, LTAs). Not all strains contain WTA, but all lactobacilli have teichoic acids (Neuhaus & Baddiley, 2003). Additionally, it has been suggested that a portion of LTA may be released through deacylation or the inside-to-outside expansion of peptidoglycan without the removal of the lipid anchor, indicating that LTAs of some strains can act as soluble factors (Lebeer et al., 2008). Both WTA and LTA are often substituted with glycosyl or D-alanyl esters. The D-Ala ester substitution requires four proteins that are encoded by the dlt operon. Two of these proteins are the D-alanyl:D-alanyl carrier protein ligase (Dcl, encoded by dltA), which activates D-alanine by use of ATP, and the D-alanyl carrier protein (Dcp), which is encoded by dltC. DltB is a transport protein predicted to be involved in the passage of the activated D-alanyl-Dcp complex across the glycerol phosphate backbone of LTA, while the DltD membrane protein facilitates the binding of Dcp for ligation with D-Ala and has thioesterase activity for removing mischarged D-

alanyl carrier proteins (Neuhaus & Baddiley, 2003; Delcour et al., 1999). It has been suggested that LTA provides the main component of the hydrophobicity of the *Lactobacillus* cell membrane, while this depends on the D-alanine ester substitutions referred above (Delcour et al., 1999). Hence, LTA appears to contribute mainly to adhesion in a nonspecific way. For example, it was shown that the inactivation of *dltD* in *L. rhamnosus* GG revealed that the D-alanylation of LTA is not required for short-term adherence to Caco-2 cells (heterogeneous human epithelial colorectal adenocarcinoma cells) (Perea Velez, Verhoeven, Draing, Von Aulock, Pfitzenmaier, Lambrichts, Grangette, Pot, Vanderleyden & De Keersmaecker, 2007), whereas the *dltD* mutation improved the biofilm formation capacity of *L. rhamnosus* GG after 72 h of growth on polystyrene (Leeber, Verhoeven, Perea Velez, et al., 2007). For *L. reuteri* 100-23, D-alanylation might be important for later events in biofilm formation. Increased repulsive electrostatic forces in the mutant due to increased negative charges might be involved in the disruption of biofilm structure (Lebeer et al., 2008).

EPS in lactobacilli are the extracellular polysaccharides that can be attached to the cell wall or be secreted into the surroundings. They are complex and diverse structures due to their modes of linkage, branching, and substitutions (De Vuyst & Degeest, 1999). In lactobacilli, there are heteropolysaccharides, composed of different sugar moieties (glucose, galactose, rhamnose, and N-acetylgalactosamine) and homopolysaccharides, glucans from sucrose by the single action of extracellular glycosyltransferases, being the first ones the most common (De Vuyst, De Vin, Vaningelgem & Degeest, 2001). Generally, EPSs are secreted or remain weakly attached to the cell wall by electrostatic interactions such as ionic, hydrogen bonds, or hydrophobic interactions (Delcour et al.,

1999; De Vuyst et al., 2001). However, phenotypic analyses of lactobacilli mutants affected in EPS biosynthesis genes have not yet been performed (Lebeer et al., 2008).

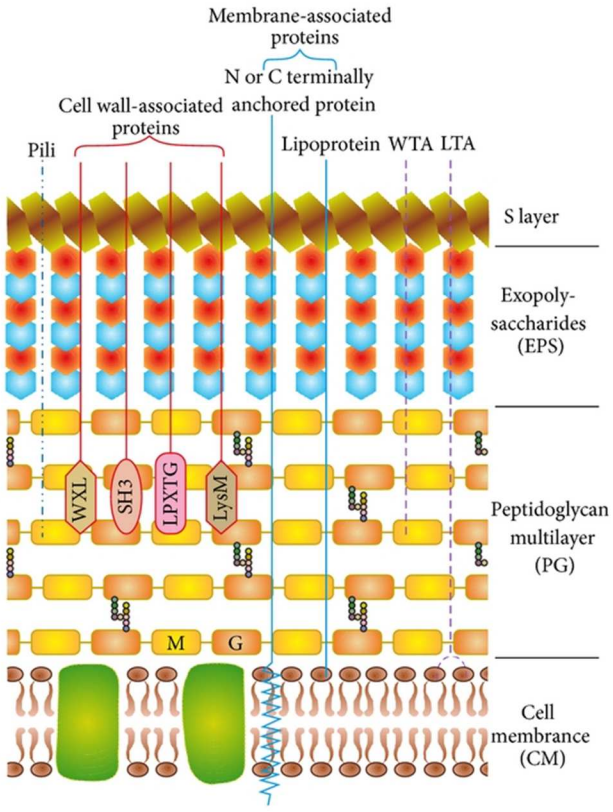

Figure 14. Cell surface architecture of Lactobacilli.

Cell surface proteins (Fig. 14) are bound to the cell wall by single N- or C-terminal transmembrane anchors, lipoprotein anchors (lipobox), LPxTG-type anchors, or other cell wall-binding domains such as LysM domains or glycine-tryptophan dipeptide motifs (Avall-Jaaskelaimen & Palva, 2005). In addition, there are other proteins that are secreted in the surroundings and are re-associated to the cell wall by electrostatic interactions (Batch, Roos, Wall, & Jonsson, 2005). Sortase-dependent proteins (SDPs) and surface-layer proteins (S-layer) are the best-known cell surface proteins in lactobacilli; SDPs comprises a group characterized by the presence of a cell wall-sorting signal at the C-

terminal that includes a pentapeptide motif (LPxTG) followed by a stretch of hydrophobic side chains and a positively charged tail (Navarre & Schneewind, 1994). S-layer proteins are small (40-60 kDa) highly basic proteins with stable structures, they are not covalently bound to the cell wall (to secondary cell wall polymers such as LTA, and neutral polysaccharides), and assemble into surface layers covering the cell wall. Not all *Lactobacillus* are surrounded by a superimposed S-layer made of proteins subunits packed into a paracrystalline hexagonal or tetragonal mono layer (Avall-Jaaskelaimen & Palva, 2005); *L. acidophilus, L. gasseri, L. johnsonii, L. brevis, L. helveticus*, and *L. crispatus* are (Boot, Kolen, Vannoort, & Pouwels, 1993; Ventura, Jankovic, Walker, Pridmore & Zink, 2002; Vidgren, Palva, Pakkanen, Lounatmaa & Palva, 1992; Callegari, Riboli, Sanders, Cocconcelli, Kok, et al., 1998; Sillanpaa, Martinez, Antikainen, Toba, Kalkkinen et al., 2000). Glycan and glycoprotein structure layers' protein have been reported for *L. buchneri* and *L. rhamnosus* GG, respectively (Avall-Jaaskelaimen & Palva, 2005; Francius, Lebeer, Alsteens, Wildling, Gruber et al., 2008).

The antimicrobial mechanism known as competitive exclusion, generally requires that the probiotic lactobacilli are administered in a preventive step, as the supplant of a pathogen by a *Lactobacillus* strain is usually not reported. Probiotics could use attachment site so that the pathogen is in competition for binding to the host mucosal interface and thereby could be inhibited from invading the mucosal layer (Lebeer et al., 2008).

QSS is a cell-to-cell signaling mechanism through which bacteria produce and respond to chemical signals called autoinducers. QSS is best studied in lactobacilli in relation to bacteriocin production. Intraspecies bacterial communication in gram-positive bacteria is mostly mediated by specific auto inducing signaling peptides that are often post

translationally modified and exported by transport systems (Sturme, Francke, Siezen, de Vos & Kleerebezem, 2007). In silico analysis predicted the presence of five QSS in *L. plantarum* WCFS1, two in the intestinal species *L. acidophilus* NCFM and *L. johnsonii* NCC533, one in the intestinal species *L. salivarius* UCC118 and the food species *L. delbrueckii* subsp. *bulgaricus* ATCC BAA-365, and none in the intestinal species *L. gasseri* ATCC 33323 (Sturme et al., 2007). The high number identified in *L. plantarum* WCFS1 could reveal the ecological flexibility of this species, which can be found on plants, in fermented foods, and in the GIT (Kleerebezem et al., 2003). However, an in vivo function for these QSS systems in the competitive ability of lactobacilli remains to be elucidated (Lebeer et al., 2008).

2.4.2. Surlactin

The biosurfactants produced by *L. casei* subsp. *casei* 393, *L. acidophilus* T-13, *L. rhamnosus* GR-1, *L. plantarum* RC-6, *L. fermentum* RC-14, and *L. fermentum* B-54 were

Table 4. Classification of surlactin along with their producing strains

Microorganism	**Composition reported**
L. acidophilus RC14, *L. casei* 70, *L. casei subsp. rhamnosus* GR-1 *L. casei subsp. rhamnosus* 36 *L. fermentum* RC-14, *L. casei Shirota, L. acidophilus* ATCC 4356	Protein-like
L. casei, L. acidophilus, L. plantarum CFR 2194, *L. agilis* CCUG31450, *L. pentosus* CECT-4023, *L. fermenti* 126, *L. rhamnosus* CCM 1825	Glycoprotein
L. brevis CV8LAC	Mixture of containing sugars
L. pentosus CECT-4023, *L. delbrueckii, L. helveticus, L. casei* MRTL3	Glycolipid
L. paracasei subsp. paracasei A20, *L. reuteri* DSM20016	Not stated

collectively named “surlactin” by Velraeds and colleagues in 1996. In their observations, they selected *L. fermentum* RC-14 as the positive control of their studies, due to its best surface tension reduction among all the strains tested (M. M. C. Velraeds, van der Mei, Reid, & Busscher, 1996). Although not all the reports refer to surlactin when mentioning biosurfactant produced by *Lactobacillus*, in this review all kinds of surfactants produced by these species will be noted as surlactin. This family of biosurfactants differs in their chemical composition (Table 4).

2.4.2.1.Surlactin physicochemical characterization

Surlactin can be produced in an intracellular or extracellular manner, or cell-bound and cell-free, respectively. Most of the commercially available biosurfactants have been reported to be released extracellularly, whereas the majority of surlactin is release intracellularly and have protein rich compositions (Table 5) (Satpute et al., 2016).

The composition of surlactin varies depending upon the producing specie; only about 50% of the studies reported it. This is because of the complex structures that are difficult to elucidate, in comparison to other biosurfactants that have been extensively analyzed. Surlactin of proteinaceous cell associated composition appears to be most frequently produced, with about 30% of reports. Whereas glycoproteins composition is mentioned in with a 7.5%, glycolipid 5%, and glycolipopeptide 5% (Satpute et al., 2016). For instance, Velraeds et al. (1996) reported that surlactin had a mixture of protein and polysaccharides when analyzed by Fourier transform infrared spectroscopy (FTIR); *L. acidophilus* RC14 and *L. fermentum* B54 were found to be the most proteinaceous (28-30% protein) by the BioRad protein assay.

Table 5. Type and composition of surlactin along with extraction methods depending upon cell-bound or cell-free biosurfactant nature

Table 5. Type and composition of surlactin along with extraction methods

Microorganism	**Composition of biosurfactant**	**Extraction method and type of biosurfactant**	**Reference**
***L. acidophilus* RC14, *L. casei* 70, *L. casei* subsp. *rhamnosus* GR-1**	Rich in protein and less content of polysaccharide, phosphate	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(M. M. C. Velraeds et al., 1996)
L. casei subsp. rhamnosus 36	Protein rich with LTA		(M. M. C. Velraeds et al., 1996)
***L. acidophilus* RC14**	Protein rich	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(M. M. Velraeds, van der Mei, Reid, & Busscher, 1997)
***L. acidophilus* RC-14**	Rich in protein and less content of polysaccharide, phosphate	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(M. M. C. Velraeds, van de Belt-Gritter, van der Mei, & Busscher, 1998)
L. fermentum RC-14, L. casei Shirota, Ll. Rhamnosus GR-1, L. rhamnosus GR-36	Proteins that bind to both collagen types III and VI,	After BS release, supernatant: filtration, dialysis and freeze-drying.	(Howard et al., 2000)
***L. casei* CECT-5275, *L. rhamnosus* CECT-288, *L. pentosus* CECT-4023 and *L. coryniformis* subsp. *torquens* CECT-25600**	Composition not stated	Culture growth media, cell-free. Centrifugation and extraction for cell-bound.	(L. Rodrigues, Moldes, Teixeira, & Oliveira, 2006)
***L. acidophilus* H-1, *L. acidophilus* 336, *L. acidophilus* Ch-1 (Biolacta Company, Olsztyn, Poland)**	Glycoprotein	After BS release, dialysis, freeze-drying. Cell-bound	(Walencka, Różalska, Sadowska, & Różalska, 2008)

Table 5. Type and composition of surlactin along with extraction methods

Microorganism	Composition of biosurfactant	Extraction method and type of biosurfactant	Reference
***L. casei* 8/4 (University of Warmia and Mazury in Olsztyn, Poland)**	Glycoprotein with phosphoric groups	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(Gołek, Bednarski, Brzozowski, & Dziuba, 2009)
***L. paracasei* subsp. *paracasei* A20**	Composition not stated	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(Gudina, Rocha, Teixeira, & Rodrigues, 2010)
***L. acidophilus* (vaginal swabs, maternity hospital in Baghdad**	Glycoprotein	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(Fouad, Khanaqa, & Munira, 2010)
***L.* spp. CV8LAC**	Various components including 5% sugars	Centrifugation, acid precipitation, freeze-drying. Cell-free	(Fraccia, Cavallo, Allegrone, & Martinotti, 2010)
***L. fermenti* 126 and *L. rhamnosus* CCM 1825**	Protein, polysaccharide and phosphate in different ratio	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(Brzozowski, Wlodzimierz, & Gołek, 2011)
L. delbrueckii	Glycolipid with carbohydrate and lipid combination of 30 and 70%, respectively	Centrifugation, extraction (chloroform and methanol), dialysis, freeze-drying. Cell-free	(Thavasi, Jayalakshmi, & Banat, 2011)
L. acidophilus	Protein-like with polysaccharides and phosphate fractions	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(Tahmourespour, Salehi, & Kermanshahi, 2011)

Table 5. Type and composition of surlactin along with extraction methods

Microorganism	Composition of biosurfactant	Extraction method and type of biosurfactant	Reference
***L. pentosus* CECT-4023**	Glycoprotein or glycolipopeptide	After BS release, supernatant: filtration. Cell-bound	(Moldes et al., 2013)
L. plantarum* CFR *2194	Glycoprotein	After BS release, supernatant: filtration. Cell-bound	(Madhu & Prapulla, 2014)
L. helveticus	Glycolipid similar to xylolipid	After BS release, supernatant: dialysis, freeze-drying. Cell-bound	(D. Sharma, Saharan, Chauhan, Bansal, & Procha, 2014)
***L. casei* MRTL3 (from raw milk)**	Glycolipid	After BS release, supernatant wash. Cell-bound	(D. Sharma & Singh Saharan, 2014)
***L. reuteri* DSM20016**	Composition not stated	After BS release, supernatant: dialysis, freeze-drying. Cell-bound	(Salehi et al., 2014)
L. pentosus	Glycolipopeptide	After BS release, centrifugation. Cell-bound	(Vecino, Barbosa-Pereira, Devesa-Rey, Cruz, & Moldes, 2015)
***L. brevis* CV8LAC**	Mixture containing sugars	Centrifugation, acidification, extraction, evaporation. Cell-bound	(Ceresa et al., 2015)
***L. agilis* CCUG31450**	Glycoprotein	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(Gudina et al., 2015)
***L. acidophilus* ATCC 4356**	Proteinaceous with polysaccharides and phosphate fractions	After BS release, supernatant: filtration, dialysis and freeze-drying. Cell-bound	(Shokouhfard, Kermanshahi, Shalandashti, Feizabadi, & Teimourian, 2015)

Additionally, the amino acid analysis in the same study indicated the presence of alanine as the free amino acid (M. M. C. Velraeds et al., 1996). Another example, Sharma et al. (2014) characterized surlactin as a glycoproteinaceus, and contained glycosyldiglycerides content also. By nuclear magnetic resonance (NMR) analysis, *Lactobacillus helveticus* MRTL 91 surfactant was a glycolipid with hexadecanoic fatty acid (C16) chain, similar to xylolipid. Moreover, total molecular weight of surlactin was found to be between 14.4-94 KDa (D. Sharma & Singh Saharan, 2014). The optimal pH identified for production was 6.0-6.3 values, even up to 7. The incubation temperature and time were 25°C for four hours with inoculation volume of 600 µliters (6x 10^7 cell/ml) per 10 mL of the media (Fouad et al., 2010). It has been reported that the lower surface tension, 39 mN/m, is from *L. acidophilus* RC14 with a CMC of 1.0 mg/ml (M. M. C. Velraeds et al., 1996). They suggested, later in 1998, that the drop-in liquid surface tension of surlactin should be between 12 and 29 mN/m and possibly even higher, to be considered as a good surfactant (M. M. C. Velraeds et al., 1998). However, other reports showed the surface tension of 39.5 mN/m for *L. pentosus* CECT-4023; 41.8 mN/m for *L. paracasei;* 47.68 mN/m for *Lactobacillus* spp. CV8LAC; 22.0 mN/m for *L. paracasei* spp. *paracasei* A20; 39.5 nM/m for *L. helveticus*. Gudina et al. (2013) detected that the use of peptone and meat extract yielded a higher production with a surface tension reduction of 24.5 mN/m (when growing *L. paracasei* spp. *paracasei* A20). The CMC values obtained from the lactobacilli that have been tested are in between the range of 106 g/mL, from *Lactobacillus* spp. CV8LAC (Fraccia et al., 2010), and up to 2.5 mg/mL from *L. paracasei* (Gudina et al., 2010), and *L. helveticus* (D. Sharma et al., 2014); both strains were isolated from dairy.

Nevertheless, some researchers are not calculating the CMC of the BS studied, and not even characterizing (e.g., physicochemical properties, chemical structure) surlactin. They are indeed interested in finding quantifiable variables that demonstrate antimicrobial activity of the BS. Although probiotic bacteria are well known because of their interference against pathogens, not all their mechanisms of action are completely understood(Wood & Warner, 2003). Probiotics produce antimicrobial compounds such as carbon peroxide, bacteriocins, organic acids, hydrogen peroxide, and BS, which prevent adhesion and cause disruption of the cell wall (Pascual et al., 2008; Merk et al., 2005; Ceresa et al., 2015). For LAB, some mechanisms are related to their byproducts (e.g. lactic acid, H_2O_2) and others have the ability to form biofilms, which co-relate the production of EPS, QSS, and anti-adhesive and co-aggregation properties that are proven to be an important factor in BS antimicrobial results (Wood & Warner, 2003).

2.4.2.2.Surlactin antimicrobial and anti-adhesion activity

Regarding the studies of surlactin, the most common topic of analysis is its evaluation as antimicrobial agent and its anti-adhesion capacity, not only in vitro but also in biotic and food contact surfaces. A summary of these reports findings is presented in Table 6; in which the activity is often related to an inhibition of pathogen adhesion rather than a direct antimicrobial activity or inhibition of the cell growth (Satpute et al., 2016). Although the mechanisms of BS antimicrobial activity are not fully elucidated, there are hypotheses based on the evidence reported from *these* studies. Desai and Banat (1997) suggested that the addition of the smaller acyl tails of the BS into the plasma membrane triggering disruptions of the plasma membrane, causing the plasma membrane to lift away from the cytoplasmic matter (Desai & Banat, 1997). In aother study, it was

proposed that the antimicrobial activity of biosurfactant could intrude the plasma membrane structure while interacting with phospholipids and other membrane proteins (Cameotra & Makkar, 2004). Ines and Dhouha (2015) reported that BS are able to form pores and disrupt the plasma membrane.

Surlactin inhibition was particularly effective against *E. faecalis, E. coli* and *Staphylococcus epidermidis.* The stationary growth phase BS from *L. acidophilus* RC14 and *L. fermentum* B54 caused a marked decrease in the initial deposition rate of *E. faecalis* whereas those released by *L. casei* subsp. *Rhamnosus* 36 did not. Additionally, surlactin has shown antiadhesive properties against *Klebsiella penumoni, P. aeruginosa* and *E. coli* (M. M. Velraeds, van de Belt-Gritter, Busscher, Reid, & van der Mei, 2000; M. M. C. Velraeds et al., 1998). Surlactin from *L. acidophilus* (isolated from vaginal swabs) has the ability, as reported by Munira et al. (2013), to inhibit the adhesion up to 60% and 55% for two different isolates of *P. aeruginosa* producing biofilms on contact lenses, and does not have an antibacterial activity. Surlactin did not show this ability against *S. aureus (Munira, Kadhim, & Maysaa, 2013)*. A study by Gudina et al. (2010) observed a total growth inhibition for *E. coli, S. agalactiae* and *S. pyogenes* with a BS concentration of 25 mg/ml. The isolated crude BS from *L. paracasei* was found to possess anti-adhesive activity against all the microorganisms assayed, the highest percentages were obtained for *S. aureus*, *S. epidermidis* and *S. agalactiae* (72 to 60%), and a low activity was observed for *P. aeruginosa* and *E. coli* (16.5 and 11.8% respectively) (Gudina et al., 2010). BS derived from *L. fermentum* exhibited the highest inhibition to the growth of *C. albicans* 22 CBS 5703, similar to *L. paracasei* ssp. *Paracasei* A20 inhibited *C. albicans, S. aureus* and *S. epidermidis* (Gudina et al., 2010).

Table 6. Antimicrobial and anti-adhesion reported properties of surlactin produced by different Lactobacillus strains.

Strain	Antimicrobial or anti-adhesion activities	Reference
***L. acidophilus* RC14**	Silicon rubber was filled with a 1.0 mg/ml surlactin solution, 18 h adsorption. Inhibited adhesion of *E. faecalis, E. coli, and S. epidermidis* and reduce 50% the adhesion of *C. albicans*	(M. M. C. Velraeds et al., 1998)
***L. fermentum* B54, *L. casei rhamnosus* 36**	70-100% inhibition of uropathogenic biofilm growth on silicone rubber in human urine	(M. M. Velraeds et al., 2000)
L. casei	Antimicrobial activity against *S. aureus, B. subtilis, Micrococcus roseus*	(Gołek et al., 2009)
***L. paracasei, L. paracasei* A20**	Growth inhibition of *E. coli, S. agalactiae*, and *S. pyogenes* at 25 mg/ml concentration	(Gudina et al., 2010)
***L.* sp. CV8LAC**	70-82% inhibition of *C. albicans* CA-2894 and DSMZ 11225 biofilms/adhesion in pre-coating and co-incubation experiments	(Fraccia et al., 2010)
***L. acidophilus* DSM 20079**	Reduction of attachment and biofilm production of *S. mutans* ATCC 35668 due reduction of gtfB and gtfC expression	(Tahmourespour et al., 2011)
***L. acidophilus* (isolated from vaginal swabs)**	Cure of infected rabbits' eyes with *P. aeruginosa* after 36 h due anti-adhesive activity of surlactin	(Munira et al., 2013)
***L. fermentum* (isolated from dairy)**	Anti-adhesive activity (85%) against *C. albicans* ATCC70014	(Gomaa, 2013)
***L. jensenii* AATCC 25258 and *L. rhamnosus* ATCC 7469**	Antimicrobial, anti-adhesive and anti-biofilm activities against *A. baumannii, E. coli* and *S. aureus* at 25-50 mg/ml. activities	(Sambanthamoorthy, Feng, Patel, Patel, & Paranavitana, 2014)
***L. casei* MRTL3**	Antimicrobial activity against *S. aureus, S. epidermidis, B. cereus, L. monocytogenes, L. innocua, Shigella flexneri, Salmonella typhi*	(D. Sharma & Singh Saharan, 2014)
***Lactobacillus agilis* CCUG31450**	Anti-adhesion activity (67%) against *S. aureus* at 10 mg/ml concentration, and antimicrobial activity (20%) at 5mg/ml	(Gudina et al., 2015)
***L. helveticus* MRTL91**	Growth inhibition (70-99%) against *E. coli, P. aeruginosa, S. typhi, S. flexneri,*	(Deepansh Sharma & Saharan, 2016)

Table 6. Antimicrobial and anti-adhesion reported properties of surlactin produced by different Lactobacillus strains.

Strain	Antimicrobial or anti-adhesion activities	Reference
	S. aureus, S. epidermidis, L. monocytogenes, L. innocua, B. cereus at 25 mg/ml surlactin. Anti-adhesion (82-87%) against *B. cereus, S. epidermidis, L. monocytogenes, S. aureus* and *L. innocua*	

For anti-adhesion activity, the highest value was recorded for *L. fermentum* BS against *C. albicans* 22CBS 5703 (84%), the lowest activity was recorded for *L. delbrueckii* BS against *Proteus vulgaris* 1227 (9%). *L. acidophilus* showed by far the best biofilm formation properties on polystyrene (Gomaa, 2013). Extracted *Lactobacillus* spp. CV8LAC BS inhibited the adhesion of two *C. albicans* pathogenic biofilm producer strains in pre-coating and co-incubation experiments at concentration of 312.5 µg/ml by 82% and 70%, respectively (Fraccia et al., 2010). The *L. acidophilus* DSM 20079 released a BS that was able to interfere in the adhesion and biofilm formation of the *S. mutans* to a glass slide. gtfB and gtfC gene expression were decreased in the presence of the BS (Tahmourespour et al., 2011). *L. jensenii* and *L. rhamnosus* showed antimicrobial activities against *A. baumannii, E. coli* and *S. aureus.* Anti-adhesive and anti-biofilm activities were also observed for the same pathogens. Additionally, analysis by electron microscope indicated that surlactin caused membrane damage for *A. baumannii* and pronounced cell wall damage in *S. aureus* (Sambanthamoorthy et al., 2014).

2.4.3. Surlactin biosynthesis

There is no literature that reports findings related to the biosynthesis mechanisms of surlactin. Based on the studies, the majority of the *Lactobacillus* spp. reported produce a cell associated BS (Table 5), whereas only six secrete it (Satpute et al., 2016).

2.4.2.3.Genes related to the probiotic action of Lactobacilli

Genomic-based strategies (i.e., dedicated mutant analysis, in silico analysis, proteomics, DD-PCR, comparative genomic hybridization, DNA microarray for transcription profiling) have been providing confirmation of some postulated roles of genes and molecules of a number of strains of lactobacilli involved in probiotic action. However, this remains a technical challenge for several lactobacilli, and the number of currently identified genetic loci hypothesized to encode attributes supporting probiotic action confirmed by mutant analysis is still lacking (Lebeer et al., 2008).

For example, after comparative genomics, the main differences among lactobacilli were found in transferable regions like prophages and insertion sequence elements but also in other regions that are predicted to encode the production of the bacteriocin plantaricin, nonribosomal peptides, or EPSs. High levels of strain-specific variation were encountered in a 600-kb region containing genes involved mainly in sugar metabolism and which represents a lifestyle adaptation island (Kleerebezem et al., 2003; Molenaar et al., 2005).

L. acidophilus and *L. reuteri* EPS biosynthesis genes have been identified, epsB, epsC, epsE, and lr0957; epsE encodes the putative priming glycosyltransferase, which catalyzes the transfer of the first sugar in EPS polymer biosynthesis (Pfeiler, Azcarate-Peril, & Klaenhammer, 2007; Whitehead, Versalovic, Ross & Britton, 2008). Nevertheless,

phenotypic analyses of dedicated *Lactobacillus* mutants affected in EPS biosynthesis genes have not yet been performed (Lebeer et al., 2008).

Other probiotic bacteria that produces biosurfactants

Lactococcus lactis 53 biosurfactant was investigated to inhibit adhesion of four bacterial and two yeast strains isolated from explanted voice prostheses to silicone rubber (L. Rodrigues, van der Mei, Teixeira, & Oliveira, 2004). In another study L. lactis BS was characterized, by NMR, as glycolipid which consist of Methyl-2-O-methyl-b-D-xylopyranoside (Fig. 15), called xylolipid. The surface tension was reduced to 57.14% at 3.5 mg/ml, measured with the drop collapsed method (Saravanakumari & Mani, 2010).

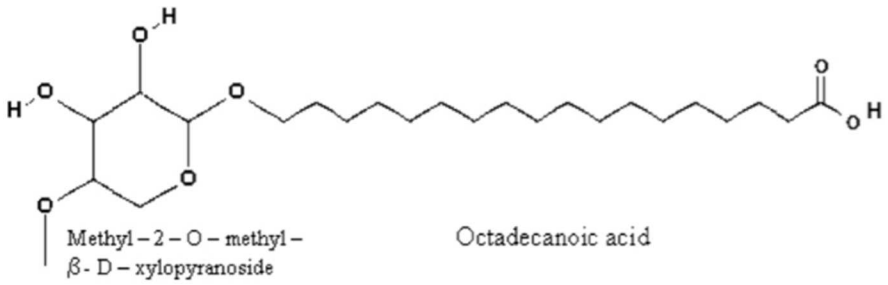

Figure 15. Structure of glycolipid biosurfactant purified from Lactococcus lactis.

Streptococcus thermophilus A biosurfactant was partially characterized by Rodrigues and colleagues (2006). A fraction rich in glycolipids was obtained by the fractionation of crude biosurfactant using hydrophobic interaction chromatography (HIC). CMC reported was 20 g/l, allowing for a surface tension value of 36 mN/m. This glycolipid was found to be an anti-adhesive and antimicrobial agent against several bacterial and yeast strains isolated from explanted voice prostheses (L. R. Rodrigues, Teixeira, van der Mei, & Oliveira, 2006).

2.5. Biosurfactant screening methods

In the nineteen sixties, a sophorolipid compound was first identified and can be synthesized by a selected number of non-pathogenic yeast species, and surfactin (*Bacillus* spp. producer) was purified and characterized by Arima et al. (1968) and others (Gorin, Spencer, & Tulloch, 1962). The primary objective in screening commercial viable BS is the discovery and optimization of good production strains with great yields (Satpute, Bhawsar, Dhakephalkar, & Chopade, 2008). Additionally, researchers are focused on finding new structures that exhibit preferred physicochemical properties, such as strong interfacial activity, low CMC, high emulsion capacity, good solubility, activity in a broad pH-range, and antimicrobial mechanisms (Walter et al., 2013). These characteristics are related to their mechanisms of action. Hence, the majority of the current screening methods are based on the physical effects of BS. There are two main screening methods of BS that are based on the interfacial or surface activity (directly and indirectly measured) and hydrophobicity of the cell surface. Furthermore, there are other methods that cannot be applied to all BS (Youssef et al., 2004).

Comparison of screening methods to detect BS production and its quality has been done (Youssef et al., 2004), concluding that it is necessary to perform a combination of techniques in order to successfully evaluate a potential BS. Drop collapse, emulsification and tensiometric evaluation are the most commonly used methods for testing BS. These processes are highly labor intensive and can take several days because they include the optimal growth of the microorganism (in order to release the BS), and then extraction and purification of the product. None of the most commonly used methods are suitable for a high-throughput screening according to Chen et al. (2007). Chen et al. stated it must

fulfill three requirements: 1) the ability to identify potential organisms; 2) the ability to assess quantitatively how effective the surfactant is; and, 3) the ability to screen many candidates quickly. Interestingly, the microplate assay, the penetration assay and the drop collapse assay can be performed in microplates, therefore used for high throughput screening (Ramkrishna, 2010b).

2.5.1. Direct surface or interfacial tension methods

2.5.1.1. Du-Nouy-Ring method

The Du-Nouy-Ring method measures the force required to detach a ring of wire from a surface where the detachment force is proportional to the interfacial tension. The use of an automated tensiometer is common, in addition to the use of Wilhelm plate instead of a ring. This method is easy to use and accurate and the disadvantages include the volume of sample required per analysis (a few milliliters) and the single sample tested per analysis (Walter, Syldatk, & Hausmann, 2010).

2.5.1.2. Pendant drop shape technique

In the pendant drop shape technique, a drop of liquid is allowed to hang from the end of a capillary; when adopting an equilibrium profile that is a unique function of the tube radius, the interfacial tension, its density and the gravitational field. Then, density and small and equatorial diameters are measured and calculated according to the equation given by Tadros (2005). This technique does not permit the simultaneous measurements (Tadros, 2005).

2.5.1.3. Stalagmometric method

Using a Trauble stalagmometer the surface tension of a liquid can be measured. This instrument is a pipette with a broad flattened tip, that permits large drops of reproducible size to form and finally drop under the gravity force. The surface tension is calculated based on the number of drops which fall per volume, the density of the sample and the surface tension of a reference liquid (e.g., water), per Dilmohamud et al. (2005) equation. Only consecutive measurements can be performed (Dilmohamud, Seeneevassen, Rughooputh et al., 2005).

2.5.2. Measurements based on surface or interfacial tension

2.5.2.1. Drop collapse assay

The drop collapse assay relies on the destabilization of liquid droplets by surfactants; drops of a cell suspension or of culture supernatant are placed on an oil coated, solid surface. The polar water molecules are repelled from the hydrophobic surface and the drops remain stable, if the liquid does not contain surfactants. On the contrary, if the liquid contains surfactants, the drops spread or even collapse because the force or interfacial tension between the liquid drop and the hydrophobic surface is reduced. The stability of drops is dependent on surfactant concentration and correlates with surface and interfacial tensions. This is a rapid assay that requires a small amount of sample and can be performed in microplates. However, it displays a relative low sensitivity since a significant concentration of surface active compounds must be present to cause a collapse of the aqueous drops on the oil or glass surfaces (Jain, Collins-Thompson, Lee et al., 1991).

2.5.2.1. Oil spreading assay

The oil spreading method is rapid and easy to prepare, requires no equipment and only a small volume of sample and can be used for lower surface activities of BSs. In this assay, 10 µl of crude oil is added to the surface of 40 ml of distilled water in a petri dish to form a thin oil layer. Then, 10 µl of culture supernatant or culture are carefully placed on the center of the oil layer. They oil is displaced and a clearing zone formed if BS is present in the sample. For pure BS, a linear correlation between quantity of surfactant and clearing zone diameter is given (Morikawa et al., 2000).

2.5.3. Cell surface hydrophobicity methods

2.5.3.1. Bacterial adhesion to hydrocarbons assay (BATH)

BATH is a photometrical method that measures the hydrophobicity of bacteria and it is based on the degree of adherence of cells to various liquid hydrocarbons. An aqueous suspension of washed microbial cells is mixed with hydrocarbon (e.g., hexadecane or octane), after mixing for 2 minutes, the two faces are separated. Then, hydrophobic cells become bound to hydrocarbon droplets and rise with the hydrocarbon, thus they are removed from the aqueous phase. The turbidity of the aqueous phase is measured and the decrease in its turbidity correlates to the hydrophobicity of the cells (Rosenberg, Gutnick & Rosenberg, 1980).

2.5.3.2. Hydrophobic interaction chromatography (HIC)

This method was developed by Smyth et al. (1978), and allows the simultaneous isolation and screening of microbes. HIC is a chromatographic procedure based on hydrophobic

interaction between the nonpolar groups on a hydrophobic chromatographic resin and the nonpolar regions of a particle. A bacterial suspension is drained into a gel bed of hydrophobized sepharose. Hydrophobic microbes are retained by the gel and the degree of adsorption of the cells to the gel can be measured by the turbidity of the eluate or by bacteria counting. The advantage of HIC is that can be used for combined strains at a time (Smyth, Jonsson, Olsson et al., 1978).

2.5.4. Other methods

2.5.4.1. Hemolysis

The blood agar method can be used for a preliminary screening of BS producing microorganisms due to the BS capacity to cause lysis of erythrocytes. Mulligan et al. (1984) developed a technique where cultures are inoculated on sheep blood agar plates and incubated for 2 days at 25°C. Lysis of the blood cells show a colorless halo around the colonies, or pure BS can be used instead. However, this method is not specific since lytic enzymes can also lead to clearing zones. Thus; this method can give false negative and false positive results, thereby other methods may be used to support this technique (Youssef et al., 2004).

A semi quantitative BS detection method was developed by Burch et al. (2010) using an airbrush to apply a fine mist of oil droplets on agar plates, surfactants can be observed instantaneously as halos around biosurfactant-producing colonies (Burch et al., 2010). This method can simultaneously assay multiple strains (already colony-grown on agar plate) within a few seconds. However, it is not clear if there is an invariant correlation

between a surfactants hydrophilic-lipophilic balance and the shape that imparts to oil droplet on an agar surface and causes either a “bright” or “dark” halo (Burch et al., 2010).

Need for new screening method for biosurfactant produced by Lactobacilli

It appears that if there is a more effective approach to determine the production of surlactin, it will be more accessible for its utilization in controlling pathogens. Although, studies have identified the genetic and enzymatic pathways responsible in BS production in some bacterial species (not for Lactobacilli), and used them as a marker in methods for its detection, the tools and genetic resources developed are still in need to prove useful application in a novel and rapid technique to overcome the time-consuming disadvantage of current methods.

CHAPTER III

MATERIALS AND METHODS

Bioinformatics is a sub discipline of biology and computer science concerned with the acquisition, storage, analysis, and dissemination of biological data, most often DNA and amino acids sequences. Bioinformatics uses computer programs for a variety of applications, including determining gene and protein functions, establishing evolutionary relationships, and predicting the three-dimensional shapes of proteins (Wong, 2016).

2.5. Retrieval of sequences

1.1.Retrieval of Lactobacillus data

Lactobacilli sequences, including complete and partial genomes, were retrieved from GenBank part of the International Nucleotide Sequence Database Collaboration, which comprises the DNA DataBank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at NCBI. These three organizations exchange data daily. A database was created on February 16, 2017 using a local server including BLAST algorithms.

1.2.Retrieval of previously identified genes for biosurfactant production

The sequences for the genes identified for BS production (Table 7) were retrieved from GenBank.

Table 7.Identified genes for biosurfactant production and their producing microorganisms

Biosurfactant name/type	Microorganism	Gene(s)	Reference
Surfactin lipopeptide/extracellular	*B. subtilis*	srfA, srfB, srfC and srfD	Peypoux et al., 1999
Lichensyn lipopeptide/extracellular	*B. licheniformis*	licAA, lic AB, licAC and licAD	Marahiel et al., 1999
Rhamnolipid glycolipid/extracellular	*P.* spp. MIS38	arfA, arfB, and arfC	Roongsawang et al., 2003
Emulsan lipopolysaccharide/ extracellular	*A. lwoffii*	wza, wzb,wzc,wzx,wzy	Nakar & Butnick, 2001
Serrawettin W1/glycolipid/extracellular	*S. marcescens*	pswP	Eberl et al 1999

1.3.Retrieval of other surfactant-related compounds

Norine is a database dedicated to NRP that contains more than 1000 peptides (http://bioinfo.lifl.fr/norine/). For each peptide, the database stores its structure and other annotations such as the biological activity, producing organisms, and bibliographical references among others. The database can be queried in order to search for peptides through their annotations as well as through their monomeric structures (Caboche et al., 2008). BioSurfDB database (www.biosurfdb.org) is a curated system including biosurfactant lists, grouped by producing organism, surfactant name, class and reference (Oliveira et al., 2015). Anti-SMASH (https://antismash.secondarymetabolites.org/#!/start) is a database that integrates and cross-links with a large number of in silico secondary metabolite analysis tools. Additionally, it allows the rapid genome-wide identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genomes (Medema et al., 2011).

2. In silico identification of gene for biosurfactant production in lactobacilli

In silico (from Latin "in silicon", due to the mass use of silicon for semiconductor computer chips) is used to refer to “performed on computer or via computer simulation”. In silico studies include drug discovery and virtual screening, cell models, genetics and proteomics (Wong, 2016).

A basic local alignment search was performed for all the genes in Table 5 with genomic DNA of all the *Lactobacillus* genomic sequences. ORF finder was used to search for long ORFs that would likely be part of a gene for potential protein encoding segments. And then web Batch conserved domains (CD) search tool from NCBI was used to identify the proteins within the operons. BLAST compares nucleotide or protein sequences to sequence databases and calculates the statistical significance (Altschul et al., 1990 & 1997). ORF finder searches for ORFs in the DNA input sequence. This program returns the range of each ORF, along with its protein translation. Batch CD-Search serves as both a web application and a script interface for a conserved domain search on multiple protein sequences, accepting up to 4,000 proteins in a single job. It has a graphical display of the result for any individual protein from the input list, or to download the results for the complete set of proteins.

3. Design of primers set

Selection of critical domain coordinates, of 500-900 bases, was based on identity percentage. Integrated DNA Technologies (http://www.idtdna.com/site) PrimerQuest tool was used to design the set of primers for Adenylation domain-NRPS, including 7 sequences of *L. plantarum.*

CHAPTER IV

RESULTS AND DISCUSSION

1. Genomic sequences

A total of 173 *Lactobacillus* species (Appendix A) with 198 different strains, including 126 completed genomes and 922 incomplete and partial genome sequences were downloaded from GenBank. The data contained 2,674,859,000 total bases on 115,787 sequences (contigs). A contig is a series of overlapping DNA sequences used to make a physical map that reconstructs the original DNA sequence of a chromosome or a region of a chromosome. A contig can also refer to one of the DNA sequences used in making such a map.

Anti-SMASH database contains secondary metabolites such as antibiotics, bacteriocins but it did not show any data regarding biosurfactants. BioSurfDB database had listed some of the BS produced by Lactobacilli, but only with the microorganism, class of BS and no structure of the compounds nor genes were available. As of other biosurfactants they provided either the link to NCBI or the sequence. However, data was consistent with that obtained from GenBank retrieval. Norine database contains 143 NRPs registered as surfactants grouped in 16 families, orfamide, amphibactin, amphisin, aquachelin,

carmabin, fengycin, iturin, lichenysin, marinobactin, putisolvin, serrawettin, surfactin, syringafactin, syringomycin, tolaasin, and viscosin. However, data regarding biosynthetic pathways and genes were not included within the structure, producing organisms, taxonomy and references. *Lactobacillus* strains and neither surfactin or any other related BS was listed in this database, that was updated on December 2016.

1.2. Sequence alignments

An alignment is the process of matching up the nucleotide or amino acid residues of two or more biological sequences to achieve maximal levels of identity and, in the case of amino acid sequences, conservation. The objective is assessing the degree of similarity and the possibility of homology. Identity is the extent to which two, nucleotide or amino acid, sequences have the same residues at the same positions in an alignment, expressed as percentage. Similarity is the extent to which nucleotide or protein sequences are related. Similarity between two sequences can be expressed as percent sequence identity or percent positive substitutions. Homology is the similarity attributed to descent from a common ancestor. Homologous biological components, such as genes, proteins, structures, are called homologs (Fassler & Cooper, 2011).

1.2.1. Lactobacillus spp. and surfactin gene cluster

BLAST results showed 101 *Lactobacillus* strain sequences producing significant alignments with surfactin regulatory gene cluster. Whereas any other identified gene showed no meaningful identity with any of the 198 lactobacillus strains blasted. *L. plantarum* showed the highest significant alignment with the identified gene cluster for BS producers, srfA (CAE02630.1), srfB (CAE02631.1), and srfC (CAE02633.1) size

3584, 3586, and 1278, respectively. The identity with srfA showed scores on the range of 1467 and 1472, E-value of 0.0 for *L. plantarum* strains K35, P86, P67, P26, RI-515, WCFS1, B21, PS128, and a score of 1074 for *L. plantarum* 19.1. There was a drastic decrease in score of 346 for *L. brevis* KB290 plasmid pKB290-3 DNA and a E-value of 1e-92. The rest of the strains had a score between 217 and 322 and E-value in the range of 2e-85 and 1e-53. The Expect value (E-value) represents the number of different alignments with scores equivalent to or better than the score of an alignment, that is expected to occur in a database search by chance. Thus, the lower the E-value, the more significant the score and the alignment (Fassler & Cooper, 2011). Similarly, srfB had scores between 1409 and 1412 with an E-value of 0.0 for the same strains, whereas the rest of the hits were between 220 and 310, and E-values of 2e-81 to 2e-54. The last subunit of the surfactin gene cluster, srfC again showed the same strains on the top 10 as the most significant alignments. However, the scores and E-values declined down to 401-424, and 1e-116 and 1e-111.

Table 8. NRPS NpsA Lactobacillus plantarum, 5289 amino acids.

Source	CDS Region in Nucleotide	Protein	Name	Organism	Strain
RefSeq	NC_004567.2 536024-551893 (+)	YP_004888577.1	NRPS NpsA	*L. plantarum*	WCFS1
RefSeq	NC_004567.2 536024-551893 (+)	WP_011101060.1	NRPS	*L. plantarum*	WCFS1
RefSeq	NZ_MKGF01000013.1 50256-66125 (-)	WP_011101060.1	NRPS	*L. plantarum*	RI-515
INSDC	AL935263.2 536024-551893 (+)	CCC78063.1	NRPS NpsA	*L. plantarum*	WCFS1

A NRPS NpsA, from *L. plantarum* was identified to have tandem domains. A domain is a discrete portion of a protein assumed to fold independently of the rest of the protein and possessing its own function. The NRPS NpsA was identified in *L. plantarum* strains WCFS1 and RI-515 (Table 8), 5289 amino acids length (sequence in FASTA format, Appendix B. FASTA format is a representation of either nucleotide sequences or peptide sequences, in which single-letter codes are used). The Batch CD-search showed five types of architecture (Fig. 16). The first one where three domain families, adenylation, thiolation (or PCP) and condensation formed five modules in seven *L. plantarum* strains. The five modules are highly similar but not identical. This is expected because of each module synthetize different amino acids and its recognition and attachment site for the following amino acid. Furthermore, the modules 1-5 are conserved among the seven *L. plantarum* strains, showing identical (99%) sequences at the nucleotide level. Whereas the second architecture observed was with *L. plantarum, L. brevis, L. reuteri* and *L. iners* having one copy of the same CDs but in different order, condensation, adenylation, and thiolation. These two types resemble NRPS type II operon. The last configuration was an assembly of polyketide synthetase (PKS); a single module was observed in *L. reuteri*

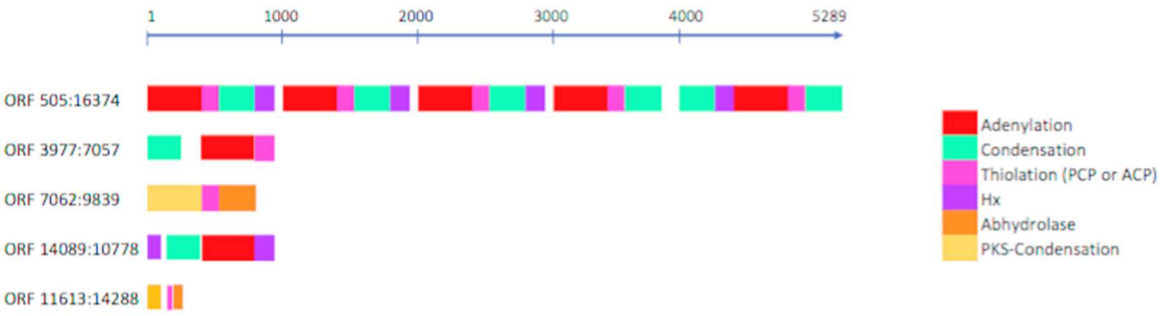

Figure 16. Multidomain organization of the gene clusters encoding NRPS and PKS from Lactobacillus

including acyl carrier protein (ACP) domain. Lastly, a combination of NRPS and PKS modules was found in *L. plantarum* and *L. reuteri* (Table 9).

Table 9. NRPS NpsA and PKS operons on Lactobacillus found when compared with surfactin gene clusters using BLAST.

Architecture	Specie	Strain	ID
NRPS (2-5 copies)	*L. plantarum*	K35	LEBT01000005.1
	L. plantarum	P86	LEBJ01000017.1
	L. plantarum	P67	LEBM01000029.1
	L. plantarum	P26	LEBQ01000025.1
	L. plantarum	RI-515	MKGF01000013.1
	L. plantarum	WCFS1	AL935263.2
	L. plantarum	PS128	LBHS01000001.1
	L. plantarum	19.1	LUXM01000033.1
NRPS (2-5 copies), single & PKS	*L. plantarum*	4_3	AYTU01000062.1
NRPS	*L. brevis*	KB290	AP012170.1
	L. iners	LEAF 2052A-d	AEKI01000023.1
	L. iners	DSM 13335	AZET01000002.1
	L. iners	DSM 13335	GG700805.1
	L. iners	AB-1	ADHG01000001.1
NRPS & PKS	*L. reuteri*	TMW1.656	JOSW02000004.1
	L. reuteri	LTH2584	JOSX01000007.1
PKS	*L. reuteri*	LTH5448	JOOG01000036.1

L. plantarum of all the 173-species compared showed significant similarity with the identified gene(s) NRPS SrfA. This finding could explain that the BS produced by these specific species follows a quite similar biosynthetic assembly line as surfactin. Whereas, the rest of the *Lactobacillus* spp. do not share a similar NRPS pathway or could be ribosomally synthesized. However, there are gene clusters that contain functionally related but non-homologous genes and that represent functional gene organizations with operon-like features (Osbourn & Field, 2009), like NRPS. In addition to the types of BSs, surfactin and surlactin, are different in composition and release mode (intra or

extracellular). Surlactin from *L. plantarum* has been reported as cell-bound whereas surfactin is extracellular.

Because of the large diversity of building blocks, over 500, for NRPS different pathways may lead to the same amino acid produced; which means operon architectures are not exclusively representative. Genes coding for NRPS are organized in operons or in clusters. The modularly organization of NRPSs allows each module for the incorporation of a specific amino acid in the final peptide product (Stachelhaus, Mootz, & Marahiel, 1999). According to Krop & Marahiel (2007), only three domains are ubiquitous in NRPS and essential for peptide elongation: adenylation, thiolation, condensation, and there is a fourth thioesterase domain (Strieker et al., 2010). The thioesterase domain is responsible for the release of the peptide by hydrolysis, macrocyclization (present in surfactin), heterocyclization or cross-linking (Grunewald & Marahiel, 2006). If NRPS NspA is confirmed for encoding BS biosynthesis, this could explain its absence in *Lactobacillus*, since most surlactin is intra-cellular produced. However, the overall identity among Te domains is only 10-15%, therefore reflecting the high degree of specialization for their catalyzed cyclization reactions (Grunewald & Marahiel, 2006). Whereas the ubiquitous domains remain present. Adenylation catalyzes two reactions. First selects the cognate building block from the pool of available substrates, followed by activation as an aminoacyl adenylate intermediate. And then, the activated aminoacyl adenylate is transferred onto the thiol group of the 4'-phosphopantetheine cofactors of the PCP, which is the only NRPS domain without autonomous catalytic activity. The PCP facilitates the ordered transport of substrates and elongation intermediates to the catalytic centers with all intermediates covalently tethered (Hur et al., 2012). This principle

facilitates substrate channeling and overcomes diffusive barriers, therefore maximizing the catalytic efficiency of the NRPS-mediated biosynthesis. Condensation domain mediates the formation of the peptide bond in NRPS. This domain catalyzes the nucleophilic attack of the down-stream PCP-bound amino acid with its α-amino group on the electrophilic thioester of the upstream PCP-bound amino acid or peptide (Strieker et al., 2010). Biochemical characterization of different condensation domains revealed that the acceptor site discriminates against amino acids of opposite stereochemistry and with no cognate side chains. In contrast, the donor site is more tolerant of the respective electrophile. Furthermore, in addition to A domains, C domains serve as a selectivity filter in NRPSs (Grunewald & Marahiel, 2006).

PKS are modular proteins implicated in the biosynthesis of complex molecules through a series of catalytic conducts. The products of these enzymes have diverse structural complexities by combinatorial use of a specific sequential order of catalytic domains (Yadav et al., 2009). Each module contains ACP, ketosynthase (KS) and acyltransferase (AT) domains that extend the linear sequence of an intermediate by two carbon atoms. The AT loads the ACP with a building block from a specific acyl-CoA, and the KS catalyzes C-C bond formation between the intermediate from the upstream module and the acyl-ACP. In addition, modules may contain domains that successively modify the β-keto group to a hydroxyl (keto reductase (KR)), a double bond (dehydratase), or a single bond (enoyl reductase). The ACP employs a phosphopantetheine (Ppant) arm and thioester bond to tether polyketide intermediates and building blocks, and transfers them to respective catalytic domains for loading, extension and keto-group processing or modification. Through direct fusion or a non-covalent docking interaction, the ACP also

transfers the fully processed intermediate to the KS in the subsequent module for further extension or to the thioesterase in the final module. (Dutta et al., 2014). Selvin et al. (2016) suggested that the biosynthesis of BS produced by marine actinobacteria might be mediated by PKS gene. Their in-silico studies showed iterative type II PKS domain gene fragments, indicating the existence of a PKS gene cluster associated with BS biosynthesis (Selvin et al., 2016). The comparative analysis of the isolated PKS II (ACS45380-ACS45382) with *Lactobacillus* showed β-ketoacyl-[ACP] synthase II, with 37-40% identity, E-value of 1e-33, and a query cover of 93%. β-ketoacyl-[ACP] synthase II is an enzyme involved in several enzymatic systems such as the production of fatty acids, polyketide antibiotics, and conidial green pigment (Garwin, Klages & Cronan, 1980; Cui, Wei, Liang, et al., 2016). Modular PKSs are thought to share a common ancestor with the mammalian fatty acid synthase. However, to achieve perfect fidelity in product formation, the sequential modular PKSs have evolved to be highly ordered in structure and function (Dutta et al., 2014). However, further in vitro analyses are needed to identify if it is related to the BS production. Additionally, this confirms that the diversity among BSs is specie and strain-dependent. Therefore, the characterization of each BS is necessary to elucidate specific properties rather than having a cocktail of strains producing the BS that has been tested.

1.2.2. Lactobacillus spp. and various identified genes for BS production

The identified genes from various bacteria encoding BS production (shown in Table 7) did not have significant identity with *Lactobacillus* species. Furthermore, the lack of data about composition and structure of surlactin makes difficult the targeting of domains.

2. Primers design

The set of designed degenerate primers for amplification of NpsA adenylation domains, each for each of the five copies, is displayed in Table 10. This set of primers is intended for PCR screening of *L. plantarum* for putative surlactin production. PCR screening should be performed only after proving that NRPS NpsA is indeed responsible for BS biosynthesis.

Table 10. List of degenerate primers designed for amplification of NRPS gene fragments in L. plantarum

Name	Primer	Sequence (5'-3')	Size	Tm[a] (°C)	GC[b] (%)
LplantA NRPS1	Forward	GGTACAACCGGACATCCTAAAG	22	62.225	50
	Reverse	CCACTCGTGCCAGAAGTAAA	20	62.005	50
LplantA NRPS2	Forward	GGTAAGCCGAAGGGAGTAATG	21	62.046	52.381
	Reverse	CGTCAGAGCGATACACATCTAAT	23	61.948	43.478
LplantA NRPS3	Forward	CAATCAACGCCTGGAAGAATTAG	23	61.834	43.478
	Reverse	CTGCACCTTGAGGCAATAAAC	21	61.739	47.619
LplantA NRPS4	Forward	TTCAGTGTGGGAGCAAGTATG	21	62.125	47.619
	Reverse	GAACACCACCCAGTGCTAAT	20	62.175	50
LplantA NRPS5	Forward	GAGCGATTGGTGAGCTATGT	20	61.919	50
	Reverse	GACCAGTTGTGCCAGATGTA	20	61.824	50

[a]Melting temperature, [b]Guanine/Cytosine content

PCR is one of the most important molecular biology assays. Small amounts of the genetic material are amplified to be able to identify, manipulate and detect target DNA. Applications include detection of infectious organisms, detection of genetic variations such as mutations among several other tasks. PCR involves three steps denaturation, annealing, and extension or elongation. In the first step the genetic material is desaturated, converting the double stranded DNA molecules to single strands. The primers are then annealed to the complementary regions of the single stranded molecules.

At elongation step, they are extended by the action of DNA polymerase. Denaturation, annealing, and elongation are temperature sensitive and generally temperatures choice are 94°C, 60°C and 70°C, respectively. Important design considerations are a key to specific amplification; size, melting temperature (T_m), GC content and specificity. It is generatlly accepted that the optimal length of PCR primers is 18-30 base pairs. This size is long enough for adequate specificity and short enough for primer to bind easily to the template at the annealing temperature. Primer melting temperature by definition is the temperature at which one half of the DNA duplex sill dissociate to become single stranded and indicates the duplex stability. Primers with melting temperatures in the range of 52-58 °C generally produce good results. Primers with melting temperatures above have a tendency for secondary annealing; therefore, should be avoided. The GC content (number of G's and C's in the primer as a percentage of the total bases) of primer should be 40-60%. The precense of G or C bases withing the last five bases from the 3' end of primers (GC clamp) helps promote specific binding at the 3' end due to the stronger bonding of G and C bases. More than 3 G's or C's should be avoided in the last 5 bases at the 3' end of the primer. Primers designed for a sequence must not amplify other genes in the mixture. The set of primers were BLASTed against the Lactobacillus non-redundant database to test the specificity. Identity percentage were 88-100 indicatng high specificity.

CHAPTER V

CONCLUSION AND FUTURE STUDIES

1. Conclusion

BSs are potential agents in several industrial and environmental processes as well as in biomedical and therapeutic applications; it is essential to make them cost competitive. Understanding of the genetic regulatory mechanisms would help to develop screening methods for BS production. Additionally, it could lead to the engineering to produce strains with better product characteristics and acquired capability of utilizing cheap industrial wastes as substrates. Furthermore, understanding biosurfactants' key role in biofilms would contribute to improving their application against pathogens and other undesired microorganisms. Probiotic surfactant-producing bacteria (i.e., *Lactobacillus)* use against foodborne pathogens is a promising tool in food industries and medical environments.

This study thus provides an overview of the role and importance of molecular genetics and gene regulation mechanisms behind the biosynthesis of surlactin. However, the genetics of surlactin synthesis, which is a primary factor determining their productivity, has not been discovered. The present study serves this purpose by comparing molecular genetic regulation for the biosynthesis of a wide variety of BSs with surlactin. It is

concluded that surlactin produced by some strains of *L. plantarum, L. inners, L. reuteri* and *L. brevis* could be nonribosomally synthesized, sharing high identity with surfactin NRPS biosynthesis pathway. PKS were also found to be potentially involved in *L. reuteri* and hybrid system with NRPS in *L. plantarum* with a possible responsibility in surlactin production. However, NRPSs are involved in the biosynthesis of not only biosurfactants, but also of a number of important bioactive secondary metabolites. These include antibiotics, bacteriocins, and some of them may also show surfactant activities.

1. Future studies

Further studies are needed to screen the *Lactobacillus plantarum* strains with NRPS NpsA for surlactin production, followed by suppression of the identified gene clusters. Thus, the genetic locus identified as potentially responsible for BS production could be tested. Chemical structure characterization of each type of surlactin produced by lactobacilli is indeed needed. Knowing the amino acid composition allows the use of predicting tools for the precursor building blocks and its enzymatic activities.

Adhesion and anti-adhesion features in lactobacilli producing biosurfactant may be worthy to analyze in relation to the cell-bound BS. It is known that these adhesion and anti-adhesion properties play an important role in adaptation to environment in different surfaces, in addition to the communal adaptation or repelling antagonistic microorganisms. Correlational studies may indicate whether these two factors, cell-bound BS and adhesion, are biosynthetically dependent or independent.

REFERENCES

A.M. Shete, G. W., I.M. Banat, and B.A. Chopade. (2006). Maping of patents on bioemulsifier and biosurfactant: A review. *Journal of Scientific & industrial Research, 65*, 91-115.

Agladze, K., Wang, X., & Romeo, T. (2005). Spatial periodicity of Escherichia coli K-12 biofilm microstructure initiates during a reversible, polar attachment phase of development and requires the polysaccharide adhesin PGA. *J Bacteriol, 187*(24), 8237-8246. doi:10.1128/jb.187.24.8237-8246.2005

Andersen, J. B., Koch, B., Nielsen, T. H., Sorensen, D., Hansen, M., Nybroe, O., . . . Givskov, M. (2003). Surface motility in Pseudomonas sp. DSS73 is required for efficient biological containment of the root-pathogenic microfungi Rhizoctonia solani and Pythium ultimum. *Microbiology, 149*(Pt 1), 37-46. doi:10.1099/mic.0.25859-0

Arima, K., Kakinuma, A., & Tamura, G. (1968). Surfactin, a crystalline peptidelipid surfactant produced by Bacillus subtilis: isolation, characterization and its inhibition of fibrin clot formation. *Biochemical and Biophysical Research Communications, 31*(3), 488-494.

Basak, S., N, M., O, R., & Kumar Mallick, S. (2013). Biofilms: A Challenge to Medical Fraternity in Infection Control. doi:10.5772/55649

Berti, A. D., Greve, N. J., Christensen, Q. H., & Thomas, M. G. (2007). Identification of a biosynthetic gene cluster and the six associated lipopeptides involved in swarming motility of Pseudomonas syringae pv. tomato DC3000. *J Bacteriol, 189*(17), 6312-6323. doi:10.1128/JB.00725-07

Bhomkar, P., Materi, W., Semenchenko, V., & Wishart, D. S. (2010). Transcriptional response of E. coli upon FimH-mediated fimbrial adhesion. *Gene Regul Syst Bio, 4*, 1-17.

Bogino, P. C., Oliva Mde, L., Sorroche, F. G., & Giordano, W. (2013). The role of bacterial biofilms and surface components in plant-bacterial associations. *Int J Mol Sci, 14*(8), 15838-15859. doi:10.3390/ijms140815838

Brint, J. M., & Ohman, D. E. (1995). Synthesis of multiple exoproducts in Pseudomonas aeruginosa is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. *Journal of Bacteriology*, 7155-7163.

Brzozowski, B., Wlodzimierz, B., & Gołek, P. (2011). Physicochemical properties of Lactobacillus biosurfactants. *Food Technol. Biotechnol, 49*(2), 177-186.

Burch, A. Y., Shimada, B. K., Browne, P. J., & Lindow, S. E. (2010). Novel high-throughput detection method to assess bacterial surfactant production. *Appl Environ Microbiol, 76*(16), 5363-5372. doi:10.1128/AEM.00592-10

Caboche, S., Pupin, M., Leclere, V., Fontaine, A., Jacques, P., & Kucherov, G. (2008). NORINE: a database of nonribosomal peptides. *Nucleic Acids Res, 36*(Database issue), D326-331. doi:10.1093/nar/gkm792

Cameotra, S. S., & Makkar, R. S. (2004). Recent applications of biosurfactants as biological and immunological molecules. *Curr Opin Microbiol, 7*(3), 262-266. doi:10.1016/j.mib.2004.04.006

Cerca, N., Maira-Litran, T., Jefferson, K. K., Grout, M., Goldmann, D. A., & Pier, G. B. (2007). Protection against Escherichia coli infection by antibody to the Staphylococcus aureus poly-N-acetylglucosamine surface polysaccharide. *Proc Natl Acad Sci U S A, 104*(18), 7528-7533. doi:10.1073/pnas.0700630104

Ceresa, C., Tessarolo, F., Caola, I., Nollo, G., Cavallo, M., Rinaldi, M., & Fracchia, L. (2015). Inhibition of Candida albicans adhesion on medical-grade silicone by a Lactobacillus-derived biosurfactant. *J Appl Microbiol, 118*(5), 1116-1125. doi:10.1111/jam.12760

Chen, J., Huang, P. T., Zhang, K. Y., & Ding, F. R. (2012). Isolation of biosurfactant producers, optimization and properties of biosurfactant produced by Acinetobacter sp. from petroleum-contaminated soil. *J Appl Microbiol, 112*(4), 660-671. doi:10.1111/j.1365-2672.2012.05242.x

Cirigliano, M. C., & Carman, G. M. (1984). Isolation of a bioemulsifier from Candida lipolytica. *Appl Environ Microbiol, 48*(4), 747-750.

Cirigliano, M. C., & Carman, G. M. (1985). Purification and characterization of liposan, a bioemulsifier from candida lipolytica. *Appl Environ Microbiol, 50*(4), 846-850.

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., & Lappinscott, H. M. (1995). MICROBIAL BIOFILMS. *Annual Review of Microbiology, 49*, 711-745. doi:10.1146/annurev.mi.49.100195.003431

Das, P., Mukherjee, S., & Sen, R. (2008). Genetic Regulations of the Biosynthesis of Microbial Surfactants: An Overview. *Biotechnology and Genetic Engineering Reviews, 25*(1), 165-186. doi:10.5661/bger-25-165

de Bruijn, I., de Kock, M. J., Yang, M., de Waard, P., van Beek, T. A., & Raaijmakers, J. M. (2007). Genome-based discovery, structure prediction and functional analysis of cyclic lipopeptide antibiotics in Pseudomonas species. *Mol Microbiol, 63*(2), 417-428. doi:10.1111/j.1365-2958.2006.05525.x

Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and molecular Biology Reviews, 61*(1), 47-64.

Dubern, J. F., Coppoolse, E. R., Stiekema, W. J., & Bloemberg, G. V. (2008). Genetic and functional characterization of the gene cluster directing the biosynthesis of putisolvin I and II in Pseudomonas putida strain PCL1445. *Microbiology, 154*(7), 2070-2083. doi:doi:10.1099/mic.0.2008/016444-0

Dunne, W. M. (2002). Bacterial Adhesion: Seen Any Good Biofilms Lately? *Clinical Microbiology Reviews, 15*(2), 155-166. doi:10.1128/cmr.15.2.155-166.2002

Dutta, S., Whicher, J. R., Hansen, D. A., Hale, W. A., Chemler, J. A., Congdon, G. R., . . . Skiniotis, G. (2014). Structure of a modular polyketide synthase. *Nature, 510*(7506), 512-517. doi:10.1038/nature13423

Eberl, L., Molin, S., & Givskov, M. (1999). Surface motility of Serratia liquefaciend MG1. *Journal of Bacteriology, 181*(6), 1703-1712.

Fischer, M., Friedrichs, G., & Lachnit, T. (2014). Fluorescence-based quasicontinuous and in situ monitoring of biofilm formation dynamics in natural marine environments. *Journals American Society for Microbiology, 80*(12), 3721-3728.

Fouad, H. K., Khanaqa, H. H., & Munira, C., I. (2010). Purification and characterization of surlactin produced by L. acidophilus. *2*.

Fraccia, L., Cavallo, M., Allegrone, G., & Martinotti, M. G. (2010). A Lactobacillus-derived biosurfactant inhibits biofilm formation of human pathogenic Candida albicans biofilm producers. *Appl Microbiol Biotechnol, 2*, 827-837.

Garcia-Medina, R., Dunne, W. M., Singh, P. K., & Brody, S. L. (2005). Pseudomonas aeruginosa acquires biofilm-like properties within airway epithelial cells. *Infect Immun, 73*(12), 8298-8305. doi:10.1128/IAI.73.12.8298-8305.2005

Gołek, P., Bednarski, W., Brzozowski, B., & Dziuba, B. (2009). The obtaining and properties of biosurfactants synthesized by bacteria of the genus Lactobacillus. *Annals of Microbiology, 59*(1), 119-126. doi:10.1007/bf03175608

Gomaa, E. Z. (2013). Antimicrobial and anti-adhesive properties of biosurfactants produced by lactobacilli isolaes, biofilm formation and aggregation ability. *J. Gen. Appl. Microbiol., 59*, 425-436.

Gomez, N. C., Ramiro, J. M., Quecan, B. X., & de Melo Franco, B. D. (2016). Use of Potential Probiotic Lactic Acid Bacteria (LAB) Biofilms for the Control of Listeria monocytogenes, Salmonella Typhimurium, and Escherichia coli O157:H7 Biofilms Formation. *Front Microbiol, 7*, 863. doi:10.3389/fmicb.2016.00863

Gorin, P. A. J., Spencer, J. F. T., & Tulloch, A. P. (1962). Hydroxy fatty acid glycosides of sophorose from Torulopsis magnoliae. *Canadian Journal of Chemistry, 39*.

Grunewald, J., & Marahiel, M. A. (2006). Chemoenzymatic and template-directed synthesis of bioactive macrocyclic peptides. *Microbiol Mol Biol Rev, 70*(1), 121-146. doi:10.1128/MMBR.70.1.121-146.2006

Gudina, E. J., Fernandes, E. C., Teixeira, J., & Rodrigues, L. (2015). Antimicrobial anad anti-adhesive activies of cell-bound biosurfactant from Lactobacillus agilis CCUG31450. *The Royal Society of Chemistry, 5*(90960-90968). doi:10.1039/C5RA11659G

Gudina, E. J., Rangarajan, V., Sen, R., & Rodrigues, L. R. (2013). Potential therapeutic applications of biosurfactants. *Trends Pharmacol Sci, 34*(12), 667-675. doi:10.1016/j.tips.2013.10.002

Gudina, E. J., Rocha, V., Teixeira, J. A., & Rodrigues, L. R. (2010). Antimicrobial and antiadhesive properties of a biosurfactant isolated from Lactobacillus paracasei ssp. paracasei A20. *Lett Appl Microbiol, 50*(4), 419-424. doi:10.1111/j.1472-765X.2010.02818.x

Guiton, P. S., Hung, C. S., Kline, K. A., Roth, R., Kau, A. L., Hayes, E., . . . Hultgren, S. J. (2009). Contribution of autolysin and Sortase a during Enterococcus faecalis DNA-dependent biofilm development. *Infect Immun, 77*(9), 3626-3638. doi:10.1128/iai.00219-09

Harmsen, M., Yang, L., Pamp, S. J., & Tolker-Nielsen, T. (2010). An update on Pseudomonas aeruginosa biofilm formation, tolerance, and dispersal. *FEMS Immunol Med Microbiol, 59*(3), 253-268. doi:10.1111/j.1574-695X.2010.00690.x

Hassan, A., Usman, J., Kaleen, F., Omair, M., Khalid, A., & Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. *Braz J Infect Dis, 15*(4), 305-311.

Henrici, A. T. (1933). Studies of freshwater bacteria. *J Bacteriol, 25*, 277-287.

Hewald, S., Josephs, K., & Bolker, M. (2005). Genetic analysis of biosurfactant production in Ustilago maydis. *Appl Environ Microbiol, 71*(6), 3033-3040. doi:10.1128/AEM.71.6.3033-3040.2005

Hewald, S., Linne, U., Scherer, M., Marahiel, M. A., Kamper, J., & Bolker, M. (2006). Identification of a gene cluster for biosynthesis of mannosylerythritol lipids in the basidiomycetous fungus Ustilago maydis. *Appl Environ Microbiol, 72*(8), 5469-5477. doi:10.1128/AEM.00506-06

Hong, S. H., Lee, J., & Wood, T. K. (2010). Engineering global regulator Hha of Escherichia coli to control biofilm dispersal. *Microb Biotechnol, 3*(6), 717-728. doi:10.1111/j.1751-7915.2010.00220.x

Howard, J. C., Heinemann, C., Thatcher, B. J., Martin, B., Gan, B. S., & Reid, G. (2000). Identification of collagen-binding proteins in Lactobacillus spp. with surface-enhanced laser desorption/ionization-time of flight ProteinChip technology. *Appl Environ Microbiol, 66*(10), 4396-4400.

Hur, G. H., Vickery, C. R., & Burkart, M. D. (2012). Explorations of catalytic domains in non-ribosomal peptide synthetase enzymology. *Nat Prod Rep, 29*(10), 1074-1098. doi:10.1039/c2np20025b

Jackson, K. D., Starkey, M., Kremer, S., Parsek, M. R., & Wozniak, D. J. (2004). Identification of psl, a locus encoding a potential exopolysaccharide that is essential for Pseudomonas aeruginosa PAO1 biofilm formation. *J Bacteriol, 186*(14), 4466-4475. doi:10.1128/jb.186.14.4466-4475.2004

Jefferson, K. K. (2004). What drives bacteria to produce a biofilm? *FEMS Microbiol Lett, 236*(2), 163-173. doi:10.1016/j.femsle.2004.06.005

Jha, S. S., Joshi, S. J., & S, J. G. (2016). Lipopeptide production by Bacillus subtilis R1 and its possible applications. *Braz J Microbiol, 47*(4), 955-964. doi:10.1016/j.bjm.2016.07.006

Karatan, E., & Watnick, P. (2009). Signals, regulatory networks, and materials that build and break bacterial biofilms. *Microbiol Mol Biol Rev, 73*(2), 310-347. doi:10.1128/mmbr.00041-08

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O. P., Leer, R., . . . Kerkhoven, R. (2003). Complete genome sequence of Lactobacillus plantarum WCFS1. *Proc Natl Acad Sci, 100*(4), 1990-1995.

Kline, K. A., Dodson, K. W., Caparon, M. G., & Hultgren, S. J. (2010). A tale of two pili: assembly and function of pili in bacteria. *Trends Microbiol, 18*(5), 224-232. doi:10.1016/j.tim.2010.03.002

Kolodkin-Gal, I., Romero, D., Cao, S., Clardy, J., Kolter, R., & Losick, R. (2010). D-amino acids trigger biofilm disassembly. *Science, 328*(5978), 627-629. doi:10.1126/science.1188628

Kostakioti, M., Hadjifrangiskou, M., & Hultgren, S. J. (2013). Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb Perspect Med, 3*(4), a010306. doi:10.1101/cshperspect.a010306

Kruijt, M., Tran, H., & Raaijmakers, J. M. (2009). Functional, genetic and chemical characterization of biosurfactants produced by plant growth-promoting Pseudomonas putida 267. *Journal of Applied Microbiology, 107*(2), 546-556. doi:10.1111/j.1365-2672.2009.04244.x

Lebeer, S., Vanderleyden, J., & De Keersmaecker, S. C. (2008). Genes and molecules of Lactobacilli supporting probiotic action. *Microbiol Mol Biol Rev, 72*(4), 728-764. doi:10.1128/MMBR.00017-08

Lemon, K. P., Higgins, D. E., & Kolter, R. (2007). Flagellar motility is critical for Listeria monocytogenes biofilm formation. *J Bacteriol, 189*(12), 4418-4424. doi:10.1128/JB.01967-06

Ma, L., Jackson, K. D., Landry, R. M., Parsek, M. R., & Wozniak, D. J. (2006). Analysis of Pseudomonas aeruginosa conditional psl variants reveals roles for the psl polysaccharide in adhesion and maintaining biofilm structure postattachment. *J Bacteriol, 188*(23), 8213-8221. doi:10.1128/jb.01202-06

Madhu, A. N., & Prapulla, S. G. (2014). Evaluation and functional characterization of a biosurfactant produced by Lactobacillus plantarum CFR 2194. *Appl Biochem Biotechnol, 172*(4), 1777-1789. doi:10.1007/s12010-013-0649-5

Malik, S., Petrova, M. I., & Claes, I. J. (2013). The Highly Autoaggregative and Adhesive Phenotype of the Vaginal Lactobacillus plantarum strain CMPG5300 is Sortase Dependent. *Appl Environ Microbiol, 79*, 4576-4585.

Martinez, J. J., Mulvey, M. A., Schilling, J. D., Pinkner, J. S., & Hultgren, S. J. (2000). Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *The EMBO Journal, 19*(12), 2803-2812.

Medema, M. H., Blin, K., Cimermancic, P., de Jager, V., Zakrzewski, P., Fischbach, M. A., . . . Breitling, R. (2011). antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res, 39*(Web Server issue), W339-346. doi:10.1093/nar/gkr466

Meena, K. R., & Kanwar, S. S. (2015). Lipopeptides as the antifungal and antibacterial agents: applications in food safety and therapeutics. *Biomed Res Int, 2015*, 473050. doi:10.1155/2015/473050

Moldes, A. B., Paradelo, R., Vecino, X., Cruz, J. M., Gudina, E. J., Rodrigues, L., . . . Barral, M. T. (2013). Partial Characterization of Biosurfactant from Lactobacillus pentosus and Comparison with Sodium Dodecyl Sulphate for the Bioremediation of Hydrocarbon Contaminated Soil. *BioMed Research International, 2013*, 6. doi:10.1155/2013/961842

Molenaar, D., Bringel, F., Schuren, F. H., de Vos, W. M., Siezen, R. J., & Kleerebezem, M. (2005). Exploring Lactobacillus plantarum Genome Diversity by Using Microarrays. *Journal of Bacteriology, 187*(17), 6119-6127. doi:10.1128/JB.187.17.6119-6127.2005

Morici, L. A., Carterson, A. J., Wagner, V. E., Frisk, A., Schurr, J. R., Honer zu Bentrup, K., . . . Schurr, M. J. (2007). Pseudomonas aeruginosa AlgR represses the Rhl

quorum-sensing system in a biofilm-specific manner. *J Bacteriol, 189*(21), 7752-7764. doi:10.1128/jb.01797-06
Morikawa, M., Daido, H., Takao, T., Murata, S., Shimonishi, Y., & Imanaka, T. (1993). A new lipopeptide biosurfactant produced by Arthrobacter sp. strain MIS38. *Journal of Bacteriology, 175*(20), 6459-6466.
Mozzi, F., Raya, R. R., & Vignolo, G. M. (2016). *Biotechnology of Lactic Acid Bacteria: Novel Applications* (2nd ed.): John Wiley & Sons, Ltd.
Munira, C., I., Kadhim, M. I., & Maysaa, K. A. (2013). The effect of surlactin produced by Lactobacillus acidophilus on eye infections bacteria in rabbits. *Baghdad Science Journal, 10*, 133-143.
Neethirajan, S., Clond, M. A., & Vogt, A. (2014). Medical Biofilms: Nanotechnology Approaches. *Journal of Biomedical Nanotechnology, 10*(10), 2806-2827.
Nielsen, T. H., Christophersen, C., Anthoni, U., & Sorensen, J. (1999). Viscosinamide, a new cyclic depsipeptide with surfactant and antifungal properties produced by Pseudomonas fluorescens DR54. *Journal of Applied Microbiology, 86*, 80-90.
O'Toole, G. A., & Kolter, R. (1998). Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. *Molecular Microbiology, 30*(2), 295-304.
O'Toole, G. A., & Wong, G. C. (2016). Sensational biofilms: surface sensing in bacteria. *Curr Opin Microbiol, 30*, 139-146. doi:10.1016/j.mib.2016.02.004
Ochsner, A., Koch, A. K., Fiechter, A., & Reiser, J. (1994). Isolation and Characterization of a Regulatory Gene Affecting Rhamnolipid Biosurfactant Synthesis in Pseudomonas aeruginosa. *Journal of Bacteriology, 176*(7), 2044-2054.
Oliveira, J. S., Araujo, W., Lopes Sales, A. I., Brito Guerra, A., Silva Araujo, S. C., de Vasconcelos, A. T., . . . Freitas, A. T. (2015). BioSurfDB: knowledge and algorithms to support biosurfactants and biodegradation studies. *Database (Oxford), 2015*. doi:10.1093/database/bav033
Osbourn, A. E., & Field, B. (2009). Operons. *Cellular and Molecular Life Sciences, 66*(23), 3755-3775. doi:10.1007/s00018-009-0114-3
Pratt, L. A., & Kolter, R. (1998). Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Mol Microbiol, 30*(2), 285-293.
Prigent-Combaret, C., & Lejeune, P. (1999). Monitoring gene expression in biofilms. *Methods Enzymol, 310*, 56-79.
Ramkrishna, S. (2010a). Advances in Experimental Medicine and Biology 672) Vanessa Walter, Christoph Syldatk, Rudolf Hausmann (auth.), Ramkrishna Sen MTech, PhD (eds.)-Biosurfactants-Springer-.pdf>.
Ramkrishna, S. (2010b). Biosurfactants. *Advances in Experimental Medicine and Biology, 672*.
Rodrigues, L., Banat, I. M., Teixeira, J., & Oliveira, R. (2006). Biosurfactants: potential applications in medicine. *J Antimicrob Chemother, 57*(4), 609-618. doi:10.1093/jac/dkl024
Rodrigues, L., Moldes, A., Teixeira, J., & Oliveira, R. (2006). Kinetic study of fermentative biosurfactant production by Lactobacillus strains. *Biochemical Engineering Journal, 28*(2), 109-116. doi:10.1016/j.bej.2005.06.001

Rodrigues, L., van der Mei, H. C., Teixeira, J. A., & Oliveira, R. (2004). Biosurfactant from Lactococcus lactis 53 inhibits microbial adhesion on silicone rubber. *Applied Microbiology and Biotechnology, 66*(3), 306-311. doi:10.1007/s00253-004-1674-7

Rodrigues, L. R., Teixeira, J. A., van der Mei, H. C., & Oliveira, R. (2006). Isolation and partial characterization of a biosurfactant produced by Streptococcus thermophilus A. *Colloids and Surfaces B: Biointerfaces, 53*(1), 105-112. doi:http://doi.org/10.1016/j.colsurfb.2006.08.009

Roongsawang, N., Hase, K., Haruki, M., Imanaka, T., Morikawa, M., & Kanaya, S. (2003). Cloning and Characterization of the gene clster encoding arthrofactin synthetase from Pseudomonas sp. MIS38. *Chem Biol., 10*, 869-880. doi:10.1016/j.chembiol.2003.09.004

Roongsawang, N., Washio, K., & Morikawa, M. (2007). In vivo characterization of tandem C-terminal thioesterase domains in arthrofactin synthetase. *Chembiochem, 8*(5), 501-512. doi:10.1002/cbic.200600465

Roongsawang, N., Washio, K., & Morikawa, M. (2010). Diversity of nonribosomal peptide synthetases involved in the biosynthesis of lipopeptide biosurfactants. *Int J Mol Sci, 12*(1), 141-172. doi:10.3390/ijms12010141

Rowe, M. C., Withers, H. L., & Swift, S. (2010). Uropathogenic Escherichia coli forms biofilm aggregates under iron restriction that disperse upon the supply of iron. *FEMS Microbiol Lett, 307*(1), 102-109. doi:10.1111/j.1574-6968.2010.01968.x

Salehi, R., Savabi, O., Kazemi, M., Kamali, S., Salehi, A. R., Eslami, G., & Tahmourespour, A. (2014). Effects of Lactobacillus reuteri-derived biosurfactant on the gene expression profile of essential adhesion genes (gtfB, gtfC and ftf) of Streptococcus mutans. *Adv Biomed Res, 3*, 169. doi:10.4103/2277-9175.139134

Sambanthamoorthy, K., Feng, X., Patel, R., Patel, S., & Paranavitana, C. (2014). Antimicrobial and antibiofilm potential of biosurfactants isolated from lactobacilli against multi-drug-resistant pathogens. *BioMed Central Microbiology, 14*.

Santos, D. K., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). Biosurfactants: Multifunctional Biomolecules of the 21st Century. *Int J Mol Sci, 17*(3), 401. doi:10.3390/ijms17030401

Saravanakumari, P., & Mani, K. (2010). Structural characterization of a novel xylolipid biosurfactant from Lactococcus lactis and analysis of antibacterial activity against multi-drug resistant pathogens. *Bioresour Technol, 101*(22), 8851-8854. doi:10.1016/j.biortech.2010.06.104

Saravanakumari, P., & Nirosha, P. (2012). Tracing of biosurfactants synthesizing genes in Bacillus spp., by in vitro and in silico techniques using srfa gene as marker. *Journal of Drug Delivery & Therapeutics, 2*(5), 67-70.

Satpathy, S., Sen, S. K., Pattanaik, S., & Raut, S. (2016). Review on bacterial biofilm: An universal cause of contamination. *Biocatalysis and Agricultural Biotechnology, 7*, 56-66. doi:http://dx.doi.org/10.1016/j.bcab.2016.05.002

Satpute, S. K., Bhawsar, B. D., Dhakephalkar, P. K., & Chopade, B. A. (2008). Assessment of different screening methods for selecting biosurfactant producing marine bacteria. *Indian Journal of Marine Sciences, 37*(3), 243-250.

Satpute, S. K., Kulkarni, G. R., Banpurkar, A. G., Banat, I. M., Mone, N. S., Patil, R. H., & Cameotra, S. S. (2016). Biosurfactant/s from Lactobacilli species: Properties,

challenges and potential biomedical applications. *J Basic Microbiol*. doi:10.1002/jobm.201600143

Sauer, K., Cullen, M. C., Rickard, A. H., Zeef, L. A., Davies, D. G., & Gilbert, P. (2004). Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. *J Bacteriol, 186*(21), 7312-7326. doi:10.1128/jb.186.21.7312-7326.2004

Scholz-Schroeder, B. K., Soule, J. D., & Gross, D. C. (2003). The sypA, sypB, and SypC synthetase genes encode 22 modules involved in the NRPS of syringopeptin by Pseudomonas synringae pv. B301D. *Molecular Plants Microbe Interactions, 16*(4), 271-280.

Sehar, S., & Naz, I. (2016). Role of the Biofilms in Wastewater Treatment. doi:10.5772/63499

Sekhon, K. K., Khanna, S., & Cameotra, S. S. (2011). Enhanced biosurfactant production through cloning of three genes and role of esterase in biosurfactant release. *Microb Cell Fact, 10*, 49. doi:10.1186/1475-2859-10-49

Sekhon, K. K., & Rahman, P. K. (2014). Rhamnolipid biosurfactants-past, present, and future scenario of global market. *Front Microbiol, 5*, 454. doi:10.3389/fmicb.2014.00454

Selvin, J., Sathiyanarayanan, G., Lipton, A. N., Al-Dhabi, N. A., Valan Arasu, M., & Kiran, G. S. (2016). Ketide Synthase (KS) Domain Prediction and Analysis of Iterative Type II PKS Gene in Marine Sponge-Associated Actinobacteria Producing Biosurfactants and Antimicrobial Agents. *Front Microbiol, 7*, 63. doi:10.3389/fmicb.2016.00063

Sharma, D., & Saharan, B. S. (2016). Functional characterization of biomedical potential of biosurfactant produced by Lactobacillus helveticus. *Biotechnology Reports, 11*, 27-35. doi:http://dx.doi.org/10.1016/j.btre.2016.05.001

Sharma, D., Saharan, B. S., Chauhan, N., Bansal, A., & Procha, S. (2014). Production and structural characterization of Lactobacillus helveticus derived biosurfactant. *ScientificWorldJournal, 2014*, 493548. doi:10.1155/2014/493548

Sharma, D., & Singh Saharan, B. (2014). Simultaneous Production of Biosurfactants and Bacteriocins by Probiotic Lactobacillus casei MRTL3. *Int J Microbiol, 2014*, 698713. doi:10.1155/2014/698713

Shokouhfard, M., Kermanshahi, R. K., Shalandashti, R. V., Feizabadi, M. M., & Teimourian, S. (2015). The inhibitory effect of a Lactobacillus acidophilus derived biosurfactant on biofilm producer Serratia marcescens. *Iranian Journal of Baisc Medical Sciences, 18*, 1001-1007.

Singh, R., Paul, D., & Jain, R. K. (2006). Biofilms: implications in bioremediation. *Trends Microbiol, 14*(9), 389-397. doi:10.1016/j.tim.2006.07.001

Soberon-Chavez, G. (2011). *Biosurfactants, from genes to applications* (G. Soberon-Chavez Ed.). Berlin: Springer.

Solano, C., Echeverz, M., & Lasa, I. (2014). Biofilm dispersion and quorum sensing. *Curr Opin Microbiol, 18*, 96-104. doi:10.1016/j.mib.2014.02.008

Strieker, M., Tanovic, A., & Marahiel, M. A. (2010). Nonribosomal peptide synthetases: structures and dynamics. *Curr Opin Struct Biol, 20*(2), 234-240. doi:10.1016/j.sbi.2010.01.009

Tahmourespour, A., Salehi, R., & Kermanshahi, R. K. (2011). Lactobacillus acidophilus-derived biosurfactant effect on GTFB and GTFC expression level in Streptococcus mutans biofilm cells. *Braz J Microbiol, 42*, 330-339.

Terraf, M. C., Juarez Tomas, M. S., Nader-Macias, M. E., & Silva, C. (2012). Screening of biofilm formation by beneficial vaginal lactobacilli and influence of culture media components. *J Appl Microbiol, 113*(6), 1517-1529. doi:10.1111/j.1365-2672.2012.05429.x

Thavasi, R., Jayalakshmi, S., & Banat, I. M. (2011). Application of biosurfactant produced from peanut oil cake by Lactobacillus delbrueckii in biodegradation of crude oil. *Bioresour Technol, 102*(3), 3366-3372. doi:10.1016/j.biortech.2010.11.071

Thumbikat, P., Berry, R. E., Zhou, G., Billips, B. K., Yaggie, R. E., Zaichuk, T., . . . Klumpp, D. J. (2009). Bacteria-induced uroplakin signaling mediates bladder response to infection. *PLoS Pathog, 5*(5), e1000415. doi:10.1371/journal.ppat.1000415

van Ditmarsch, D., & Xavier, J. B. (2011). High-resolution time series of Pseudomonas aeruginosa gene expression and rhamnolipid secretion through growth curve synchronization. *BMC Microbiol, 11*, 140. doi:10.1186/1471-2180-11-140

Van Houdt, R., & Michiels, C. W. (2010). Biofilm formation and the food industry, a focus on the bacterial outer surface. *J Appl Microbiol, 109*(4), 1117-1131. doi:10.1111/j.1365-2672.2010.04756.x

Vasseur, P., Vallet-Gely, I., Soscia, C., Genin, S., & Filloux, A. (2005). The pel genes of the Pseudomonas aeruginosa PAK strain are involved at early and late stages of biofilm formation. *Microbiology, 151*(Pt 3), 985-997. doi:10.1099/mic.0.27410-0

Vecino, X., Barbosa-Pereira, L., Devesa-Rey, R., Cruz, J. M., & Moldes, A. B. (2015). Optimization of extraction conditions and fatty acid characterization of Lactobacillus pentosus cell-bound biosurfactant/bioemulsifier. *J Sci Food Agric, 95*(2), 313-320. doi:10.1002/jsfa.6720

Velraeds, M. M., van de Belt-Gritter, B., Busscher, H. J., Reid, G., & van der Mei, H. C. (2000). Inhibition of uropathogenic biofilm growth on silicone rubber in human urine by lactobacilli--a teleologic approach. *World J Urol, 18*(6), 422-426.

Velraeds, M. M., van der Mei, H. C., Reid, G., & Busscher, H. J. (1997). Inhibition of initial adhesion of uropathogenic Enterococcus faecalis to solid substrata by an adsorbed biosurfactant layer from Lactobacillus acidophilus. *Urology, 49*(5), 790-794. doi:10.1016/s0090-4295(97)00065-4

Velraeds, M. M. C., van de Belt-Gritter, B., van der Mei, H. C., & Busscher, G. H. J. (1998). Interference uropathogenic bacteria by L. acidophilus biosurfactant. *J Med Microbiol, 47*, 1081-1085.

Velraeds, M. M. C., van der Mei, H. C., Reid, G., & Busscher, G. H. J. (1996). Physicochemical and biochemical characterization of biosurfactants released by Lactobacillus strains. *Colloids and Surfaces, 8*, 51-61.

Wagner, K., Friedrich, S., Stang, C., Bley, T., Schilling, N., Bieda, M., . . . Boschke, E. (2014). Initial phases of microbial biofilm formation on opaque, innovative anti-adhesive surfaces using a modular microfluidic system. *Engineering in Life Sciences, 14*(1), 76-84. doi:10.1002/elsc.201200035

Waksman, G., & Hultgren, S. J. (2009). Structural biology of the chaperone-usher pathway of pilus biogenesis. *Nat Rev Microbiol, 7*(11), 765-774. doi:10.1038/nrmicro2220

Walencka, E., Różalska, S., Sadowska, B., & Różalska, B. (2008). The influence of Lactobacillus acidophilus-derived surfactants on staphylococcal adhesion and biofilm formation. *Folia Microbiologica, 53*(1), 61. doi:10.1007/s12223-008-0009-y

Walter, V., Syldatk, C., & Hausmann, R. (2010). Screening concepts for the isolation of biosurfactant producing microorganisms. *Adv Exp Med Biol, 672*, 1-13.

Wang, N., Lu, S. E., Yang, Q., Sze, S. H., & Gross, D. C. (2006). Identification of the syr-syp box in the promoter regions of genes dedicated to syringomycin and syringopeptin production by Pseudomonas syringae pv. syringae B301D. *J Bacteriol, 188*(1), 160-168. doi:10.1128/JB.188.1.160-168.2006

Wang, X., Preston, J. F., 3rd, & Romeo, T. (2004). The pgaABCD locus of Escherichia coli promotes the synthesis of a polysaccharide adhesin required for biofilm formation. *J Bacteriol, 186*(9), 2724-2734.

Wei, Y. H., Wang, L. C., Chen, W. C., & Chen, S. Y. (2010). Production and characterization of fengycin by indigenous Bacillus subtilis F29-3 originating from a potato farm. *Int J Mol Sci, 11*(11), 4526-4538. doi:10.3390/ijms11114526

Welch, R. A., Burland, V., Plunkett, G., Redgord, P., Roesch, P., Rasko, D., . . . Blattner, F. R. (2002). Extensive mosaic structure revealed by the complete genome sequence of uropathogenic Escherichia coli. *Proc Natl Acad Sci, 99*(26), 17020-17024.

Winkelströter, L. K., Tulini, F. L., & De Martinis, E. C. P. (2015). Identification of the bacteriocin produced by cheese isolate Lactobacillus paraplantarum FT259 and its potential influence on Listeria monocytogenes biofilm formation. *LWT - Food Science and Technology, 64*(2), 586-592. doi:10.1016/j.lwt.2015.06.014

Wong, L. (1998). Biofilms in Food Processing L. mono.pdf>.

Wood, B. J., & Warner, P. J. (2003). *Genetics of Lactic Acid Bacteria* (Vol. 3). Scotland: Springer Science Business Media, LLC.

Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M., & McInerney, M. J. (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. *J Microbiol Methods, 56*(3), 339-347. doi:10.1016/j.mimet.2003.11.001

Zhang, J., Quigley, N., & Gross, D. C. (1997). Analysis of the syrP gene, which regulates syringomycin synthesis by Pseudomonas syringae pv. syringae. *Appl Environ Microbiol, 63*(7), 2771-2778.

Zhang, X., Fan, X., Solaiman, K. Y., Ashby, R. D., Liu, Z., Mukhopadhyay, S., & Yan, R. (2016). Inactivation of Escherichia coli O157:H7 in vitro and on the surface of spinach leaves by biobased antimicrobial surfactants. *Food Control, 60*, 158-165. doi:http://dx.doi.org/10.1016/j.foodcont.2015.07.026

APPENDICES

Appendix A Lactobacilli genomes retrieved from NCBI Database, 173 (Qty.)

Lactobacillus acetotolerans	*Lactobacillus farraginis*
Lactobacillus acidifarinae	*Lactobacillus fermentum*
Lactobacillus acidipiscis	*Lactobacillus floricola*
Lactobacillus agilis	*Lactobacillus florum*
Lactobacillus algidus	*Lactobacillus fructivorans*
Lactobacillus alimentarius	*Lactobacillus frumenti*
Lactobacillus amylolyticus	*Lactobacillus fuchuensis*
Lactobacillus amylophilus	*Lactobacillus futsaii*
Lactobacillus amylotrophicus	*Lactobacillus gallinarum*
Lactobacillus amylovorus	*Lactobacillus gasseri*
Lactobacillus animalis	*Lactobacillus gastricus*
Lactobacillus antri	*Lactobacillus ghanensis*
Lactobacillus apinorum	*Lactobacillus gigeriorum*
Lactobacillus apis	*Lactobacillus ginsenosidimutans*
Lactobacillus apodemi	*Lactobacillus gorillae*
Lactobacillus aquaticus	*Lactobacillus graminis*
Lactobacillus aviarius	*Lactobacillus hammesii*
Lactobacillus backii	*Lactobacillus hamsteri*
Lactobacillus bifermentans	*Lactobacillus harbinensis*
Lactobacillus bombicola	*Lactobacillus hayakitensis*
Lactobacillus brantae	*Lactobacillus heilongjiangensis*
Lactobacillus brevis	*Lactobacillus helsingborgensis*
Lactobacillus buchneri	*Lactobacillus helveticus*
Lactobacillus cacaonum	*Lactobacillus herbarum*
Lactobacillus camelliae	*Lactobacillus hilgardii*
Lactobacillus capillatus	*Lactobacillus hokkaidonensis*
Lactobacillus casei	*Lactobacillus hominis*
Lactobacillus ceti	*Lactobacillus homohiochii*
Lactobacillus coleohominis	*Lactobacillus hordei*
Lactobacillus collinoides	*Lactobacillus iners*
Lactobacillus composti	*Lactobacillus ingluviei*
Lactobacillus concavus	*Lactobacillus intestinalis*
Lactobacillus coryniformis	*Lactobacillus jensenii*
Lactobacillus crispatus	*Lactobacillus johnsonii*
Lactobacillus crustorum	*Lactobacillus kalixensis*
Lactobacillus curieae	*Lactobacillus kefiranofaciens*
Lactobacillus curvatus	*Lactobacillus kefiri*
Lactobacillus delbrueckii	*Lactobacillus kimbladii*
Lactobacillus dextrinicus	*Lactobacillus kimchicus*
Lactobacillus diolivorans	*Lactobacillus kimchiensis*

Lactobacillus equi	*Lactobacillus kisonensis*
Lactobacillus equicursoris	*Lactobacillus kitasatonis*
Lactobacillus equigenerosi	*Lactobacillus koreensis*
Lactobacillus fabifermentans	*Lactobacillus kullabergensis*
Lactobacillus Lactobacillus agilis,	*Lactobacillus rennini*
Lactobacillus Lactobacillus delbrueckii	*Lactobacillus reuteri*
Lactobacillus Lactobacillus mucosae,	*Lactobacillus rossiae*
Lactobacillus lindneri	*Lactobacillus ruminis*
Lactobacillus malefermentans	*Lactobacillus saerimneri*
Lactobacillus mali	*Lactobacillus sakei*
Lactobacillus manihotivorans	*Lactobacillus salivarius*
Lactobacillus mellifer	*Lactobacillus sanfranciscensis*
Lactobacillus mellis	*Lactobacillus saniviri*
Lactobacillus melliventris	*Lactobacillus satsumensis*
Lactobacillus mindensis	*Lactobacillus secaliphilus*
Lactobacillus mucosae	*Lactobacillus selangorensis*
Lactobacillus murinus	*Lactobacillus senioris*
Lactobacillus nagelii	*Lactobacillus senmaizukei*
Lactobacillus namurensis	*Lactobacillus sharpeae*
Lactobacillus nantensis	*Lactobacillus shenzhenensis*
Lactobacillus nasuensis	*Lactobacillus silagei*
Lactobacillus nodensis	*Lactobacillus siliginis*
Lactobacillus odoratitofui	*Lactobacillus similis*
Lactobacillus oeni	*Lactobacillus sp.*
Lactobacillus oligofermentans	*Lactobacillus spicheri*
Lactobacillus oris	*Lactobacillus sucicola*
Lactobacillus oryzae	*Lactobacillus suebicus*
Lactobacillus otakiensis	*Lactobacillus sunkii*
Lactobacillus ozensis	*Lactobacillus taiwanensis*
Lactobacillus panis	*Lactobacillus thailandensis*
Lactobacillus pantheris	*Lactobacillus tucceti*
Lactobacillus parabrevis	*Lactobacillus ultunensis*
Lactobacillus parabuchneri	*Lactobacillus uvarum*
Lactobacillus paracasei	*Lactobacillus vaccinostercus*
Lactobacillus paracollinoides	*Lactobacillus vaginalis*
Lactobacillus parafarraginis	*Lactobacillus versmoldensis*
Lactobacillus parakefiri	*Lactobacillus vini*
Lactobacillus paralimentarius	*Lactobacillus wasatchensis*
Lactobacillus paraplantarum	*Lactobacillus xiangfangensis*
Lactobacillus pasteurii	*Lactobacillus zeae*
Lactobacillus paucivorans	*Lactobacillus zymae*
Lactobacillus pentosus	
Lactobacillus perolens	
Lactobacillus plantarum	
Lactobacillus pobuzihii	
Lactobacillus pontis	
Lactobacillus psittaci	
Lactobacillus rapi	

Appendix B, FASTA sequence YP_004888577.1 nonribosomal peptide synthetase NpsA [Lactobacillus plantarum WCFS1]

MLNTIITRFEEQVEKVPEQNAVVFGQKKLNYRQLDKKSNIVANYLIKNCQVKHGDIVPLLLDRSENMIVA
IIAVLKAGAAYTALSKQYPQNRIDFIREQTNAAIIVDDKLLSQALVGTDSSNPDCKHGIQDLAYVVYTSG
TTGHPKGVLHSNLSVTSHIDSYWKAIGLSDEHYNMLFLVNYVFSVATTQIFGALLHGDTLVISNSNAVED
IDHLERYINTEKINYFQCTPSLANSIDFSRLTSVKTVAVAGEKIPRSLFTNTHDNQVTLVNVYGQSEFHA
GTTNIINTVDDINKIGHPVNGMRAYVVDGKMNEVTEGQIGEICFSGNQLANGYLNLELETQEHFIDNPFD
KGVLCATGDLVKKLPNNEFEFIGRKDFQLNINGIRTEPAEIETQLLTVAEIRDVVVTSYKNQTLIAYYVS
DAPLNESAIKDAVKNKLSSYMQPEYYKWMKAFPLNENGKIDRKKLPDINIRRVGYVAPKTEIEKALAQLY
ERALNVDHVGKNDNFFQLGANSLQAMQLANAIVAVSGKRLTAENIYNNQPLGKLAKFIDTYNESKLTTIQ
QSKSRNIFQMSPAEKRMFVLYELNHDSTDYNEQTVLNSSTRLDENRLKRALKALVLRHEILRTAFYRQND
HYMQKVLSNNQLDFTVIDKEISNYQELVKPFDLEAGETMRIRLIKGEKVDKLFVDKHHIITDGTSETVFY
TELEKLYRGEKLDPVKFQYKDYSEWFNELDVSDDKQWWNRQLIDYQRLELATDYPWSKNHVAVGTTVYKK
FDADMVAKLRNLAHETSSSEYMILFSVLSAFLSKTYHSNDFILGTVASGRVDAATENMLGMFINTLPVRV
NIKPNLTVLEYLAMMKKTLLASLAHQNYQFEDIVHDLHATSDVGNPMFDCMFVFQNTKNMHHRFDAPAVK
DSYETTESKFALTFEIFENDNDMELHLNYDSTLFERPTVDMLVHSLTIMLENFVQSLNNKLSKLSMITQI
EKNQLLTFPKTDNHESVIDLFEQQVMKHPNALALEFDDKKWTYAELNRRVNRVANYLVKKLDVKSEQKIP
LLLRRTDKMVVAILSVLKAGAAYVPVSLKYPSERINFITKACNAQFVIDDQFMERDFPRDDENLQMRIEQ
NQLAYLIFTSGTSGNPKGVMVEHHNLSNYVLEVSRMKNSGMHEGMKNGAFFEYVFDSSVHDLIRPFVLGE
SVVVLDTDLIFDIDKFISTLNQYQINAIGMTPSLAARVDLNRVSSLEYIYCGGEAITRDVINKYADTPIQ
LNNCYGPTETTVLSFANNDVHDLSIGRPIGGVDAYVLDDNRQLLPAGAVGNLYIGGAQVTRGYLNRAEET
EKHYINNPFGKGIIYDTGDLVRRLYDDSFQYFGRKDFQVKIRGYRVELGEIEKQLQAVEGIEQAKVIAKD
GNLIAYYISKSSIDSDDLYNQLSKSLADYMVPSMYMHMLDFPLTINGKLDVRALPEPVQQEEYVAPRNRR
EKEIADAFAKVLNLNSVSVKANFFRLGGNSIIAISLANMINVSVKTIFDCKTVANLAMQHDSIQKISKQK
FLHEEDQNLSFAQERLFFIDQLEGGTNAYNIPMLLDLQENVDHVKLEKSIQTVIQRHEVLRTVIRGSYQQ
VMTSPVKITHEPINIDEFFAYKFHLSSEIPIRINIYHNQLAINVHHIAFDGWSTDIFLTELTDLYNGKDL
PRKTVQYKDFAKWQINNQTQDMLKPQRSYWLKELNGYSPIDFPTDYVRPQRFDYTGDELNYHLSDGLIQQ
LNQVARMHDTSFYCVALSAFILLLSAYSNQNDVVVGTPIAGRHIQGTEDMIGFFVNSLAIRTTFDNGIKF
TDLLSKVTRKIAISQANQDLPFEQLVEALHIKKDMSRNPIFQIMFSIEDNMDSLIGKNALFNSLNQNLSL
KSSKFDFSLTYRGNNLNLTYATKLFKRETILNIVKTYELILKQIANEPSRRLDSIQLSMQEIACQHENYP
EKSLANLFEEQVLRTPDQVAINFKQYQLTYGELNIRANKVAHSLIDQGIKPGMHVPILLPRNERFVIAIL
GVLKAGANYVPLSLDYPKERVDYILDKIHANLVIDDEFQVTSDDGNNLALNIPTDSLAYIIFTSGTTGKP
KGVMVEQRGVVNTIYNHIQLLGAQSKLRMTHFANFVFDVSVLELFYGLLTGANIYLLDNLIRVDYQLLKQ
FVIKNKISLMILPPAVLNAEDLLPVNKLVVAGESTPEEIYKAYEKNHTQMFNAYGPTEVTVIGTVKEYES
GMSSNNIGQALKNMTACALDNQNRVVPIGAIGELCIGGPGVARGYISDRGKTEKAFINHPRLGRLYRTGD
MVKQLPSGDFIYLGRNDFQVKIRGFRVELGEIEARLMEQPSITRCLVRVHGTNLIAYYQGKLEHTLEKQL
PSYMVPSNYVHLDKFPMTINGKIDLRKLPEPEIEHAKFVAPKTAREKQIAEEICGLLNLERVSVLDDFYE
LGGNSILALKLANHINLQVKQIFDSRNIRELAKMKSNYVRVTKGNFEKVSDQKLSFAQERLWFVEQFEDN
LSAYNVPVVLNLKKDVSKEKIESALKSLVQRHEILRTCIKGDYQIVTNQELQITHDCIDVNEYLNRPFNL
TKNIPIRVNLYARKLVIDIHHMAFDGWSTEVFLRELHQLYYQEPLVSLTVQYKDFAQWQRKYVASSAVQE
QLDFWKKELSGYETLNLPADYERPKNFTYRGKTLQVPFNNKLEHELLNFAKKQKTSLYTVMLSVLDLMLS
RFSYQNDIIVGTPLANRNIEGTEDLIGFFINTLPIRTKVDEEQSFKELLSSNSLKVQGVQNMQDIPFEQI
VKSLNVERDSSRNAIFQVLFSVQDFSKEIKNSNLFSGINQGLTGNSAKYDLSVMVENGYISFNYCSDIYK
KTTISSMLNTYMTLLEKLLVQNNVPMQNVGKVDVVARGDVVNYPKKTVVELFKEQVAKTPQNVAIEYQNI
QLTYETFDKLTNKFANSLLNAGIRPGDKVPLIMKRSEKMSIAIWGVLKAGCAYVPVSPEFPEERKQFILK
QINAKVIVDDNYIIPKECSTLAPKYRPKLSDLAYIIFTSGTTGKPKGVMIEHGGLSNRIQWMNATYPITE
KDRVYQKTNFVFDVSVWEQVWALLEGARIVFALEGGHKDPVYLANEIDNKNITVMHFVPSMLDAFLETLD
VYRSDDTLPNFKLTSLKYVFCSGEALNINSVKLFRKLMPATRLFNLYGPTEASIDVTYFDCNKDNLNKVL
IGKPVANTNCYVLSRTDHLLPVGAIGELALGGVQLARGYINQPELTAQKFVINAKLGRIYKTGDLVRLLS
NGNIEYLGRNDMQVKVRGLRIELGEVETRLIEIKGITKAIVLAVNQQLVAYYISGNQLSEESIKQQLSTT
LPDYMVPSAFVKLNAFPLTFNGKLDRRALPKPTLNEKGFIEPKTQSEFELQRIFAEVLGINVSQVSVTES
FFRLGGDSIKAIQLSNRIEQQLHQTITIKQIFATKSIRNMAMLCGHKIVDKLISEQGTLTGEVQLSSIQK
WFFDEVKSSRFIQAFKIKLPSNVDYKRLKVALTELVNYHDVLRMRFDGQKQMYSKKIESVKLQFVKAESQ
LTDSQVSFDLNGILYRFVVDEQDNTLIVICHHLIIDTVSWQILADDLNSLYNHAILPAKGTSYRQWITYT
KKHPSEFKPVTIGKYNNDFQQEGESQTVIVSFDRKITDALLKRANRIYHTKINDVLLTALARSLKVITKT
DINYVRLESHGRAELSDQLNVHRTVGWFTAMYPQTISTDLLETKHYTSQVRDYGVGYGERFGIHDDKLPG

Appendix B, FASTA sequence YP_004888577.1 nonribosomal peptide synthetase NpsA [Lactobacillus plantarum WCFS1] (Continued)

ITFNYLGQLNNGELNPWSVIPEDTVVGKQASTSDFLQINGAISEQKLSFEFVGKQSEVSVLAKQYYAELG
KLIEDLSTAERTYLTVEDIEVDISKKNLDSLQQEQELQTILPANKLQEGFIYQSLNNDTNDDAYVCSYIF

DYDQPINVQMYRRAWKLAQDHFPSLRLALSSEYGTLLQVIPIHGYLDFNFVTGESVENVVHNQRIMGFDL
KKGSLLRVCLVKNDANEYTCVLTNHHAISDGWSNPVLLNYVHTMYANLMQDKVTEFSDDSAYVNAQRYLS
QPEHIDDEYWQKNLSEPVHPDLNGLFKLQAHEVKLEEVKRITDPKTKLYRISGELYRQLKRFTKENGITT
SIVLQYAWHKLLSIYGGVSSTTVGVVNAGRNIPIEKIEDSVGLYIRTLPIQFSHTDKSINYQLHKLQDIN
NECMMHSNVSLAGLQRNGTRLFDTLFVYENYPVSTDSSSSMLKVHNFRAQEKLDYPLTVMLSELSDSLSF
SLMFAGEIFAEATIDHLFAMVQRLVSQIVKGVNSFTFIEKAPNFGTAYYPEKTIAEEFEQQVREHPNNIA
LDFKNIRYTFEELNQRANRVAHTLVEDYDIKLGDRVPLLLPKSENTIIAILAILKAGAVYVPMAVTFPKE
RIKYIVEKVEAKLVIDSEFMAQSFSNLKNNLNLAVKPNDLAYIIFTSGTTGQPKGVMVEHRNFIIYLSNI
LAAIKKTGTTNIEFGCIAEYVFDIFGTEVFGQLLRGKTVNLFAGEPEDFPQFMASHDVTTLQSTPGRISY
FFQDNDSQILNTSLTTIMVGGEKMNAAFAKRFDNINLINIYGPTEGTVWTSMKRIESNYSNIGQPFPNYT
HLVLDRKKRLLPQGAVGELYISGPQLSRGYYGQPELTQHAFLNNPYNDQHLSEYSRIYKTGDIVRVLSNG
EFELIGRNDFQVKIRGFRIELGEIESAMLRVPGVKQVLALALGKEGSKYLGVYYVSNQEIARKDIERVIS
QYLTDYMMPSGYQHISEFPLTINGKIDRRALPEISYDNGVIYVEPQNSTEEQVKQIVCDLLTLKLDNTSM
LENFFTIGGDSIKVIKLISILKNHFGKQVTIKEIFKAKTLKNIAELVANKNINDAEKSLSVTKQTFKDVT
EQCLSSAQASYQKEPTNSYTNVKIAFKLKPNVDVERLIIAIEKEVKRQQILRSKIYDGFQIVDDTPFTVK
QEKIDRNRYFSHVFNLQQEIPIKANIYNGEFTCVIDHIAFDGWSTSIFLDEIEAFYRGKDLPELPYQYKD
FAKCQQNFLNGTQAKRQIDYWQHELSCYQPLLLPVDQTQIESNQLGDDVYIRLDDELYHELKVLVVNEGT
TMHNLLLTVYFVLLSRVTRNNAISIAVPTVGRNVTGVENLIGLFINQFLITIDLDSIKTFSDLLKIVDEK
MIAAQNNQDIPLPTVLEKLNISVTGNRAYFGIQGFKREALKHSSLFTSIPEMNQETVKDAFTDLAIFVWG
QTIDINYAQSLFTRSKVEKFAGVYKDVLKQMIENIDGEI

VITA

Pryscila Velasco Vazquez

Candidate for the Degree of

Master of Science

Thesis: Identification of candidate genes for biosurfactant production in *Lacobacillus* species

Major Field: Food Science

Biographical:

Education:

Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in August, 2017.

Completed the requirements for the Bachelor of Science in Environmental Systems Engineering at Universidad Politecnica de Durango, Durango, Mexico in 2013.

Experience:

Certified in Human Food and Animal Food by the Food Safety Preventive Controls Alliance (FSPCA), as a FSPCA Preventive Controls Qualified Individual, who can manage a food safety preventive controls program in accordance with the Hazard-Analysis and Preventive Controls Rule of the US Food and Drug Administration.