DISSERTATIONES
BIOLOGICAE
UNIVERSITATIS
TARTUENSIS
339

ANDRIO LAHESAARE

The role of global regulator Fis in regulating the expression of *lapF* and the hydrophobicity of soil bacterium *Pseudomonas putida*

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
339

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
339

ANDRIO LAHESAARE

The role of global regulator Fis in regulating the expression of *lapF* and the hydrophobicity of soil bacterium *Pseudomonas putida*

UNIVERSITY OF TARTU
Press

Institute of Molecular and Cell Biology, University of Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Philosophy in genetics on May 4, 2018 by the Council of Institute of Molecular and Cell Biology, University of Tartu

Supervisor: Riho Teras, PhD, Docent, University of Tartu, Estonia

Opponent: Carla C. C. R. de Carvalho, PhD, Principal Investigator, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

Commencement: Room No 105, Riia 23B, Tartu, on June 21st 2018, at 10.15

The University of Tartu grants the publication of this dissertation.

ISSN 1024-6479
ISBN 978-9949-77-744-0 (print)
ISBN 978-9949-77-745-7 (pdf)

University of Tartu Press
www.tyk.ee

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. **Moor H, Teppo A, Lahesaare A, Kivisaar M, Teras R.** Fis overexpression enhances *Pseudomonas putida* biofilm formation by regulating the ratio of LapA and LapF. Microbiology. 2014 Dec;160(Pt 12): 2681–93.

II. **Lahesaare A, Moor H, Kivisaar M, Teras R.** *Pseudomonas putida* Fis binds to the *lapF* promoter in vitro and represses the expression of LapF. PLoS One. 2014 Dec 29;9(12).

III. **Lahesaare A, Ainelo H, Kivisaar M, Heipieper HJ, Teras R.** LapF and Its Regulation by Fis Affect the Cell Surface Hydrophobicity of *Pseudomonas putida*. PLoS One. 2016 Nov 3;11(11).

My contribution to the publications is following:
Ref I – Performed some of the experiments (flow cytometry analysis and purification of *P. putida* Fis) and contributed to the manuscript editing.

Ref II – Participated in construction of plasmids and strains, performing *in vitro* experiments and contributed to the writing and editing of the manuscript.

Ref III – Constructed plasmids and strains, performed most of the experiments and wrote the manuscript.

ABBREVIATIONS

Å	ångström (SI-unit equal to 10^{-10} m)
Bap	biofilm-associated protein
c-di-GMP	cyclic di-GMP
EPS	exopolysaccharides
Fis	factor for inversion stimulation
IPTG	isopropyl β-D-1-thiogalactopyranoside
LapA	large adhesion protein A
LapF	large adhesion protein F
LB	lysogeny broth
LPS	lipopolysaccharides
LTA	lipoteichoic acids
RNAP	RNA polymerase
WT	wild-type

INTRODUCTION

Bacteria have developed several mechanisms to cope with the changing conditions of the surrounding environment. The charge and other characteristics of cell surface can passively help to deal with unfavourable conditions or can be involved in the switch between two different life-forms: free-swimming aka planktonic lifestyle and surface-attached aka biofilm aka sessile lifestyle. The transition from planktonic to sessile happens when bacteria attach to the surface and/or each-other. In this process, the adhesion abilities to different surfaces play an important role in bacterial survival. In most cases, the adhesion properties of bacteria are defined by cell surface hydrophobicity. The hydrophobicity of cell surface may also play a protective role for bacteria. For example, the surface of *P. putida* becomes more hydrophobic in the presence of different kind of environmental stressors including toxic organic solvents (Heipieper *et al.* 2007). In this way the cells increase the aggregation with each-other, thus minimizing accessibility of their surface to toxic compounds. Cell surface hydrophobicity can be affected by several factors including lipopolysaccharides (LPS), lipoteichoic acids (LTA), S-layer proteins and adhesins. In gram-negative bacteria these components are mostly located on the outer membrane, which is the first barrier of bacteria to its surrounding environment. The components and structure of outer membrane can be reorganised in response to environmental factors. For enabling all these changes, bacteria must rearrange its physiology by altering a large number of genes. That kind of regulation is often executed by global transcription regulators. In this thesis, I will focus on the global transcription regulator Fis' (factor for inversion stimulation) role in affecting the transcription of biofilm-related gene *lapF* and influencing the hydrophobicity of soil bacterium *Pseudomonas putida*.

P. putida is a cosmopolitan bacterium able to colonize the rhizosphere of plant roots and dislodge the pathogenic microbes also inhabiting the same environment. The overexpression of *fis* is shown to increase *P. putida* biofilm formation and therefore it may be an important factor for plant root colonization (Jakovleva *et al.* 2012).

The initial aim of this study was to determine the factors through which Fis affects the biofilm of *P. putida*. After finding the regulative connection between Fis and the biofilm-related adhesins LapA and LapF, the aim of this thesis further focused on LapF – the second largest adhesin of *P. putida*. The role of LapF in *P. putida*'s biofilm formation has been briefly studied before. The involvement of LapF in cell-cell interaction in mature biofilm has been shown previously (Martinez-Gil *et al.* 2010), although, we did not see any effect of LapF in the Fis-induced biofilm of *P. putida*. As cells growing in a biofilm are shown to be more hydrophobic, we decided to investigate the possibility of LapF being involved in the cell surface hydrophobicity of *P. putida* and further study the role of Fis in the regulation of this process. Thus the aim of this study was driven by the initial finding of the connection between Fis and LapF, which

surprisingly did not involve biofilm formation by *P. putida*. Therefore the intriguing questions were raised: how Fis regulates *lapF*, could LapF be engaged in the surface hydrophobicity and if yes, then what would be the purpose of it regarding the survival and adaptation of soil bacterium *P. putida*?

In the first part of this thesis, I give an overview of the current knowledge about the bacterial response to environmental changes, cell surface hydrophobicity and its factors, biofilm formation, including the role of adhesins LapA and LapF in it and transcription regulator Fis. The experimental part of the thesis focuses on the characterization of the mechanisms involved in the regulation of hydrophobicity of *P. putida* by Fis via regulating the transcription of adhesion protein LapF. Additionally, I will propouse a model of biofilm formation, and hydrophobicity regulation systems in *P. putida* in light of my experimental results and previously published data.

REVIEW OF LITERATURE

1. Bacterial adhesion

A critical approach for bacteria to adapt to the surrounding environment is to switch between planktonic and sessile lifestyles. Planktonic cells move freely; however, appropriate conditions can trigger attachment to abiotic or biotic surfaces, and bacteria switch over to sessile growth, also called biofilm. The variety of surfaces and environments that bacteria are able to occupy are almost infinite. The planktonic bacteria can adhere to metal, plastic, soil particles, medical implant materials or eukaryotic tissues (Costerton *et al.* 1999). The first colonists usually form a weak reversible attachment, which can become stronger and more permanent, when cells start to anchor themselves more and more on the surface and to one another, using surface proteins to bind other cells in a process called cell adhesion (Garrett *et al.* 2008).

The physicochemical interactions between bacteria and surface involve attractive forces which usually can be either electrostatic or chemical forces such as van der Waals bonds, hydrogen bonds or hydrophobic interactions. More closely the interactions between cell and its substrate depend on the attractive and repulsive forces between the ion layer of bacteria and the charge of the surface. Therefore, the attachment of a cell with a negative charge is stronger to a positively charged surface and *vice versa* (Montville and Schaffner 2003). Several bacteria possess large surface proteins with a net negative charge contributing to adhesion to different substrates, thereby initiating biofilm formation (Soni *et al.* 2008).

1.1. Importance of bacterial cell surface hydrophobicity

Cell surface hydrophobicity has been shown to affect the interaction to abiotic surfaces for species like *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus* (Yousefi Rad *et al.* 1998). Despite the fact that hydrophobicity regulates bacterial physiology only passively, its influence on the competitiveness of bacteria is remarkable. For example, the *P. putida* toluene tolerant strain IH-2000, which surface has a lower hydrophobicity than the surface of toluene-sensitive mutant, exhibits decreased consumption of aromatic compounds *p*-xylene and cyclohexane (Kobayashi *et al.* 1999). Furthermore, some gram-positive bacteria, like *Bacillus licheniformis* S86 decrease the level of their hydrophobicity in the presence of toxic organic compounds like 3-methyl-1-butanol and therefore show low affinity against this compound (Torres *et al.* 2011). Bacterial surface hydrophobicity can influence even the effectiveness of bacterial motility in the soil. For example, compared to hydrophilic strain LAM1 the *Pseudomonas fluorescens* hydrophobic strain LAM2 shows a much higher adherence and migration abilities in different types of soils: clay loam, sandy loam and sandy soil (van Loosdrecht *et al.* 1987,

Singh *et al.* 2002). On the contrary, the hydrophobic bacterial cell surface has been shown to conduce to cell aggregation in a mixed population habitating soil (Liu *et al.* 2009). This can facilitate degradation of aromatic compounds such as phenol, pyridine or its derivatives via different metabolic pathways (Adav *et al.* 2008). In 1987 van Loosdrecht *et al* found that cell surface hydrophobicity is the main dominant characteristic of bacteria that assure adhesion (van Loosdrecht *et al.* 1987). Meaning that the hydrophobicity of the cell surface and the ability to regulate it is an essential factor for bacterial adaptation to different environmental conditions (Segura *et al.* 1999, Heipieper *et al.* 2007).

The hydrophobicity of cell surface may play a protective role for bacteria. For example, in response to different kind of environmental stressors including osmotic stress, heat shock, and solvents, the bacterial surface becomes more hydrophobic (Heipieper *et al.* 2007). In gram-negative bacteria the stress causing agents usually strike the outer membrane first. Therefore it is logical that surface changes are involved in stress response.

One of the mechanisms related to the changes in the surface of gram-negative bacteria is the formation of outer membrane vesicles (Baumgarten *et al.* 2012). *P. putida* strain DOT-T1E has been shown to release membrane vesicles within 10 minutes after encountering different stressors: toxic concentrations of long-chain alcohols, EDTA, NaCl and heat shock (Neumann *et al.* 2006, Heipieper *et al.* 2007, Baumgarten *et al.* 2012). The vesicles are often associated with the release of virulence factors of *P. aeruginosa*, which help the bacteria to survive in human lung epithelium (Kulp and Kuehn 2010). Another function of membrane vesicles occurs in the cell aggregation, where it helps bacteria to become more tolerant against antibiotics and biocides (Beveridge *et al.* 1997). The stress-induced release of membrane vesicles and increase of cell surface hydrophobicity consequently enhance the aggregation of bacteria (Neumann *et al.* 2006, Heipieper *et al.* 2007, Baumgarten *et al.* 2012). A potential mechanism or phenomenon for this is called depletion attraction, which means the aggregation of large particles (bacteria) increases the free movement of small particles (Dorken *et al.* 2012). The particles in a colloid cannot approach the surface of other particles no more than the distance of their own radius. Therefore every particle is surrounded by a so-called depletion zone and the free movement of particles reduces when the number of particles increases in the medium (Dorken *et al.* 2012). Thus, if two large particles get closer to each other, their depletion zones overlap, creating more space for smaller particles in a medium. In other words, the entropy of the smaller particles in a system increases and therefore the aggregation of hydrophobic bacteria is favored (Dorken *et al.* 2012).

To sum up, the hydrophobicity of cell surface plays an important role for microorganisms in adhesion and attachment to various surfaces, in biofilm formation as well as in tolerance against toxic organic compounds.

1.2. Bacterial surface hydrophobicity factors

Cell surface is the first barrier of bacteria. For example, the cell surface of all gram-negative bacteria consists of an outer membrane that can quickly react to environmental conditions and reorganise the structure of membrane components. Lipopolysaccharides (LPS), adhesins, lipoteichoic acids, S-layer proteins and other proteins are located on the outer membrane of cells and therefore are the first contact elements between bacteria and its surrounding environment. Surface hydrophobicity of cells is influenced by various factors. Their contribution and properties to hydrophobicity can also differ.

One of the main factors influencing bacterial surface hydrophobicity is lipopolysaccharides (LPS) on the outer membrane of gram-negative bacteria (Fig 1). LPS consist of hydrophobic fatty acid chain harbouring lipid A – a glucosamine disaccharide, which differs from a typical phospholipid by possessing six unsaturated fatty acid chains instead of two saturated or unsaturated chains. LPS also comprises of a heterogeneous core oligosaccharide linked to lipid A and the O-antigen, which is an immunogenic oligosaccharide consisting of 1-40 repeating subunits (Fig 1). These are the features that make the asymmetric outer membrane bilayer more hydrophobic compared to a typical phospholipid bilayer, by having strong lateral connections between LPS molecules and low fluidity (Neidhardt and Curtiss 1996). In *Legionella pneumophila*, the O-antigen of LPS forms a homopolymer lacking free hydroxyl-groups, making the surface more hydrophobic and therefore can assist the attachment of bacteria to target cells (Zahringer *et al.* 1995).

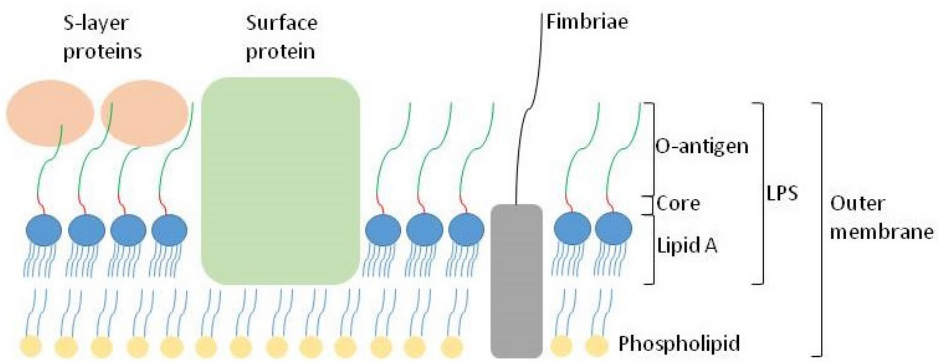

Figure 1. The localization of hydrophobicity factors (LPS, fimbriae, surface proteins and S-layer proteins) in the outer membrane of gram-negative bacteria.

In addition, lipid A also plays an important, yet indirect part in providing hydrophobic barrier to bacterial cells. For example, the repression of lipid A biosynthesis reduces the production of lipopolysaccharides, which therefore is shown to increase the permeability of hydrophobic antibiotics and can lead to

gram-negative bacteria becoming susceptible to them (Wyckoff *et al.* 1998). Consequently the role of LPS in cell surface hydrophobicity occurs mainly through changing the overall barrier of cell surface, which hinders the movement of different compounds between cell and its environment.

Similarly to LPS, S-layer proteins can also influence the hydrophobicity by contributing to the general surface hydrophobicity and protective barrier of the cells (Fig 1). Similarly to gram-negative bacteria most members of gram-positive *Bacillaceae* family are also covered with S-layer, which form a porous crystalline layer containing high amounts of hydrophobic amino acids (U. B. Sleytr *et al.* 1993). S-layers can function as protective coats or structural elements promoting adhesion and target surface recognition (Uwe B. Sleytr *et al.* 1994). Furthermore, the S-layer proteins have been reported to be involved in the surface hydrophobicity of *Lactobacillus acidophilus* ATCC 4356 (Smit *et al.* 2001) or *Bacillus cereus* (Kotiranta *et al.* 1998). Studies have shown that the changes in S-layer influence the surface hydrophobicity of *B. cereus*, which protect bacteria against the ingestion of polymorphonuclear leukocytes (Kotiranta *et al.* 1998).

Another factor demonstrated to be involved in general surface hydrophobicity and protection of bacterial cells is mycolic acids (MA). The cell envelope of gram-positive *Rhodococcus opacus* is described to possess long chain α-alkyl-β-hydroxy fatty acids, also known as mycolic acids (Asselineau and Lederer 1950). Mycolic acids, forming an outer lipid layer of the cell wall, are one of the main factors to provide tolerance for *R. opacus* and *Mycobacterium tuberculosis* against antibiotics and other stressful conditions (Alvarez *et al.* 2004, Barkan *et al.* 2009). Recently it was demonstrated that if grown in liquid medium and treated with toxic concentrations of NaCl or 4-chlorophenol the cells of *R. opacus* PWD4 respond by decreasing the average chain length of mycolic acids and the average amount of double-bonds in them. This was shown to be in correlation with higher saturation of membrane fatty acids and increased hydrophobicity of cell surface of *R. opacus* PWD4 (de Carvalho *et al.* 2016).

In addition to the factors influencing the general cell surface hydrophobicity, other factors, such as hydrophobic fimbrial adhesins can also affect the attatchment of bacteria to surfaces (Fig 1) (Zahringer *et al.* 1995, Sidhu and Olsen 1997, Higashi *et al.* 1998). For instance, enteric bacteria use fimbriae for adhesion to host cells via interaction of fimbrial lectin to carbohydrate of host cells (Isberg and Barnes 2002). This interaction can be specific however adhesion can be reduced by electrostatic repulsion that exists between the host cell and the surface of bacteria. These fimbriae contain a high number of hydrophobic amino acids that probably help overcome the initial electrostatic repulsion barrier (Corpe 1980, Rosenberg and Kjelleberg 1986).

In common with fimbriae, several proteins have been shown to be connected with cell surface hydrophobicity which helps mediate the adhesion to surfaces. For example, a yeast protein, CSH1p was the first described hydrophobic protein on the surface of *Candida albicans*. The hydrophobicity of cells lacking *csh*

gene reduces 75% along with the adhesion to the extracellular matrix protein fibronectin (Singleton *et al.* 2005). However, it is suggested that CSH1p has a pleiotropic nature and its pathogenic contribution (e.g., adhesion to fibronectin) and lower cell surface hydrophobicity are independent of one-another (Singleton *et al.* 2005).

In *C. albicans* another hydrophobicity-related adhesin is Als3 – agglutinin-like sequence (ALS) family protein which contributes to yeast aggregation and adhesion to epithelium (Dranginis *et al.* 2007, Aoki *et al.* 2012). The enhanced exposure of Als3 on the surface of *C. albicans* provides hydrophobic hyphae and causes stronger adhesion to the hydrophobic substrate (Beaussart *et al.* 2012).

One more cell wall protein of yeast – Rbt1 is described to affect the adhesion of the cells via hydrophobicity. The N-terminal region of Rbt1 in *C. albicans* is demonstrated to be the key-factor for promoting the hydrophobicity of cell surface and therefore the adherence to polystyrene (Monniot *et al.* 2013). It is also demonstrated that in the central part of the protein, a domain consisting of 42 amino acids is responsible for the cell-cell interactions by promoting the aggregation of hyphea (Monniot *et al.* 2013). Furthermore, while Rbt1 protein shows similar anchorage in the cell wall of both cell types as yeast and as hyphae, then Rbt1 proteins are more exposed to the surface and unmasked in hyphae. This means that significant changes in the structure of cell walls, which could occur in the fimbriae layer, might allow to cover the surface proteins in yeast cells and expose in hyphae cells (Monniot *et al.* 2013). In 1997 Braun and Johnson characterized a gene, *TUP1*, which deletion caused *C. albicans* cells to grow in a filamentous state in the absence of any environmental inducing signal (Braun and Johnson 1997). A few years later it was demonstrated that *TUP1* represses the expression of hyphal-specific *rbt* gene family, including the cell-wall protein Rbt1 (Braun *et al.* 2000). Although these given examples of proteins involved in hydrophobicity and adhesion illustrate the mechanisms studied in yeast *C. albicans*, which cell wall structure is different from that of bacteria. Nevertheless, these examples enlighten the importance of hydrophobicity in microorganisms in general and explain the factors involved in this. However, in bacteria, there are also several proteins described to be involved in adhesion and hydrophobic interactions.

It was only recently demonstrated that *Helicobacter pylori* protein Hsp60 can function as a chaperone in acidic conditions, whereas the interactions between Hsp60 and other proteins undergoing acid-induced denaturation are occurring due to hydrophobic surfaces (Mendoza *et al.* 2017). A hydrophobic reporter probe 1,1`-bis(4-anilino) naphthalene-5,5`-disulfonic acid (bisANS) was used, which is non-fluorescent in an aqueous environment, but becomes fluorescent in contact with proteins having hydrophobic surfaces (Hawe *et al.* 2008). Measurements with bisANS showed high fluorescence in contact with Hsp60, demonstrating the hydrophobic surface of Hsp60. Furthermore, the fluorescence of bisANS was found to increase even more in moderately lower pH conditions (Mendoza *et al.* 2017). With this ability Hsp60, functioning as a

chaperone could protect other proteins from acidic environment, like in gastric epithelium of humans, which *H. pylori* colonizes.

The first large bacterial adhesion protein Bap (biofilm-associated protein) was described in *Staphylococcus aureus* (Cucarella *et al.* 2001). Disrupting the *S. aureus bap* gene decreases the surface hydrophobicity and subsequently reduces both, initial attachment to polystyrene and diminishes intercellular adhesion (Cucarella *et al.* 2001). *Pseudomonas fluorescens* and *Pseudomonas putida* have similar large adhesins: LapA in both species and LapF in *P. putida* (Hinsa *et al.* 2003). These large adhesins share common structural and characteristic features: high molecular weight, tandem repeats, cell-surface location and have relevant role in bacterial adhesion and biofilm formation (Lasa and Penades 2006).

P. putida utilizes these large extracellular adhesins, LapA and LapF, for attachment to abiotic and biotic surfaces (Yousef and Espinosa-Urgel 2007). LapF consists of 6310 amino acids and has only one large repeat domain, comprising of 64 repeating sequences, each of them with a length of 83-91 amino acids (Martinez-Gil *et al.* 2010). Whereas *P. putida* LapA consists of 8682 amino acids with two repeat domains, including 37 repeated hydrophobic sequences, each of comprising 100 amino acids. The accumulation of LapA protein on the cell-surface interface leads to the adhesion of bacteria to hydrophobic substrate (Espinosa-Urgel *et al.* 2000, Hinsa *et al.* 2003, Fuqua 2010, El-Kirat-Chatel *et al.* 2014). Therefore, it is suggested that LapA plays a crucial part in attachment and thus, is mostly responsible for biofilm initiation (Martinez-Gil *et al.* 2010). Whereas LapF is produced later, therefore, provides cell-cell interactions that lead to the formation of a mature biofilm (Martinez-Gil *et al.* 2010).

To conclude, the factors influencing cell surface hydrophobicity can act via providing the general hydrophobic barrier to the cells mediated by LPS and S-layer proteins. Hydrophobicity factors can also act via adhesion and attachment of cells to the surface and each-other, in which adhesive fimbriae and different membrane proteins having hydrophobic characteristics are shown to be involved.

1.3. The regulation of cell surface hydrophobicity

Bacteria can quickly change cell surface properties by physiologically regulated mechanisms. However, the control of so-called hydrophobicity factors' expression is one potential regulation mechanism as well, although much slower compared to physiologically regulated mechanisms.

One of the physiological regulation methods for bacteria to change their surface hydrophobicity is to alter the molecular weight of the LPS layer. Baumgarten *et al.* in 2012 analysed the LPS composition of *P. putida* DOT-T1E in response to *n*-alkanols, which increase the cell surface hydrophobicity of DOT-T1E (Baumgarten *et al.* 2012). They used micellar electrokinetic chromato-

graphy (MEKC) to analyse the composition of LPS and found that decrease of LPS' molecular mass correlates to the increase of hydrophobicity and vesiculation. Similarly to *P. putida*, the loss of high-molecular-weight LPS has been linked to the formation of outer membrane vesicles in *P. aeruginosa* (Kadurugamuwa and Beveridge 1995, Makin and Beveridge 1996). The change of cell surface's hydrophobicity has been shown to be reversible. When *P. putida* DOT-T1E was treated with 1-octanol, the increase of cell surface hydrophobicity was quick. But the surface hydrophobicity started to gradually decrease when the cells were pelleted, washed and resuspended in fresh medium without 1-octanol (Baumgarten *et al.* 2012). It was proposed that the decrease in hydrophobicity is slow due to *de novo* synthesis of high molecular weight LPS.

In addition to physiological adjustment of LPS, the regulation can occur by alternative sigma factors controlling the expression of hydrophobicity-related genes. In 2014 Klein *et al* demonstrated that the lack of *yciM* (*lapB*) increases the envelope stress response of *E. coli*, which control the main steps of LPS biosynthesis and assembly (Klein *et al.* 2014). LapB is a lipopolysaccharide assembly protein B encoded by *lapB* gene, formerly known as *yciM* (Klein *et al.* 2014). The defects caused by lack of the LapB have been shown to be suppressed by alternative sigma factor RpoE and two-component system CpxRA, which contribute to envelope stress response (Klein *et al.* 2014). The heat-shock inducible alternative sigma factor σ^E (RpoE) and two-component signal transduction system CpxRA have both been demonstrated to be adjusted to extracytoplasmic stimuli (Mecsas *et al.* 1993, Snyder *et al.* 1995). For example, the level of proteins on the outer membrane influences the activity of RpoE (Mecsas *et al.* 1993). In *E. coli* the lack of *rpoE* can cause defects in outer membrane permeability, which has been linked to increased sensitivity to detergents and hydrophobic substances (Raina *et al.* 1995). One of the transcription targets for RpoE is *degP*, which product is a periplasmic protease specified for the degradation of various atypical extracytoplasmic polypeptides (Strauch and Beckwith 1988). The *degP* locus is also activated by the response regulator CpxR of the two-component system CpxRA (Raina *et al.* 1995). CpxR is activated by the inner-membrane sensor CpxA which in turn gets stimulated by the overproduction of NlpE – an outer-membrane located lipoprotein in *E. coli (Snyder et al. 1995)*. These examples suggest that both sigma-factor RpoE and two-component system CpxRA play a central role in envelope stress response, which could be the key factor in covering the defects of decreased hydrophobicity caused by the lack of LPS in the cell surface of bacteria.

Another example of hydrophobicity regulation involving alternative sigma factor is related to the expression control of large adhesion protein Bap, which is involved in adhesion, biofilm formation and virulence of *S. aureus* (Tormo *et al.* 2005, Tormo *et al.* 2007). Bap is shown to be positively regulated by SarA (Tormo *et al.* 2005, Tormo *et al.* 2007). SarA is a DNA-binding protein which generally activates the *agr* promoters, which produce Agr proteins. Agr in turn controls the expression of several virulence factors in response to increasing cell density (Novick 2003). Though, SarA is also able to activate some virulence

genes independently from *agr* (Novick 2003). For example, SarA binds the *bap* promoter independently from *agr*, therefore SarA is a positive regulator for Bap-mediated biofilm of *S. aureus* (Tormo *et al.* 2005, Tormo *et al.* 2007). One of the two alternative sigma factors described in *S. aureus* – σ^B affects the expression of multiple genes, including virulence genes (Horsburgh *et al.* 2002). It has been demonstrated that the lack of σ^B impaires the Bap-mediated biofilm (Marti *et al.* 2010). The extracellular proteases Aur and SspA, which are capable of degrading Bap, have been shown to be overexpressed in σ^B mutant, thus increasing the degradation of Bap protein and reducing protein-dependent biofilm formation of *S. aureus* (Marti *et al.* 2010). In the same work it was also demonstrated that in the σ^B gene and *agr* double-mutant, where the extracellular proteases are not being overexpressed, the biofilm formation is restored. This suggests that in *S. aureus* a protein-mediated biofilm could be controlled by a pathway, where global transcription regulators play a central role via regulating the expression of extracellular proteases (Marti *et al.* 2010).

Global transcription regulators have also been shown to be involved in the regulation of lipoteichoic acid (LTA)-related hydrophobicity in gram-positive bacteria. In addition, affecting hydrophobicity of *Streptococcus pyogenes,* LTA also helps to mediate the adhesion to the host cells (Hasty *et al.* 1992). The LTA of *S. pyogenes* forms complexes with surface proteins in a way where fatty acids of LTA can be exposed to the surface of bacteria (Ofek *et al.* 1983). The LTA complex with surface proteins is therefore responsible for mediating or inhibiting the adhesion of *S. pyogenes* to host cell receptors. A group of proteins of *S. pyogenes* called M-protein family has been demonstrated to be involved in anchoring LTA to the surface of the cells resulting in a more hydrophobic surface and enhanced biofilm formation (Courtney *et al.* 2009). M-proteins are in turn regulated by a global transcription regulator Mga (Caparon and Scott 1987). Specific binding of Mga to the promoter regions of genes encoding for M-proteins helps to strengthen the binding of RNA polymerase and therefore increases the transcription (McIver *et al.* 1995). The 45-bp Mga binding consensus (N_5 AGGTCAA(C)AAAGNT N_4 AA N_5 AAAAANCTGG(A)T(C)CTTTA) comprises of two highly conserved regions called C_L and C_R separated by an 11-bp sequence of more variable nucleotides with conserved adenine-pair in the middle (McIver *et al.* 1995). The C_R region of Mga binding site overlaps the -35 sequence of promoters, thus showing this region as a binding site for both Mga and RNA polymerase. Furthermore, *mga* promoter itself is positively autoregulated by Mga (Okada *et al.* 1993). In a serotype M6 of Group A streptococcus strain JRS4 it is shown that the expression of *mga* and the genes under its control *emm* and *scpA* are active in logarithmic phase but are turned off upon entry into stationary growth phase (McIver and Scott 1997). One possible advantage for expressing these genes only in exponential phase is to save energy in starvation conditions, as proposed by the authors (McIver and Scott 1997). The products of *emm* and *scpA* genes could be very stable on the surface of Group A streptococcus, and the sufficient amount of these proteins may be

enough for the time when bacteria reach to stationary growth phase, and the further transcription may not be needed (McIver and Scott 1997).

Previous examples of the regulation of hydrophobicity factors included mostly the control on transcription level. However, the regulation of hydrophobicity factor's expression can also happen via modification of translational efficiency. For example, the translation of *slpA* mRNA encoding S-protein in *Lactobacillus acidophilus* ATCC 4356 depends on the secondary structure of 5`end of mRNA, which exposes the ribosome binding site (Boot *et al.* 1996). As shown by reporter gene analysis in *Lactobacillus casei*, the cutting of the leader sequence of mRNA leads to decrease in SlpA production (Boot *et al.* 1996). This leader sequence in 5' end of mRNA is untranslated; however, it is necessary to ensure the half-life of mRNA (Boot *et al.* 1996).

In general, the regulation of cell surface hydrophobicity could occur via quick physiological changes or through different gene expression pathways, involving alternative sigma factors, two-component signal transduction systems or global transcription regulators. All these complex regulation cascades depend on the environmental conditions and signals received by bacterial cells, which respond to it by activating or repressing the right genes at the right time.

2. Bacterial biofilm

Biofilm is a phenotype of bacterial population that has several functions. Bacteria can protect themselves from rough environment by forming biofilm or on the other hand the formation of biofilm could help them to settle down where conditions are better facilitating their existing as a community (Jefferson 2004). In biofilm bacteria can withstand hazardous abiotic factors like UV (Espeland and Wetzel 2001), high osmotic stress, dryness (Le Magrex-Debar *et al.* 2000), antibiotics and antimicrobial agents (Stewart and Costerton 2001) much better compared to planktonic cells. The matrix produced in biofilm acts as a barrier between bacteria and environment, which helps bacteria to tolerate stress better (Donlan and Costerton 2002).

On the other hand, biofilm formation does not occur only in response to stress conditions. An example would be bacterial biofilm on the roots of plants. In the rhizosphere, an ecological niche is formed, where nutrients are more freely available, and microorganisms have developed specific mechanisms to exploit this niche. Plant roots excrete a variety of components, such as amino acids, organic acids, simple sugars, carbohydrates and enzymes (Lynch and Whipps 1990). These exudates are released mainly through the tips of the roots, which are constantly growing. Therefore to take advantage of this nutritional paradise, in contrast to biofilm formation, microorganisms must be able to migrate along with the growth of roots. This means that for successful adaptation to different environmental conditions, bacteria have to be able to rapidly change their lifestyle and disperse from biofilm to recolonize new locations. For example, in *P. putida* it has been demonstrated that (among other factors)

nutrient deprivation, more specifically carbon starvation causes biofilm to disperse (Gjermansen *et al.* 2005).

In nature biofilm usually consist of bacteria from multiple species, which compete, cooperate and communicate with each other (Amann *et al.* 1995). For instance, the fermentative bacteria produce different acids and alcohols. These are in turn used as substrates by acetogenic bacteria, and after that methanogenic microorganisms can get energy by converting carbon dioxide, acetate, and hydrogen into methane (Davey and O'Toole G 2000). Another example of biofilm formation unrelated to stress has been demonstrated in *E. coli*, where bacteria can form biofilm in response to an increase in cell density through quorum sensing. A signal autoinducer 2 (AI-2) enhances the initial stages of biofilm formation in *E. coli* via stimulating the motility-related genes *motA* and *fliA* (Gonzalez Barrios *et al.* 2006). Therefore AI-2 increases the initial attachment of *E. coli* cells to the surface and thus improves the first steps needed for biofilm formation (L. A. Pratt and Kolter 1998).

In natural biofilms, microorganisms also compete with each-other for nutrients and try to inhibit the growth of other species in the community. For example, *Streptococcus oligofermentans* produces hydrogen peroxide (H_2O_2) from peptone by using L-amino acid oxidase to inhibit the growth of *Streptococcus mutans* in multi-species biofilms growing in the peptone-rich environment (Tong *et al.* 2008). Marine bacterium *Pseudoalteromonas tunicate* is shown to produce protein AlpP which has antibacterial properties and therefore living in the same environment can repress the growth of another marine bacterium such as *Cellulophaga fucicola* and *Alteromonas* sp (Rao *et al.* 2005).

The human society has learned to benefit from the bacterial biofilm. For example, microorganisms are used for bioremediation to remove contaminants like oil spills (Radwan *et al.* 2002), nitrogen compounds (Li *et al.* 2003) or industrial waste (Sekoulov and Brinke-Seiferth 1999). However, the formation of biofilms can also cause problems for humans. Hospital-related infections are one of the examples. Surgical instruments, like drips, scalpels, and catheters, are common sources of biofilm growth and subsequent infection. Biofilm forming methicillin-resistant *Staphylococcus aureus* (MRSA) is particularly important due to its resistance to multiple antibiotics, whereas frequent sources of MRSA are the patients themselves (Godwin *et al.* 2001, Salgado *et al.* 2003). Another critical area is the oil industry, where biofilm formation by sulphate-reducing bacteria (SRB) causes pipe and rig corrosion, blockage of filtration systems and oil spoilage (Santegoeds *et al.* 1998).

3. The regulation of biofilm-related adhesins LapA and LapF

Probably due to their different functions in biofilm, the synthesis of LapA and LapF are regulated differently (Fuqua 2010). The expression of *lapF* is controlled by stationary phase sigma RpoS in *P. putida* (Martinez-Gil *et al.*

2010), but the regulation of *lapA* expression seems to be more complicated. In *P. putida* the transcription of *lapA* is shown to be under control of 6 promoters, whereas only 3 of them display a moderate RpoS-dependence (Ainelo *et al.* 2017). Moreover, Fis activates the transcription of *lapA* by binding to 2 different sites identified in the promoter region of *lapA* (Ainelo *et al.* 2017). In *P. putida* the two-component signal transduction system GacS/GacA seems to be involved in biofilm formation, influencing the expression of both – LapA and LapF (Fig 2) (Martinez-Gil *et al.* 2014). As shown in *P. aeruginosa*, when *gacS* is mutated, the detachment from biofilm increases (Petrova and Sauer 2009).

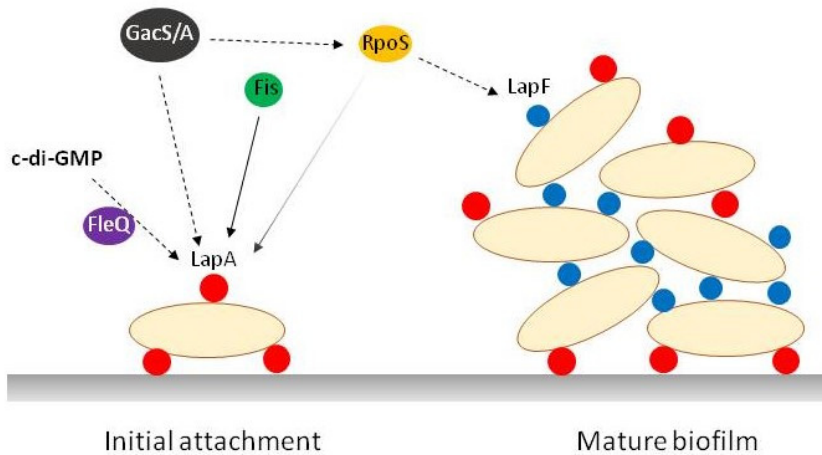

Figure 2. A potential model for a regulatory network of *lapA* and *lapF* transcription in the biofilm formation process of *P. putida*. Large ellipses demonstrate bacterial cells, red and blue circles on the surface of cells indicate LapA and LapF respectively. Direct positive effects are indicated with solid lines, dotted lines demonstrate activation of transcription without any indication of specific binding sites in front of genes. Gradient line represents partial regulation of *lapA* by RpoS, which means that only 3 out of 6 *lapA* promoters seem to be RpoS-dependent.

Part of the GacS/A signal transduction pathway is small RNA-binding transcriptional regulators. In *P. putida* these small regulators, like RsmA and RsmE repress diguanylate cyclase *cfcR* translation through regulation of *rpoS* expression (Huertas-Rosales *et al.* 2017). Furthermore, CfcR seems to be the key generator of the free pool of c-di-GMP in stationary phase *P. putida* and also a positive regulator for biofilm when Rsm proteins are absent (Huertas-Rosales *et al.* 2017). The secondary messenger c-di-GMP affects the expression of adhesine genes *lapA* and *lapF* in opposite ways. In response to artificially increased c-di-GMP, the transcription from *lapA* promoter increases (Martinez-Gil *et al.* 2014). On the other hand, the expression of *lapA* through c-di-GMP seems to require a flagellar regulator FleQ (Fig 2). In *P. aeruginosa* FleQ has been demonstrated to be involved in the expression of biofilm-related components,

like exopolysaccharides (Baraquet *et al.* 2012). C-di-GMP represses the ATPase activity of FleQ, therefore causing a down-regulation of flagellar gene expression. In contrast, the absence of c-di-GMP influences FleQ to activate Pel polysaccharide expression in *P. aeruginosa* (Hickman and Harwood 2008, Baraquet *et al.* 2012). In *P. fluorescens* F113, the swimming motility is shown to be regulated by GacS/GacA via controlling FleQ (Navazo *et al.* 2009). Furthermore, using β-galactosidase assay and RT-PCR Martinez-Gil *et al* in 2014 found that the GacS/A two-component system is modulating the expression of RpoS and is therefore indirectly involved in the regulation of *lapF* expression.

The expression of *lapF* is negatively regulated in early stages of biofilm of the *P. putida* strain, which overproduces c-di-GMP. This indicates that LapF is not needed in initial steps of biofilm formation (Fig 2). It is previously shown, that the lack of either adhesin LapA or LapF results in higher expression of exopolysaccharides, hinting that there might be some internal mechanism for balancing the biofilm structural components (Martinez-Gil *et al.* 2013). So far, the assessment of *lapF* transcription using β-galactosidase assay has demonstrated, that LapF is extensively expressed in the stationary phase but not in exponentially growing cells (Martinez-Gil *et al.* 2010, Martinez-Gil *et al.* 2014).

The expression of LapF and exposition to the surface of bacteria are suggested to be separate from LapA. LapF transportation to the cell surface is probably mediated by ABC-type transporter encoded by the other genes of the operon LapHIJ. However, there is no direct evidence that LapHIJ are involved in the transport of LapF (Fuqua 2010, Martinez-Gil *et al.* 2010).

LapF is important for *P. putida* to colonize plant roots. The *lapF*-mutants are unable to infect the roots properly: low infection ability appears both alone and in collaboration with the wild-type strain. Microscopy analysis shows that although *lapF* mutants are unable to form biofilm on plant roots alone, they are still viable in biofilm with the wild-type strain. This implies for the necessity of LapF in cell-cell interactions, as the LapF-nonproducing mutants can at least benefit from LapF expressed by wild-type strain (Martinez-Gil *et al.* 2010).

4. Global transcription regulator Fis

Fis (factor for inversion stimulation) is a small homodimeric protein found in both gammaproteobacteria families *Enterobacteriaceae* and *Pseudomonadaceae* (Beach and Osuna 1998, Boswell *et al.* 2004). Fis is thoroughly studied in *E. coli*, but there is a lot less information about Fis in genus *Pseudomonas.* Fis potein is conserved between Enterobacteria and Pseudomonases (Azam and Ishihama 1999). The protein identity is 64.8 %, and the similarity is 81.7 % between Fis in *E. coli* K12 and *P. putida* KT2440 (protein alignment tool http://cmr.jcvi.org). Despite the similarity of the proteins, the DNA-binding properties and therefore also the target genes can still differ in different orga-

nisms (Osuna *et al.* 1995, Bradley *et al.* 2007). For example, *Salmonella enterica* serovar Typhimurium LT2 and *E. coli* K12, which often share a similar environment and possess similar regulatory mechanisms, have different Fis regulons for their virulence genes (Wang *et al.* 2013). It has been demonstrated that *E. coli* Fis alone is responsible for the regulation of 76 genes directly and 15 genes in collaboration with other transcriptional factors (Martinez-Antonio and Collado-Vides 2003). According to microarray analysis performed by Bradley *et al*, *E. coli* Fis regulates indirectly the expression of 231 genes altogether. These genes can be divided into 15 different categories by their function, including motility, stress, biosynthesis of nucleotides and amino acids, energy metabolism, nutrient transportation etc (Bradley *et al.* 2007). Furthermore, Fis dictates the transcription of the genes encoding for four transcription factors and sigma-factors σ^{70}, σ^{38} and σ^{32} (Martinez-Antonio and Collado-Vides 2003).

In *E. coli* Fis regulates its transcription both directly and indirectly. Fis protein binds to the promoter of *fis* gene, blocking the binding of RNA polymerase and thereby the transcription of itself (Ninnemann *et al.* 1992). There are six different Fis binding sites in the promoter region of *fis* and two of them overlap the binding site for RNA polymerase (Ball *et al.* 1992). The indirect regulation of Fis takes place through the change of DNA topology, increasing the negative supercoiling (Schneider *et al.* 2000). Also, the expression of *fis* has been shown to be regulated by another global transcription factor – IHF. In *E. coli* IHF binds to the promoter of *fis* and increases the transcription from it up to 4 times (T. S. Pratt *et al.* 1997). Furthermore, cAMP-receptor protein (CRP) also directly regulates the expression of *fis.* In 2001 Nasser *et al* demonstrated the involvement of CRP in the formation of nucleoprotein complexes that are able to activate or repress the transcription of *fis* (Nasser *et al.* 2001).

Fis can be both, the activator or repressor of transcription, depending on the binding position on the promoter region of target genes. Prokaryotic transcription factors are generally classified into class I and class II activators (Fig 3) (Smolke 2009). Class I activator`s binding sites are usually located more than -61 bp from the transcription start site, and they secure the transcription activation by binding to the RNA polymerase (RNAP) α-subunit`s C-terminus (Fig 3) (Smolke 2009). Class II activators tend to bind the DNA near the -35 element of promoters and strengthen the RNAP binding via the N-terminus of RNAP α-subunit (Fig 3) (Smolke 2009). Class I and class II activators can also co-activate the same promoters. For example in *E. coli proP* P2 promoter is activated by CRP, acting as a class I activator by binding to the position at -121.5 bp from the transcription start site, while Fis binds to -41.5 position, acting as a class II activator (McLeod *et al.* 2002). Some transcription activators can also bind DNA far away from the transcription start site, thus activating the transcription by bending the DNA (Browning *et al.* 2004). Transcription repression usually occurs via binding between the -10 and -35 elements of promoter region and therefore blocking the binding of RNAP (Fig 3) (Smolke 2009).

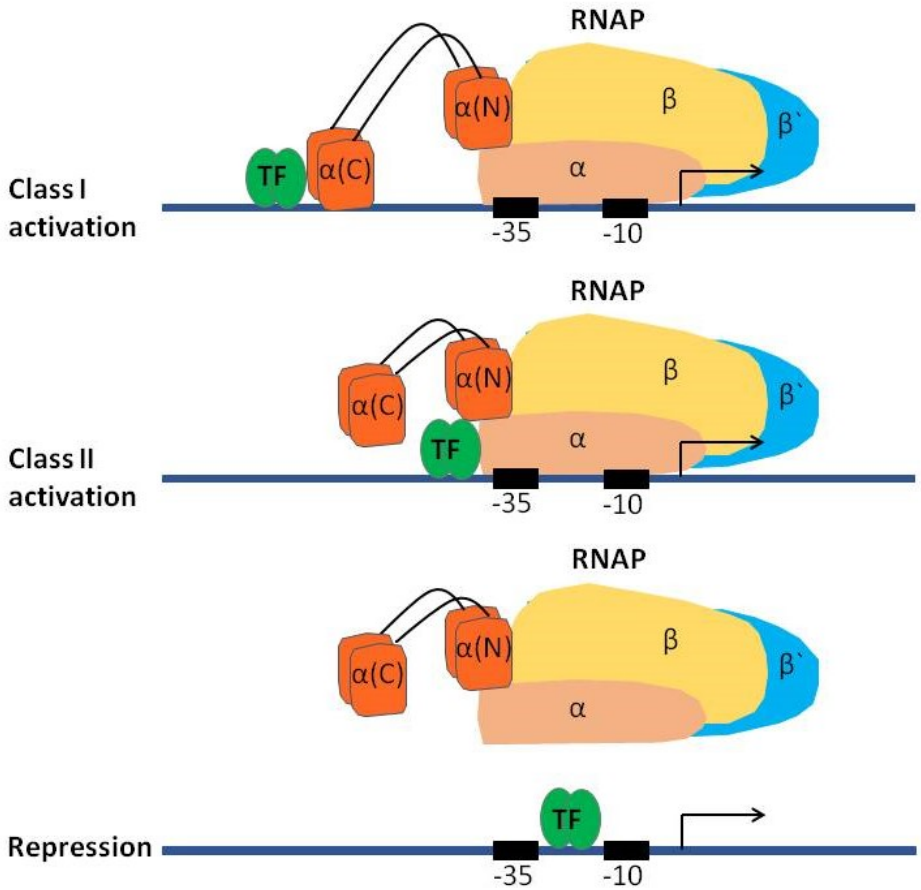

Figure 3. An illustration of class I and class II activation and repression mechanisms of transcription factors interacting with RNA polymerase (RNAP) inspired by Smolke *et al* 2009. Class I and II activation by TF occur via mediating RNAP to the right position and repression via TF happens by blocking the binding of RNAP. TF represents transcription factor, -10 and -35 mark the promoter elements, the arrow show the transcription start site and RNAP different subunits are α, β, β`, whereas α(C) and α(N) represent the C and N terminus of α-subunit.

Although Fis is important for bacteria in many processes, it does not seem to be essential for *Enterobacteriaceae* family members like *E. coli* or *S. enterica* serovar Typhimurium. Both bacteria have been shown to be viable and well growing after *fis* deletion, although the lag-phase of *fis* null-mutant may be prolonged (Johnson *et al.* 1988, Osuna *et al.* 1995, Bradley *et al.* 2007). Contrary to preceding members of *Enterobacteriaceae*, *fis* seems to be essential for the species from the genus *Pseudomonas*, as the cells cannot tolerate the lack of functional Fis protein (Liberati *et al.* 2006, Teras *et al.* 2009, Yeung *et al.* 2009). Liberati *et al* (2006) conducted an experiment, where they constructed a

non-redundant library of *P. aeruginosa* PA14 transposon mutants. This library consisted of nonessential PA14 genes with single transposon insertions chosen from an extensive library of transposon mutants. Insertions of 4468 genes were obtained, which make up 75 % of the genome of PA14. Only one mutant carried an insertion in the *fis* gene, and even this was located in a stop codon of the *fis* gene (Liberati *et al.* 2006). In 2009 Yeung *et al* used the very same transposon mutant in their experiments and demonstrated a significant reduction in growth rate in liquid swarming medium (Yeung *et al.* 2009). This suggests that *fis* is a potentially essential gene in *P. aeruginosa* PA14 (Liberati *et al.* 2006). Similarly, in our laboratory, the attempts for creating a *fis* knockout mutant in *P. putida* have not been successful (Teras *et al.* 2009).

The studies of expression regulation of *fis* in *E. coli* have shown, that the amount of Fis increases rapidly near to maximum of 50 000 molecules after inoculated with rich medium (Ball *et al.* 1992, Ali Azam *et al.* 1999). After growth in logarithmic phase for 90 minutes the amount of Fis starts to decrease till in stationary phase, there are less than 100 molecules of Fis in each cell (Ball *et al.* 1992, Ali Azam *et al.* 1999). Such rapid changes in protein level for bacteria could be important for growth phase-dependent regulation of different biological processes. The changes in the protein and mRNA level of *fis* appear similarly, but the changes in mRNA level start approximately 15 minutes earlier. Thus the most critical regulatory phase for *fis* expression in *E. coli* is transcription (Ninnemann *et al.* 1992).

4.1. DNA-binding properties of Fis

Fis is able to bind specific DNA by bending it up to 90° (Azam and Ishihama 1999). It has been showed that Fis participates in certain site-specific DNA recombination processes as well as suppressing DNA supercoiling in *E. coli*, which affects the under-winding of DNA strands and therefore indirectly the expression of genes. Fis can modulate the topology of DNA directly or indirectly via regulation of topoisomerase genes. For example, Fis binds the promoter of gyrase gene and represses the transcription from it (Travers *et al.* 2001).

In *E. coli* Fis, there are 4 tyrosine residues in positions 38, 51, 69 and 95, each of them is responsible for specific intra- and intermolecular interactions. Two of them – Tyr51 and Tyr95 are both participating in hydrogen bonding-salt bridge networking and therefore play a significant role in stabilization and flexibility of Fis protein (Boswell *et al.* 2004).

By the crystal structures of the Fis protein of *E. coli,* it is demonstrated, that Fis consists of 4 α-helixes and β-sheets, that form excrescent hairpin structures (Kostrewa *et al.* 1991, Hancock *et al.* 2013). The α-A helix in the N-terminus of the protein is essential for recombination and α-C, and α-D helixes in the C-terminus form a helix-turn-helix motif, needed for the binding with DNA (Yuan *et al.* 1991). Tyrosine residues responsible for recognizing specific DNA sequences are located in α-D helixes (Feldman-Cohen *et al.* 2006). In Fis-dimer

the distance between DNA-binding α-helixes is only 25 Å, which does not change in the binding process. Thus, Fis can only bind DNA by bending it (Kostrewa *et al.* 1991, Merickel *et al.* 2002).

The DNA-binding consensus of Fis in *E. coli* is GNTYAAAWTTTRANC, where Y = pyrimidine, R = purine, W = A or T and N = any nucleotide (Shao *et al.* 2008). The most critical nucleotides for high-affinity binding of Fis are G, A, T, and C in positions 1, 5, 11 and 15 respectively. Furthermore, the binding is shown to be stronger in the occurrence of A, and T nucleotide repeats in the middle of binding consensus and adjacent to it (Shao *et al.* 2008). In *E. coli* Fis, first of all, prefers to bind to DNA, which has very good similarity to consensus. When such sites are saturated with Fis, free Fis binds to less conserved DNA (Feldman-Cohen *et al.* 2006).

4.2. The role of Fis in regulating bacterial physiology

The whole process of adapting to different conditions in surrounding environment requires changes in bacterial physiology, which can be maintained by altering the expression of large number of genes (Ramos-Gonzalez *et al.* 2005, Matilla *et al.* 2007). Fis is a global regulator modulating the physiological state of bacteria by regulating the expression of multiple growth-related genes, chemotaxis, response to environmental changes, motility and biofilm (Ishihama 2009).

In *Enterobacteriaceae,* Fis is mainly known to be active in logarithmic phase. Therefore Fis has an opposite function to the stationary phase-specific sigma factor RpoS. In *E. coli* Fis controls the expression of rRNAs and tRNAs, which are needed in maximum quantities in fast-growing bacteria. On the other hand, the gene promoters needed for growth are inactivated in stationary phase, and RpoS upregulates the ones that are needed for more stressful conditions (Xu and Johnson 1995).

Fis can affect biofilm formation in both directions: activating or repressing it. In enteroaggregative *Escherichia coli* (EAEC) Fis has been shown to facilitate biofilm formation by regulating the biosynthesis of aggregative adherence fimbriae (AAF/II), which are important for EAEC to be able to form biofilm in the human intestinal mucosa. Fis regulates the transcription of 3 genes required for AAF/II fimbriae synthesis: *aggR* encoding for synthesis activator, *aafD* encoding for chaperon and *aafA* encoding for a subunit of fimbriae (Sheikh *et al.* 2001). The example of repression of biofilm by Fis is *Dickeya dadantii*. The main component of the biofilm matrix of *D. dadantii* is cellulose, produced by *bcs* operon, which transcription is repressed by Fis, therefore inhibiting the formation of biofilm (Prigent-Combaret *et al.* 2012). Fis also represses the synthesis of curli – adhesive fimbriae in enteropathogenic *E. coli* (EPEC) strain E2348/69. Fis inhibits the expression of the main subunit of curli and therefore reduces biofilm formation (Saldana *et al.* 2009). In *P. putida fis* overexpression has been shown to repress the motility of bacteria and enhance biofilm formation (Jakovleva *et al.* 2012, Ainelo *et al.* 2017).

THE AIM OF THE THESIS

This work was initiated by the finding that *fis* overexpression enhances biofilm formation in *P. putida* (Jakovleva *et al.* 2012). The colonization experiments on barley roots demonstrated that *fis*-overexpressing *P. putida* is unable to move on the barley roots, instead the bacteria stay in the region where they were at the beginning of the colonization (Jakovleva *et al.* 2012). Motility being the opposite phenotype of biofilm formation raised a question, is Fis affecting biofilm indirectly or directly via regulating the transcription of biofilm-related genes. To answer the question we decided to study how Fis is taking part of regulation of genes involved in biofilm formation. We found that the gene expression of biofilm-related adhesins LapA and LapF are controlled by Fis. Regarding the circumstance that biofilm-forming cells have more hydrophobic surface, it was only logical to investigate the possibility that LapA and LapF are involved in influencing the cell surface hydrophobicity of *P. putida*. The aim of this work was focused more on studying the Fis regulation mechanism of adhesin-coding gene *lapF*, because of a controversial finding that the absence of *lapF* had no effect on Fis-induced biofilm formation of *P. putida*. Therefore we wanted to elucidate the potential purpose of LapF in the adaptation and survival processes of *P. putida*.

RESULTS AND DISCUSSION

1. The lack of LapF has no effect on *P. putida* biofilm grown in LB medium (Ref I)

To identify the biofilm-related genes that could be affected by Fis, mutagenesis with transposon mini-Tn*5* was conducted with *P. putida fis*-overexpression strain F15. This strain had an extra gene copy of *fis* under the control of *tac* promoter induced by IPTG (Jakovleva *et al.* 2012). Transconjugants with recovered motility were selected as the cells with opposite phenotype to biofilm formation. The improved motility was studied on the King B medium with IPTG in comparison with F15. The localization analysis of 76 colonies with mini-Tn*5* insertions in F15 strain revealed 68 independent hits in the *lap* genes (Ref I). Among them, 56 independent insertions were found in *lapA*, the gene of biggest adhesin in *P. putida*. Whereas no insertions were found in the gene of the second extracellular adhesin – *lapF*. In general, this test-system with mini-Tn5 could not ascertain the biofilm related genes which are repressed by *fis*-overexpression, as gene is already repressed and insertion did not activate genes.

Subsequently, we focused our research on *lap* genes and measured the biofilm formation of these transposon mutants compared with wild-type strain PSm and *fis*-overexpression strain F15. The biofilm formation of three independent *lapA*-transposon mutants in F15 exhibited approximately 2-times weaker biofilm formation compared with original F15 (Ref I, Table 2). To further confirm the *lap*-related biofilm regulation by Fis, we constructed *lapA* as well as *lapF*-deletion mutants of PSm and F15 strains (Ref I, Table 1). We decided to further investigate the biofilm involvement of both biggest adhesins in Fis-overexpression conditions, despite no findings of insertions in the *lapF* gene with transposon mutagenesis. When the deletion of *lapA* reduced biofilm formation in all constructed PSm and F15 mutants compared to wild-type strains, then the elimination of *lapF* had no effect on *P. putida* biofilm formation in LB-medium compared to wild-type PSm (Ref I, Fig 4). Also, the biofilm of the *lapF*-deletion mutant in F15 increased similarly to original F15, which further confirmed that the lack of LapF has no effect in Fis-induced biofilm formation of *P. putida* in LB medium. The results of wild-type strain are in good accordance with previously published data that LapF-deficiency has a negative impact on *P. putida* biofilm formation only in glucose minimal medium but not in complex medium LB (Martinez-Gil *et al.* 2010). Although, it is proposed that LapF is playing a role in cell-cell interactions in mature biofilm (Martinez-Gil *et al.* 2010).

2. Overexpression of *fis* represses the expression of *lapF* (Ref I)

Previous works have shown that *lapF* mutant was less capable of both individual and competitive plant root colonization, thus LapF could be important in the mature biofilm formation and can also contribute to colonization ability (Martinez-Gil *et al.* 2010). Therefore, the expression of *lapF* was assessed as a potential gene that can be involved in adhesion without Fis regulation.

At first, the amounts of adhesines LapA and LapF were quantified with the background of *fis* overexpression in different growth phases of *P. putida*. The cells were grown in LB medium for 2.5h and 18h, and the proteins from cell lysates were separated and quantified using silver stained SDS-polyacrylamide gels. In fast-growing cells, the amount of LapA was not affected and LapF was not detectable at all in wild-type nor *fis*-overexpression strains (Ref I, Fig 5). This corresponds to previously published data, where transcription activity measurements showed no expression of *lapF* in logarithmic growth phase *P. putida* cells (Martinez-Gil *et al.* 2014). In 18-hours-grown cells, the overexpression of *fis* increased LapA 1.6 times, but surprisingly the amount of LapF was decreased approximately 4 times by *fis* overexpression compared to the wild-type cells. This clearly suggests that Fis affects the expression of both adhesins LapA and LapF in *P. putida*. Also, it could indicate, that the mature biofilm of *P. putida* is mainly affected by LapA and the dispersion of wild-type biofilm, without *fis* overexpression, may be due to decreased levels of LapA. However, the results indicating the regulation of *lapF* expression by Fis centered the focus of this thesis to LapF and to the ascertainment of specific control of *lapF*`s transcription by Fis and LapF's role in *P. putida*.

3. Fis binds to the promoter of *lapF* (Ref II)

RpoS-dependency of *lapF*'s transcription was previously published by Martinez-Gil and coworkers (Martinez-Gil *et al.* 2010), although, without the exact position of *lapF*'s promoter. Thus, at first, the location of *lapF*'s promoter was needed to be identified. Therefore, the assumption was to find a recognisable -10 element of the RpoS-dependent promoter upstream of *lapF* gene. We used RACE method to map the *lapF* mRNA 5`ends and found 120 bp upstream of *lapF* starting codon one mRNA 5` end. Six nucleotides upstream of the founded mRNA 5` end we identified a putative -10 promoter sequence (Ref II, Fig 1).

For determination of potential Fis binding sequence(s), we used *in silico* prediction of Fis binding sites on upstream (-500 bp) and downstream (+100 bp) region of the *lapF* gene. Surprisingly no Fis-binding sites were found on an upstream region of *lapF*, but one binding site Fis-F1 was predicted approximately 65 bp downstream of *lapF* starting codon (Ref II, Fig 1). Verification of predicted Fis-binding site was conducted *in vitro* by DNaseI footprint

analysis, which did not confirm the binding of Fis to the predicted Fis-F1 sequence (data not shown). Instead, this assay revealed another Fis-binding site Fis-F2, upstream of the *lapF* gene (Ref II, Fig 1). Attractively the localization of Fis-F2 overlapped a putative -10 promoter sequence, which confirms the hypothesis of the binding of Fis to the promoter of *lapF* and by counteracting the binding of RNA polymerase. Therefore, Fis could directly repress the transcription of *lapF*. Fis binding to the Fis-F2 site was further confirmed by mutating the most critical nucleotides for Fis binding in Fis-F2 sequence. With these mutations, Fis was not able to bind to the Fis-F2-mut sequence *in vitro* (Ref II, Fig 1), which confirms the binding of Fis to the promoter region of *lapF*.

4. The overexpression of *fis* represses the transcription of *lapF* (Ref II)

To elucidate the direct effect of Fis on the transcription of the *lapF* gene, the promoter region of *lapF* was cloned into a promoter probe test system and the activity of *lapF* promoter was measured as β-galactosidase activity *in vivo*, where wild-type (PSm) and *fis*-overexpressing F15 strains were grown into stationary phase. The promoter region of *lapF* containing native Fis-binding site Fis-F2 or mutated version Fis-F2mut was cloned in front of reporter gene *lacZ*. F15 cells harbouring the native Fis-F2 binding site showed a drastic decrease in β-galactosidase activity when *fis* was overexpressed by the addition of 1 mM IPTG (Ref II, Fig 3). When Fis-binding site Fis-F2 was mutated, the over-expression of *fis* did not decrease the β-galactosidase activity anymore, compared with F15 without IPTG (Ref II, Fig 3), indicating the essential nature of this binding sequence for Fis-mediated repression. However, the mutations in Fis-F2 increased the LacZ activity in general. One possible explanation for this could be the introduced mutations in Fis-F2-mut, which diminished Fis binding but also changed the promoter sequence enough to result in higher transcription of *lacZ* gene and therefore β-galactosidase activity. Furthermore the transcription regulation of *lapF* could be carried out mutually by Fis and RpoS. As the trigger of exponential growth, Fis could be the repressor of *lapF* in fast-growing cells and RpoS could be the activator of *lapF* in stationary phase cells. Although this brings up a question of the necessity to repress the expression of *lapF* in bacterial logarithmic growth phase.

5. LapF provides the hydrophobicity to the surface of P. putida cells in stationary growth phase (Ref III)

From our studies, LapF has no *fis*-overexpression related effect on *P. putida* biofilm formation in LB medium, yet Fis still significantly reduces the amount of LapF. Moreover, the adhesin LapA seems to be the primary factor for *P. putida* biofilm formation. While LapA is shown to be important in the early attachment of cells to different types of surfaces, then LapF is described to be more significant in a mature biofilm for cell-cell connections (Fuqua 2010, Martinez-Gil *et al.* 2010). Also, *P. putida* cell surface growing in a biofilm is more hydrophobic (Baumgarten *et al.* 2012, Montag *et al.* 2012, Ruhs *et al.* 2014). Therefore it was intriguing to study the role of adhesins LapA and LapF in the regulation of *P. putida* cell surface hydrophobicity.

The hydrophobicity of the cells was measured as a contact angle between a water droplet and a filter paper covered with cell lawn. To elucidate the effect of adhesins on hydrophobicity, we measured the contact angles of deletion mutants of PSm Δ*lapA* and PSm Δ*lapF* strains grown for 3 h and 18 h in LB medium. As expected, the wild-type cells of stationary phase were more hydrophobic than cells in logarithmic phase (Ref III, Fig 2A), which correlates to the finding, that stressed cells exhibit more hydrophobic surface (Baumgarten *et al.* 2012). The most surprising result was the remarkable decrease in contact angles (from 76° to 47°) of the stationary phase cells lacking *lapF* comparing to wild-type strain PSm (Ref III, Fig 2A). At the same time, the lack of *lapA* did not show any difference in hydrophobicity compared to wild-type cells. Correspondingly, the role of LapA in attachment to surfaces has been shown to be independent of hydrophobicity, as it is capable of binding both hydrophilic and hydrophobic surfaces (Boyd *et al.* 2014).

The finding of LapF`s participation in cell surface hydrophobicity of *P. putida* brought up a potential role of Fis in regulating hydrophobicity via LapF. For verifying that hypothesis, we constructed three types of strains carrying *lapF* promoter area modification in chromosome (Ref III, Fig 1). F15KmFm, where Fis binding site Fis-F2 upstream of the *lapF* gene was mutated, therefore decreasing the direct repression of *lapF* by Fis. PSmlapF3 and F15lapF3, where native promoter region of *lapF* was deleted from the chromosome and replaced by P_{tac} promoter inducible with IPTG for *lapF* overexpression. And third, PSmKm and F15Km, as so-called wild-type control strains for the first two. The contact angle measurements of these strains confirmed that LapF is an important factor for hydrophobicity in stationary phase *P. putida* cells grown in LB-medium (Ref III, Fig 2 and 6). The wild-type originated strain PSmlapF3 is not able to express *lapF* without IPTG (Ref III, Fig 3) and is less hydrophobic than wild-type cells (Ref III, Fig 2). Though, the PSmlapF3 cell surface hydrophobicity is possible to induce by adding IPTG to medium. IPTG triggers *lapF* expression in PSmlapF3 cells, and the cells become more hydrophobic (Ref III, Fig 2C). Additionally, it is possible to induce *lapF* expression as well as the higher hydrophobicity of both logarithmic and stationary phase growing

PSmlapF via IPTG supplementation, when *lapF* is natively downregulated (Ref III, Fig 2).

In *fis* overexpression conditions as with F15-originated strains, it is clear that Fis regulates the hydrophobicity of *P. putida* via regulating the expression of *lapF*. This is in good agreement with our findings by Fis binding to the Fis-F2 site on the promoter of *lapF* and therefore suppressing the transcription from *lapF* gene (Ref II), whereas mutating the Fis-F2 abolishes Fis' repression. This is further confirmed by the contact angle measurement result of F15KmFm in stationary phase, where mutations in Fis-F2 binding site diminishes Fis binding, thus providing *P. putida* cells LapF-mediated hydrophobicity (Ref III, Fig 6). In other words, mutating the Fis-F2 binding site changes the phenotype of the cells, which confirms the involvement of Fis in direct regulation of hydrophobicity of *P. putida* via LapF.

In *S. aureus* a biofilm-associated protein Bap, which carries similar structural features with LapF, has also been shown to be involved in the regulation of hydrophobicity. *S. aureus* cells with a mutation in *bap* gene show decreased surface hydrophobicity compared to wild-type cells and therefore reduced the initial attachment to polystyrene as well as intercellular adhesion (Cucarella *et al.* 2001). In *S. aureus* Bap is involved in several stages of biofilm, starting from initial attachment, whereas LapF has been shown to be important only in mature biofilm (Cucarella *et al.* 2001, Martinez-Gil *et al.* 2010). Moreover, in *S. aureus,* the surface adhesion proteins are usually expressed in stationary and early exponential phase, while in *P. putida* the LapF is only expressed in stationary phase cells. This suggests that the two characteristically similar adhesins in different species have potentially different functions.

5.1. LapF-provided hydrophobicity as a potential defensive factor for *P. putida* (Ref III)

It is known that LapF is not essential for *fis*-induced biofilm in *P. putida* and the main adhesin for biofilm is LapA, which can bind to both hydrophobic and hydrophilic surfaces (Boyd *et al.* 2014, Moor *et al.* 2014). Cells growing in biofilm tend to be more hydrophobic, although the lack of *lapF* in *P. putida* makes cell surface more hydrophilic than wild-type cells, these hydrophilic cells can still form biofilm (Ref II, Ref III). What could be the reason of hydrophobic surface for *P. putida* and is LapF needed for biofilm formation or for something else? To answer these questions, we decided to test the viability of *P. putida* cells with and without *lapF* against hydrophilic and hydrophobic compounds. Methanol as a representative of hydrophilic and 1-octanol as a representative of hydrophobic chemicals was used in different concentrations to assess the effect on *P. putida* wild-type cells and cells lacking *lapF*. Stationary phase LB-grown bacteria, where LapF provides a hydrophobic surface, have more cells with intact membrane compared to cells lacking LapF, when treated with hydrophilic methanol (Ref III, Table 4). Similarly, when lacking *lapF,* there are more cells

with intact membrane when treated with hydrophobic 1-octanol, suggesting that LapF might play a role in defence mechanisms of *P. putida* against hydrophilic compounds. Furthermore, this means that Fis could have an important part in defensive regulation also, as the main repressor of *lapF* in *P. putida*.

P. putida is a rhizospheric bacterium (Lugtenberg and Bloemberg 2004) and therefore has to deal with reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) produced by plant roots (Apel and Hirt 2004, Matilla *et al.* 2007). Plants secrete ROS through their roots into rhizosphere for protection against pathogenic microorganisms. Plant growth promoting bacteria like *P. putida* also have to cope with this. Previously described results with methanol as an example of hydrophilic toxic compound, showed that *P. putida* cells over-expressing *lapF* had more hydrophobic surface and therefore were protected against methanol treatment compared with cells lacking *lapF* (Ref III). Thus these results suggest the possibility that LapF may protect the cell against other hydrophilic toxic compounds like H_2O_2. It has been previously described that the ability of LapF-deficient *P. putida* strain to colonize plant roots has dropped compared to the wild-type strain (Martinez-Gil et al. 2010). Therefore, LapF may also protect the rhizospheric *P. putida* against ROS and facilitate colonization, although, the most essential defensive factors against ROS are oxido-reductases that reduce ROS directly, for example, catalases (Kat), catalase-peroxidases (CP), superoxide dismutases (SOD) (Passardi *et al.* 2007). This possibility enlightens the role of adhesine LapF, not only being involved in mature biofilm formation (Martinez-Gil *et al.* 2010) but also playing a part in situations, where bacteria need to protect themselves against hydrophilic compounds or improve the ability to absorb nutrients from the environment for a better adaptation in changing conditions.

6. Model for the potential regulatory mechanism of LapF and Fis in *P. putida*

Here I propose a potential scheme for Fis-mediated regulation of LapF in *P. putida* cell aggregation and hydrophobicity regulation (Fig 4). Fis is a negative regulator of LapF, which expression is thereby repressed in the exponential growth phase. When cells enter the stationary phase, different stressors including nutrient deficiency start to set in. Stationary phase specific sigma factor RpoS activates and induces the expression of *lapF* as soon as Fis levels start to decrease and RpoS level increases. LapF provides *P. putida* a more hydrophobic surface that can passively protect cells against toxic hydrophilic compounds. When cells are confronted with toxic substances, the hydrophobicity of the cell surface increases and bacteria start to aggregate (Baumgarten *et al.* 2012). In this way, they could reduce the surface area of the cells exposed to the toxic compounds.

The ability of better defense against different kinds of chemicals and compounds found in the rhizosphere helps bacteria to compete with other micro-

organisms, especially plant pathogenic bacteria. In that way, bacteria acquire a better recolonization ability, which could improve the survival of bacteria on plant roots.

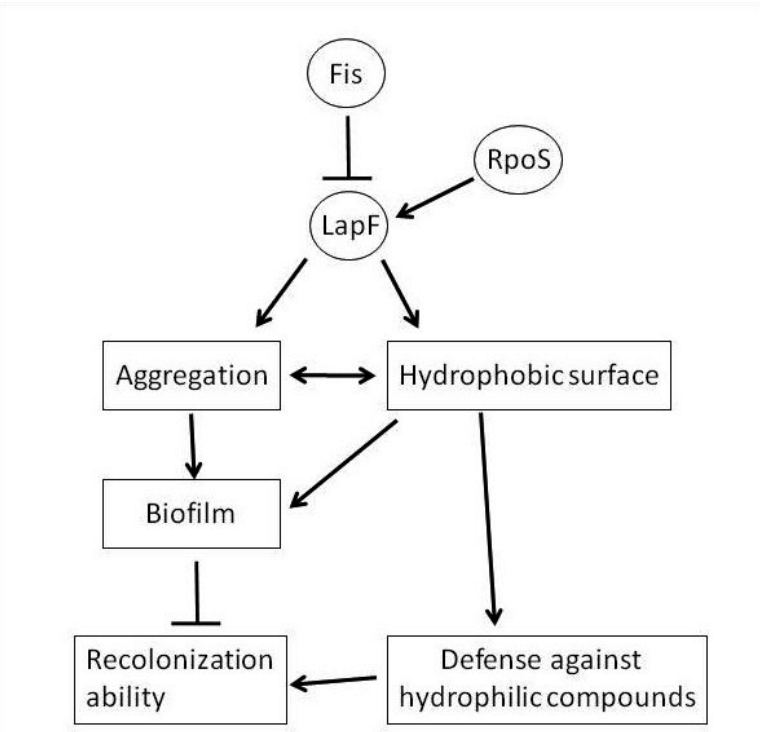

Figure 4. Proposed scheme for Fis-dependent regulation of *P. putida* biofilm formation and hydrophobicity via regulating the expression of the adhesine gene *lapF*. Solid lines with arrows indicate positive regulation and solid lines with blunt ends mark repression.

CONCLUSIONS

Pseudomonas putida is a soil bacterium mainly residing in the rhizosphere of plants. That kind of habitation is strongly dependent on the roots exudates, which include both nutrients and toxic compounds for bacteria. In order to survive and outcompete other microorganisms in the rhizosphere *P. putida* must be able to quickly adapt to the changes in its surrounding environment. For example staying put by forming biofilm or moving freely to recolonize new parts of plant roots for better conditions. Often, such extensive changes involve global transcription regulators, which are able to control the expression of several genes from different categories. Global regulator Fis is previously shown to enhance the biofilm formation of *P. putida* and reduce its recolonization ability on barley roots.

The first contact with environment happens through the surface of bacteria, which has developed the ability to change its composition and properties for better adaptation. One of the mechanisms for this is to alter the hydrophobicity of the cell surface. It can be done via different factors, like lipopolysaccharides, lipoteichoic acids, fimbriae, S-layer proteins or several surface proteins. Prior this work, a surface protein LapF, had only been described as a biofilm-related protein, having a role in cell-cell connections in the mature biofilm. Also, there was no detailed information published about the transcription regulation of *lapF* in *P. putida*. The aim of this research was to study and enlighten the role of LapF in *P. putida,* and it`s transcriptional regulation by global regulator Fis and the potential involvement of Fis and LapF in altering the hydrophobicity of *P. putida*`s surface. The main findings of this work can be summarized as follows:

- The absence of LapF does not affect the ability to form biofilm of *P. putida* in LB medium. This finding is in good accordance with previous work showing the LapF is needed for mature biofilm only in minimal medium and not in LB medium. However, it may suggest that LapF might have additional properties for *P. putida* besides biofilm formation.
- Fis regulates the expression of *lapF*. This thesis is the first to present that Fis binds to the promoter sequence of *lapF* by covering the -10 element of *lapF* promoter and therefore represses the transcription. In *P. putida lapF* is shown to be activated in stationary phase by sigma factor RpoS and no expression of *lapF* is detected in the logarithmic phase. Therefore it seems that Fis and RpoS might act counteractively in regulating the expression of *lapF* – repression by Fis in logarithmic phase and activation by RpoS in stationary phase.
- The stationary phase cells of *P. putida* expressing *lapF* are more hydrophobic than cells without LapF.
- LapF might have a potential role as a protective factor for *P. putida*. More hydrophobic bacteria can be more aggregative and therefore might help the cells reduce the surface exposed to the environment. This can be a passive protection mechanism against toxic organic compounds. The results of

measuring the ability of *P. putida* to tolerate different toxic compounds, like hydrophobic 1-octanol and hydrophilic methanol or hydrogen peroxide in the presence or absence of LapF, might suggest an additional role of LapF in *P. putida*.

SUMMARY IN ESTONIAN

Globaalse regulaatorvalgu Fis-i roll *lapF* geeni ekspressiooni reguleerimisel ja rakupinna hüdrofoobsuse mõjutamisel mullabakteris *Pseudomonas putida*

Bakteritel on pikaaegse evolutsiooni käigus välja kujunenud omadused ja mehhanismid selleks, et olla kohanemisvõimelised ja jääda ellu mitmesugustes keskkonnatingimustes. Vastavalt vajadusele muudavad bakterid oma eluvormi, ujudes vabalt keskkonnas ringi või olles paiksed ja moodustades koos teiste bakteritega biofilmi. Üleminek liikuvalt eluviisilt paiksele algab tavaliselt bakterite kinnitumisega elusale või elutule pinnale.

Üks peamisi bakteriraku pinna omadusi, mis aitab kinnitumisele kaasa, on hüdrofoobsus. Hüdrofoobsema pinnaga rakud agregeeruvad omavahel paremini, tänu millele nad vähendavad eksponeeritavat rakupinda kahjulikele ainetele. Näiteks mullabakter *Pseudomonas putida* muudab oma rakupinda hüdrofoobsemaks kokkupuutel erinevate toksiliste ainetega. Bakterite pinna hüdrofoobsust võivad mõjutada mitmesugused rakumembraaniga seotud faktorid, sh lipopolüsahhariidid, S-kihi valgud ja ka adhesiinvalgud. Nende faktorite olemasolu või puudumine rakupinnal aitab bakteril vastavalt vajadusele suurendada või vähendada oma pinna hüdrofoobsust. See omakorda soodustab keskkonnaga kohanemist ning vajadusel kaitseb rakku toksiliste ainete eest. Suuremahuliste füsioloogiliste muutuste läbiviimiseks on bakteril vaja aktiveerida või maha suruda palju geene, mida viivad läbi globaalsed regulaatorid. Käesolevas töös kirjeldatakse *P. putida* pinna hüdrofoobsufaktorit LapF-i ning selle geeni ekspressiooni regulatsiooni Fis-ga.

P. putida on kosmopoliitne bakter, mis tihti koloniseerib taimejuuri ja soodustab taimede kasvu. Üldiselt mullabakterid eelistavad elada risosfääris, sest taimed eritavad juurte kaudu bakterite jaoks kergesti kasutatavaid süsinikallikaid. Samas, taimed võivad juurte kaudu ümbritsevasse mulda eritada ka toksilisi ühendeid. Seega bakterid peavad risosfääris kohanema nii kasvu soodustavate kui ka pärssivate tingimustega. Näiteks, *P. putida* puhul oleme varem näidanud, et üleekspresseeritud *fis*-i korral jäävad bakterid paikseks, moodustades rohkem biofilmi, ja nende liikumine on pärsitud. Liikumine on omakorda oluline taimejuurte uute osade koloniseerimiseks. Seega, Fis mõjutab oluliselt *P. putida* konkurentsi- ja kohanemisvõimet. Uurides Fis-st sõltuvaid geene, mis osalevad biofilmi reguleerimisel, leidsime, et Fis repressеerib *lapF*-i transkriptsiooni, samas oli teada, et LapF on oluline taime juurte koloniseerimiseks. Võttes arvesse, et biofilmi moodustavad bakterid võivad olla hüdrofoobsemad, huvitas meid just LapF-i potentsiaalne roll *P. putida* pinna hüdrofoobsuse mõjutamisel. Enne käesoleva töö ilmumist ei ole varem avaldatud seoseid LapF-i ja *P. putida* pinna hüdrofoobsuse vahel ega ole ka teada detailsemat informatsiooni *lapF* geeni ekspressiooni regulatsiooni kohta. Saadud tulemused võib kokku võtta järgnevalt:

- LapF-i puudumine ei mõjuta *P. putida* biofilmi moodustamisvõimet toitainerikkas LB-söötmes. Saadud tulemus langeb hästi kokku varem avaldatuga, kus on näidatud, et LapF on oluline ainult minimalssöötmes kasvanud rakkude biofilmi moodustumiseks ning seda biofilmi hilistes etappides.
- Globaalne regulaatorvalk Fis seondub *lapF* geeni promootorile, takistades sellega RNA polümeraasi seondumist ja *lapF*-i transkriptsiooni. Kuna Fis-i hulk on kõrge bakteripopulatsiooni eksponentsiaalses kasvufaasis, siis Fis käitub antagonistlikult statsionaarse sigmafaktori RpoS-ga, mis aktiveerib *lapF*-i transkriptsiooni.
- *P. putida*, mis toodab LapF-i, on statsionaarses kasvufaasis hüdrofoobsem võrreldes rakkudega, kus puudub LapF. LapF-st tulenev hüdrofoobsem rakupind võib omada potentsiaalset kaitsvat rolli *P. putida*'le. LapF-ga *P. putida*'l on soodustatud rakkude agregeerumine, tänu millele vähendatakse toksilistele ainetele kättesaadavat rakupinda. Kirjeldatud kaudne kaitsemehhanism võib olla üks seni avastamata LapF-i rolle mullabakteris *P. putida*.

REFERENCES

Adav SS, Lee DJ, Show KY and Tay JH (2008) Aerobic granular sludge: recent advances. *Biotechnol Adv* **26**(5): 411–423.

Ainelo H, Lahesaare A, Teppo A, Kivisaar M and Teras R (2017) The promoter region of lapA and its transcriptional regulation by Fis in Pseudomonas putida. *PLoS One* **12**(9): e0185482.

Ali Azam T, Iwata A, Nishimura A, Ueda S and Ishihama A (1999) Growth phase-dependent variation in protein composition of the *Escherichia coli* nucleoid. *J Bacteriol* **181**(20): 6361–6370.

Alvarez HM, Silva RA, Cesari AC, Zamit AL, Peressutti SR, Reichelt R, Keller U, Malkus U, Rasch C, Maskow T, Mayer F and Steinbuchel A (2004) Physiological and morphological responses of the soil bacterium *Rhodococcus opacus* strain PD630 to water stress. *FEMS Microbiol Ecol* **50**(2): 75–86.

Amann RI, Ludwig W and Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* **59**(1): 143–169.

Aoki W, Kitahara N, Miura N, Morisaka H, Kuroda K and Ueda M (2012) Profiling of adhesive properties of the agglutinin-like sequence (ALS) protein family, a virulent attribute of *Candida albicans*. *FEMS Immunol Med Microbiol* **65**(1): 121–124.

Apel K and Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* **55**: 373–399.

Asselineau J and Lederer E (1950) Structure of the mycolic acids of Mycobacteria. *Nature* **166**(4227): 782–783.

Azam TA and Ishihama A (1999) Twelve species of the nucleoid-associated protein from *Escherichia coli*. Sequence recognition specificity and DNA binding affinity. *J Biol Chem* **274**(46): 33105–33113.

Ball CA, Osuna R, Ferguson KC and Johnson RC (1992) Dramatic changes in Fis levels upon nutrient upshift in *Escherichia coli*. *J Bacteriol* **174**(24): 8043–8056.

Baraquet C, Murakami K, Parsek MR and Harwood CS (2012) The FleQ protein from *Pseudomonas aeruginosa* functions as both a repressor and an activator to control gene expression from the pel operon promoter in response to c-di-GMP. *Nucleic Acids Res* **40**(15): 7207–7218.

Barkan D, Liu Z, Sacchettini JC and Glickman MS (2009) Mycolic acid cyclopropanation is essential for viability, drug resistance, and cell wall integrity of *Mycobacterium tuberculosis*. *Chem Biol* **16**(5): 499–509.

Baumgarten T, Sperling S, Seifert J, von Bergen M, Steiniger F, Wick LY and Heipieper HJ (2012) Membrane vesicle formation as a multiple-stress response mechanism enhances *Pseudomonas putida* DOT-T1E cell surface hydrophobicity and biofilm formation. *Appl Environ Microbiol* **78**(17): 6217–6224.

Baumgarten T, Vazquez J, Bastisch C, Veron W, Feuilloley MG, Nietzsche S, Wick LY and Heipieper HJ (2012) Alkanols and chlorophenols cause different physiological adaptive responses on the level of cell surface properties and membrane vesicle formation in *Pseudomonas putida* DOT-T1E. *Appl Microbiol Biotechnol* **93**(2): 837–845.

Beach MB and Osuna R (1998) Identification and characterization of the fis operon in enteric bacteria. *J Bacteriol* **180**(22): 5932–5946.

Beaussart A, Alsteens D, El-Kirat-Chatel S, Lipke PN, Kucharikova S, Van Dijck P and Dufrene YF (2012) Single-molecule imaging and functional analysis of Als adhesins

and mannans during *Candida albicans* morphogenesis. *ACS Nano* **6**(12): 10950–10964.
Beveridge TJ, Makin SA, Kadurugamuwa JL and Li Z (1997) Interactions between biofilms and the environment. *FEMS Microbiol Rev* **20**(3–4): 291–303.
Boot HJ, Kolen CP, Andreadaki FJ, Leer RJ and Pouwels PH (1996) The *Lactobacillus acidophilus* S-layer protein gene expression site comprises two consensus promoter sequences, one of which directs transcription of stable mRNA. *J Bacteriol* **178**(18): 5388–5394.
Boswell S, Mathew J, Beach M, Osuna R and Colon W (2004) Variable contributions of tyrosine residues to the structural and spectroscopic properties of the factor for inversion stimulation. *Biochemistry* **43**(10): 2964–2977.
Boyd CD, Smith TJ, El-Kirat-Chatel S, Newell PD, Dufrene YF and O'Toole GA (2014) Structural features of the *Pseudomonas fluorescens* biofilm adhesin LapA required for LapG-dependent cleavage, biofilm formation, and cell surface localization. *J Bacteriol* **196**(15): 2775–2788.
Bradley MD, Beach MB, de Koning AP, Pratt TS and Osuna R (2007) Effects of Fis on *Escherichia coli* gene expression during different growth stages. *Microbiology* **153**(Pt 9): 2922–2940.
Braun BR, Head WS, Wang MX and Johnson AD (2000) Identification and characterization of TUP1-regulated genes in *Candida albicans*. *Genetics* **156**(1): 31–44.
Braun BR and Johnson AD (1997) Control of filament formation in *Candida albicans* by the transcriptional repressor TUP1. *Science* **277**(5322): 105–109.
Browning DF, Beatty CM, Sanstad EA, Gunn KE, Busby SJ and Wolfe AJ (2004) Modulation of CRP-dependent transcription at the *Escherichia coli* acsP2 promoter by nucleoprotein complexes: anti-activation by the nucleoid proteins FIS and IHF. *Mol Microbiol* **51**(1): 241–254.
Caparon MG and Scott JR (1987) Identification of a gene that regulates expression of M protein, the major virulence determinant of group A streptococci. *Proc Natl Acad Sci U S A* **84**(23): 8677–8681.
Corpe WA (1980). Microbial surface components involved in the adsorbtion of microorganisms onto surfaces. Adsorption of Microorganisms to Surfaces. Marshall GBaKC. New York, Wiley-Interscience**:** pp. 105–144.
Costerton JW, Stewart PS and Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. *Science* **284**(5418): 1318–1322.
Courtney HS, Ofek I, Penfound T, Nizet V, Pence MA, Kreikemeyer B, Podbielski A, Hasty DL and Dale JB (2009) Relationship between expression of the family of M proteins and lipoteichoic acid to hydrophobicity and biofilm formation in *Streptococcus pyogenes*. *PLoS One* **4**(1): e4166.
Cucarella C, Solano C, Valle J, Amorena B, Lasa I and Penades JR (2001) Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *J Bacteriol* **183**(9): 2888–2896.
Davey ME and O'Toole G A (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev* **64**(4): 847–867.
de Carvalho C, Fischer MA, Kirsten S, Wurz B, Wick LY and Heipieper HJ (2016) Adaptive response of *Rhodococcus opacus* PWD4 to salt and phenolic stress on the level of mycolic acids. *AMB Express* **6**(1): 66.
Donlan RM and Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* **15**(2): 167–193.

Dorken G, Ferguson GP, French CE and Poon WC (2012) Aggregation by depletion attraction in cultures of bacteria producing exopolysaccharide. *J R Soc Interface* **9**(77): 3490–3502.

Dranginis AM, Rauceo JM, Coronado JE and Lipke PN (2007) A biochemical guide to yeast adhesins: glycoproteins for social and antisocial occasions. *Microbiol Mol Biol Rev* **71**(2): 282–294.

El-Kirat-Chatel S, Boyd CD, O'Toole GA and Dufrêne YF (2014) Single-Molecule Analysis of *Pseudomonas fluorescens* Footprints. *ACS nano* **8**(2): 1690–1698.

Espeland EM and Wetzel RG (2001) Complexation, Stabilization, and UV Photolysis of Extracellular and Surface-Bound Glucosidase and Alkaline Phosphatase: Implications for Biofilm Microbiota. *Microb Ecol* **42**(4): 572–585.

Espinosa-Urgel M, Salido A and Ramos JL (2000) Genetic analysis of functions involved in adhesion of *Pseudomonas putida* to seeds. *J Bacteriol* **182**(9): 2363–2369.

Feldman-Cohen LS, Shao Y, Meinhold D, Miller C, Colon W and Osuna R (2006) Common and variable contributions of Fis residues to high-affinity binding at different DNA sequences. *J Bacteriol* **188**(6): 2081–2095.

Fuqua C (2010) Passing the baton between laps: adhesion and cohesion in *Pseudomonas putida* biofilms. *Mol Microbiol* **77**(3): 533–536.

Garrett TR, Bhakoo M and Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science* **18**(9): 1049–1056.

Gjermansen M, Ragas P, Sternberg C, Molin S and Tolker-Nielsen T (2005) Characterization of starvation-induced dispersion in *Pseudomonas putida* biofilms. *Environ Microbiol* **7**(6): 894–906.

Godwin PG, Choyce A and McCarthy S (2001) The prevalence of MRSA carriage measured over five years in a District General Hospital. *J Hosp Infect* **47**(1): 73–75.

Gonzalez Barrios AF, Zuo R, Hashimoto Y, Yang L, Bentley WE and Wood TK (2006) Autoinducer 2 controls biofilm formation in *Escherichia coli* through a novel motility quorum-sensing regulator (MqsR, B3022). *J Bacteriol* **188**(1): 305–316.

Hancock SP, Ghane T, Cascio D, Rohs R, Di Felice R and Johnson RC (2013) Control of DNA minor groove width and Fis protein binding by the purine 2-amino group. *Nucleic Acids Res* **41**(13): 6750–6760.

Hasty DL, Ofek I, Courtney HS and Doyle RJ (1992) Multiple adhesins of streptococci. *Infect Immun* **60**(6): 2147–2152.

Hawe A, Sutter M and Jiskoot W (2008) Extrinsic fluorescent dyes as tools for protein characterization. *Pharm Res* **25**(7): 1487–1499.

Heipieper HJ, Neumann G, Cornelissen S and Meinhardt F (2007) Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. *Appl Microbiol Biotechnol* **74**(5): 961–973.

Hickman JW and Harwood CS (2008) Identification of FleQ from *Pseudomonas aeruginosa* as a c-di-GMP-responsive transcription factor. *Mol Microbiol* **69**(2): 376–389.

Higashi JM, Wang IW, Shlaes DM, Anderson JM and Marchant RE (1998) Adhesion of *Staphylococcus epidermidis* and transposon mutant strains to hydrophobic polyethylene. *J Biomed Mater Res* **39**(3): 341–350.

Hinsa SM, Espinosa-Urgel M, Ramos JL and O'Toole GA (2003) Transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas fluorescens* WCS365 requires an ABC transporter and a large secreted protein. *Mol Microbiol* **49**(4): 905–918.

Horsburgh MJ, Aish JL, White IJ, Shaw L, Lithgow JK and Foster SJ (2002) sigmaB modulates virulence determinant expression and stress resistance: characterization of a functional rsbU strain derived from *Staphylococcus aureus* 8325–4. *J Bacteriol* **184**(19): 5457–5467.

Huertas-Rosales O, Romero M, Heeb S, Espinosa-Urgel M, Camara M and Ramos-Gonzalez MI (2017) The *Pseudomonas putida* CsrA/RsmA homologues negatively affect c-di-GMP pools and biofilm formation through the GGDEF/EAL response regulator CfcR. *Environ Microbiol* **19**(9): 3551–3566.

Isberg RR and Barnes P (2002) Dancing with the host; flow-dependent bacterial adhesion. *Cell* **110**(1): 1–4.

Ishihama A (2009) The Nucleoid: an Overview. *EcoSal Plus* **3**(2).

Jakovleva J, Teppo A, Velts A, Saumaa S, Moor H, Kivisaar M and Teras R (2012) Fis regulates the competitiveness of *Pseudomonas putida* on barley roots by inducing biofilm formation. *Microbiology* **158**(Pt 3): 708–720.

Jefferson KK (2004) What drives bacteria to produce a biofilm? *FEMS Microbiol Lett* **236**(2): 163–173.

Johnson RC, Ball CA, Pfeffer D and Simon MI (1988) Isolation of the gene encoding the Hin recombinational enhancer binding protein. *Proc Natl Acad Sci U S A* **85**(10): 3484–3488.

Kadurugamuwa JL and Beveridge TJ (1995) Virulence factors are released from *Pseudomonas aeruginosa* in association with membrane vesicles during normal growth and exposure to gentamicin: a novel mechanism of enzyme secretion. *J Bacteriol* **177**(14): 3998–4008.

Klein G, Kobylak N, Lindner B, Stupak A and Raina S (2014) Assembly of lipopolysaccharide in *Escherichia coli* requires the essential LapB heat shock protein. *J Biol Chem* **289**(21): 14829–14853.

Kobayashi H, Takami H, Hirayama H, Kobata K, Usami R and Horikoshi K (1999) Outer membrane changes in a toluene-sensitive mutant of toluene-tolerant *Pseudomonas putida* IH-2000. *J Bacteriol* **181**(15): 4493–4498.

Kostrewa D, Granzin J, Koch C, Choe HW, Raghunathan S, Wolf W, Labahn J, Kahmann R and Saenger W (1991) Three-dimensional structure of the *E. coli* DNA-binding protein FIS. *Nature* **349**(6305): 178–180.

Kotiranta A, Haapasalo M, Kari K, Kerosuo E, Olsen I, Sorsa T, Meurman JH and Lounatmaa K (1998) Surface structure, hydrophobicity, phagocytosis, and adherence to matrix proteins of *Bacillus cereus* cells with and without the crystalline surface protein layer. *Infect Immun* **66**(10): 4895–4902.

Kulp A and Kuehn MJ (2010) Biological functions and biogenesis of secreted bacterial outer membrane vesicles. *Annu Rev Microbiol* **64**: 163–184.

Lasa I and Penades JR (2006) Bap: a family of surface proteins involved in biofilm formation. *Res Microbiol* **157**(2): 99–107.

Le Magrex-Debar E, Lemoine J, Gelle MP, Jacquelin LF and Choisy C (2000) Evaluation of biohazards in dehydrated biofilms on foodstuff packaging. *Int J Food Microbiol* **55**(1–3): 239–243.

Li BY, Liang YZ, Hu Y, Du YP, Song YQ and Cui H (2003) Evaluation of gas chromatography-mass spectrometry in conjunction with chemometric resolution for identification of nitrogen compounds in crude oil. *Talanta* **61**(6): 803–809.

Liberati NT, Urbach JM, Miyata S, Lee DG, Drenkard E, Wu G, Villanueva J, Wei T and Ausubel FM (2006) An ordered, nonredundant library of *Pseudomonas*

aeruginosa strain PA14 transposon insertion mutants. *Proc Natl Acad Sci U S A* **103**(8): 2833–2838.
Liu XW, Sheng GP and Yu HQ (2009) Physicochemical characteristics of microbial granules. *Biotechnol Adv* **27**(6): 1061–1070.
Lugtenberg BJJ and Bloemberg GV (2004). Life in the rhizosphere. *Pseudomonas*. Ramos J. New York, Kluwer Academic / Plenum Publishers. **Vol.1** pp. 403- 430.
Lynch JM and Whipps JM (1990) Substrate flow in the rhizosphere. *Plant and Soil* **129**(1): 1–10.
Makin SA and Beveridge TJ (1996) *Pseudomonas aeruginosa* PAO1 ceases to express serotype-specific lipopolysaccharide at 45 degrees C. *J Bacteriol* **178**(11): 3350–3352.
Marti M, Trotonda MP, Tormo-Mas MA, Vergara-Irigaray M, Cheung AL, Lasa I and Penades JR (2010) Extracellular proteases inhibit protein-dependent biofilm formation in *Staphylococcus aureus*. *Microbes Infect* **12**(1): 55–64.
Martinez-Antonio A and Collado-Vides J (2003) Identifying global regulators in transcriptional regulatory networks in bacteria. *Curr Opin Microbiol* **6**(5): 482–489.
Martinez-Gil M, Quesada JM, Ramos-Gonzalez MI, Soriano MI, de Cristobal RE and Espinosa-Urgel M (2013) Interplay between extracellular matrix components of *Pseudomonas putida* biofilms. *Res Microbiol* **164**(5): 382–389.
Martinez-Gil M, Ramos-Gonzalez MI and Espinosa-Urgel M (2014) Roles of cyclic Di-GMP and the Gac system in transcriptional control of the genes coding for the *Pseudomonas putida* adhesins LapA and LapF. *J Bacteriol* **196**(8): 1484–1495.
Martinez-Gil M, Yousef-Coronado F and Espinosa-Urgel M (2010) LapF, the second largest *Pseudomonas putida* protein, contributes to plant root colonization and determines biofilm architecture. *Mol Microbiol* **77**(3): 549–561.
Matilla MA, Espinosa-Urgel M, Rodriguez-Herva JJ, Ramos JL and Ramos-Gonzalez MI (2007) Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. *Genome Biol* **8**(9): R179.
McIver KS, Heath AS, Green BD and Scott JR (1995) Specific binding of the activator Mga to promoter sequences of the emm and scpA genes in the group A streptococcus. *J Bacteriol* **177**(22): 6619–6624.
McIver KS and Scott JR (1997) Role of mga in growth phase regulation of virulence genes of the group A streptococcus. *J Bacteriol* **179**(16): 5178–5187.
McLeod SM, Aiyar SE, Gourse RL and Johnson RC (2002) The C-terminal domains of the RNA polymerase alpha subunits: contact site with Fis and localization during co-activation with CRP at the *Escherichia coli* proP P2 promoter. *J Mol Biol* **316**(3): 517–529.
Mecsas J, Rouviere PE, Erickson JW, Donohue TJ and Gross CA (1993) The activity of sigma E, an *Escherichia coli* heat-inducible sigma-factor, is modulated by expression of outer membrane proteins. *Genes Dev* **7**(12B): 2618–2628.
Mendoza JA, Weinberger KK and Swan MJ (2017) The Hsp60 protein of helicobacter pylori displays chaperone activity under acidic conditions. *Biochem Biophys Rep* **9**: 95–99.
Merickel SK, Sanders ER, Vazquez-Ibar JL and Johnson RC (2002) Subunit exchange and the role of dimer flexibility in DNA binding by the Fis protein. *Biochemistry* **41**(18): 5788–5798.
Monniot C, Boisrame A, Da Costa G, Chauvel M, Sautour M, Bougnoux ME, Bellon-Fontaine MN, Dalle F, d'Enfert C and Richard ML (2013) Rbt1 protein domains

analysis in *Candida albicans* brings insights into hyphal surface modifications and Rbt1 potential role during adhesion and biofilm formation. *PLoS One* **8**(12): e82395.
Montag D, Frant M, Horn H and Liefeith K (2012) Dependence of the initial adhesion of biofilm forming *Pseudomonas putida* mt2 on physico-chemical material properties. *Biofouling* **28**(3): 315–327.
Montville R and Schaffner DW (2003) Inoculum size influences bacterial cross contamination between surfaces. *Appl Environ Microbiol* **69**(12): 7188–7193.
Moor H, Teppo A, Lahesaare A, Kivisaar M and Teras R (2014) Fis overexpression enhances *Pseudomonas putida* biofilm formation by regulating the ratio of LapA and LapF. *Microbiology* **160**(Pt 12): 2681–2693.
Nasser W, Schneider R, Travers A and Muskhelishvili G (2001) CRP modulates fis transcription by alternate formation of activating and repressing nucleoprotein complexes. *J Biol Chem* **276**(21): 17878–17886.
Navazo A, Barahona E, Redondo-Nieto M, Martinez-Granero F, Rivilla R and Martin M (2009) Three independent signalling pathways repress motility in Pseudomonas fluorescens F113. *Microb Biotechnol* **2**(4): 489–498.
Neidhardt FC and Curtiss R (1996) *Escherichia coli* and *Salmonella* : cellular and molecular biology. Washington, D.C., ASM Press.
Neumann G, Cornelissen S, van Breukelen F, Hunger S, Lippold H, Loffhagen N, Wick LY and Heipieper HJ (2006) Energetics and surface properties of *Pseudomonas putida* DOT-T1E in a two-phase fermentation system with 1-decanol as second phase. *Appl Environ Microbiol* **72**(6): 4232–4238.
Ninnemann O, Koch C and Kahmann R (1992) The *E.coli* fis promoter is subject to stringent control and autoregulation. *EMBO J* **11**(3): 1075–1083.
Novick RP (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol Microbiol* **48**(6): 1429–1449.
Ofek I, Whitnack E and Beachey EH (1983) Hydrophobic interactions of group A streptococci with hexadecane droplets. *J Bacteriol* **154**(1): 139–145.
Okada N, Geist RT and Caparon MG (1993) Positive transcriptional control of mry regulates virulence in the group A streptococcus. *Mol Microbiol* **7**(6): 893–903.
Osuna R, Lienau D, Hughes KT and Johnson RC (1995) Sequence, regulation, and functions of fis in *Salmonella typhimurium*. *J Bacteriol* **177**(8): 2021–2032.
Passardi F, Zamocky M, Favet J, Jakopitsch C, Penel C, Obinger C and Dunand C (2007) Phylogenetic distribution of catalase-peroxidases: Are there patches of order in chaos? *Gene* **397**(1–2): 101–113.
Petrova OE and Sauer K (2009) A novel signaling network essential for regulating *Pseudomonas aeruginosa* biofilm development. *PLoS Pathog* **5**(11): e1000668.
Pratt LA and 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.
Pratt TS, Steiner T, Feldman LS, Walker KA and Osuna R (1997) Deletion analysis of the fis promoter region in *Escherichia coli*: antagonistic effects of integration host factor and Fis. *J Bacteriol* **179**(20): 6367–6377.
Prigent-Combaret C, Zghidi-Abouzid O, Effantin G, Lejeune P, Reverchon S and Nasser W (2012) The nucleoid-associated protein Fis directly modulates the synthesis of cellulose, an essential component of pellicle-biofilms in the phytopathogenic bacterium *Dickeya dadantii*. *Mol Microbiol* **86**(1): 172–186.
Radwan SS, Al-Hasan RH, Salamah S and Al-Dabbous S (2002) Bioremediation of oily sea water by bacteria immobilized in biofilms coating macroalgae. *International Biodeterioration & Biodegradation* **50**(1): 55–59.

Raina S, Missiakas D and Georgopoulos C (1995) The *rpoE* gene encoding the sigma E (sigma 24) heat shock sigma factor of *Escherichia coli*. *EMBO J* **14**(5): 1043–1055.

Ramos-Gonzalez MI, Campos MJ and Ramos JL (2005) Analysis of *Pseudomonas putida* KT2440 gene expression in the maize rhizosphere: in vivo [corrected] expression technology capture and identification of root-activated promoters. *J Bacteriol* **187**(12): 4033–4041.

Rao D, Webb JS and Kjelleberg S (2005) Competitive interactions in mixed-species biofilms containing the marine bacterium *Pseudoalteromonas tunicata*. *Appl Environ Microbiol* **71**(4): 1729–1736.

Rosenberg M and Kjelleberg S (1986). Hydrophobic interactions: Role in bacterial adhesion. Advances in Microbial Ecology. Marshall KC. New York, Plenum Press. **9:** pp. 353–393.

Ruhs PA, Bocker L, Inglis RF and Fischer P (2014) Studying bacterial hydrophobicity and biofilm formation at liquid-liquid interfaces through interfacial rheology and pendant drop tensiometry. *Colloids Surf B Biointerfaces* **117**: 174–184.

Saldana Z, Xicohtencatl-Cortes J, Avelino F, Phillips AD, Kaper JB, Puente JL and Giron JA (2009) Synergistic role of curli and cellulose in cell adherence and biofilm formation of attaching and effacing *Escherichia coli* and identification of Fis as a negative regulator of curli. *Environ Microbiol* **11**(4): 992–1006.

Salgado CD, Calfee DP and Farr BM (2003) Interventions to prevent methicillin-resistant *Staphylococcus aureus* transmission in health care facilities: What works? *Clinical Microbiology Newsletter* **25**(18): 137–144.

Santegoeds CM, Ferdelman TG, Muyzer G and de Beer D (1998) Structural and Functional Dynamics of Sulfate-Reducing Populations in Bacterial Biofilms. *Applied and Environmental Microbiology* **64**(10): 3731–3739.

Schneider R, Travers A and Muskhelishvili G (2000) The expression of the *Escherichia coli* fis gene is strongly dependent on the superhelical density of DNA. *Mol Microbiol* **38**(1): 167–175.

Segura A, Duque E, Mosqueda G, Ramos JL and Junker F (1999) Multiple responses of gram-negative bacteria to organic solvents. *Environ Microbiol* **1**(3): 191–198.

Sekoulov I and Brinke-Seiferth S (1999) Application of biofiltration in the crude oil processing industry. *Water Science and Technology* **39**(8): 71–76.

Shao Y, Feldman-Cohen LS and Osuna R (2008) Functional characterization of the *Escherichia coli* Fis-DNA binding sequence. *J Mol Biol* **376**(3): 771–785.

Sheikh J, Hicks S, Dall'Agnol M, Phillips AD and Nataro JP (2001) Roles for Fis and YafK in biofilm formation by enteroaggregative *Escherichia coli*. *Mol Microbiol* **41**(5): 983–997.

Sidhu MS and Olsen I (1997) S-layers of *Bacillus* species. *Microbiology* **143 (Pt 4)**: 1039–1052.

Singh T, Srivastava AK and Arora DK (2002) Horizontal and vertical movement of Pseudomonas fluorescens toward exudate of *Macrophomina phaseolina* in soil: influence of motility and soil properties. *Microbiological Research* **157**(2): 139–148.

Singleton DR, Fidel PL, Jr., Wozniak KL and Hazen KC (2005) Contribution of cell surface hydrophobicity protein 1 (Csh1p) to virulence of hydrophobic *Candida albicans* serotype A cells. *FEMS Microbiol Lett* **244**(2): 373–377.

Sleytr UB, Messner P, Pum D and Sara M (1993) Crystalline bacterial cell surface layers. *Mol Microbiol* **10**(5): 911–916.

Sleytr UB, SÁRa M, Messner P and Pum D (1994) Application Potential of 2D Protein Crystals (S-Layers)a. *Annals of the New York Academy of Sciences* **745**(1): 261–269.

Smit E, Oling F, Demel R, Martinez B and Pouwels PH (2001) The S-layer protein of *Lactobacillus acidophilus* ATCC 4356: identification and characterisation of domains responsible for S-protein assembly and cell wall binding. *J Mol Biol* **305**(2): 245–257.

Smolke C (2009) The Metabolic Pathway Engineering Handbook: Fundamentals, CRC Press.

Snyder WB, Davis LJ, Danese PN, Cosma CL and Silhavy TJ (1995) Overproduction of NlpE, a new outer membrane lipoprotein, suppresses the toxicity of periplasmic LacZ by activation of the Cpx signal transduction pathway. *J Bacteriol* **177**(15): 4216–4223.

Soni KA, Balasubramanian AK, Beskok A and Pillai SD (2008) Zeta potential of selected bacteria in drinking water when dead, starved, or exposed to minimal and rich culture media. *Curr Microbiol* **56**(1): 93–97.

Stewart PS and Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. *Lancet* **358**(9276): 135–138.

Strauch KL and Beckwith J (1988) An *Escherichia coli* mutation preventing degradation of abnormal periplasmic proteins. *Proc Natl Acad Sci U S A* **85**(5): 1576–1580.

Zahringer U, Knirel YA, Lindner B, Helbig JH, Sonesson A, Marre R and Rietschel ET (1995) The lipopolysaccharide of *Legionella pneumophila* serogroup 1 (strain Philadelphia 1): chemical structure and biological significance. *Prog Clin Biol Res* **392**: 113–139.

Teras R, Jakovleva J and Kivisaar M (2009) Fis negatively affects binding of Tn*4652* transposase by out-competing IHF from the left end of Tn*4652*. *Microbiology* **155**(Pt 4): 1203–1214.

Tong H, Chen W, Shi W, Qi F and Dong X (2008) SO-LAAO, a novel L-amino acid oxidase that enables *Streptococcus oligofermentans* to outcompete *Streptococcus mutans* by generating H2O2 from peptone. *J Bacteriol* **190**(13): 4716–4721.

Tormo MA, Knecht E, Gotz F, Lasa I and Penades JR (2005) Bap-dependent biofilm formation by pathogenic species of *Staphylococcus*: evidence of horizontal gene transfer? *Microbiology* **151**(Pt 7): 2465–2475.

Tormo MA, Ubeda C, Marti M, Maiques E, Cucarella C, Valle J, Foster TJ, Lasa I and Penades JR (2007) Phase-variable expression of the biofilm-associated protein (Bap) in *Staphylococcus aureus*. *Microbiology* **153**(Pt 6): 1702–1710.

Torres S, Pandey A and Castro GR (2011) Organic solvent adaptation of Gram positive bacteria: applications and biotechnological potentials. *Biotechnol Adv* **29**(4): 442–452.

Travers A, Schneider R and Muskhelishvili G (2001) DNA supercoiling and transcription in *Escherichia coli*: The FIS connection. *Biochimie* **83**(2): 213–217.

van Loosdrecht MC, Lyklema J, Norde W, Schraa G and Zehnder AJ (1987) Electrophoretic mobility and hydrophobicity as a measured to predict the initial steps of bacterial adhesion. *Applied and Environmental Microbiology* **53**(8): 1898–1901.

Wang H, Liu B, Wang Q and Wang L (2013) Genome-Wide Analysis of the *Salmonella* Fis Regulon and Its Regulatory Mechanism on Pathogenicity Islands. *PLOS ONE* **8**(5): e64688.

Wyckoff TJ, Raetz CR and Jackman JE (1998) Antibacterial and anti-inflammatory agents that target endotoxin. *Trends Microbiol* **6**(4): 154–159.

Xu J and Johnson RC (1995) Identification of genes negatively regulated by Fis: Fis and RpoS comodulate growth-phase-dependent gene expression in *Escherichia coli*. *J Bacteriol* **177**(4): 938–947.

Yeung AT, Torfs EC, Jamshidi F, Bains M, Wiegand I, Hancock RE and Overhage J (2009) Swarming of *Pseudomonas aeruginosa* is controlled by a broad spectrum of transcriptional regulators, including MetR. *J Bacteriol* **191**(18): 5592–5602.

Yousef F and Espinosa-Urgel M (2007) In silico analysis of large microbial surface proteins. *Res Microbiol* **158**(6): 545–550.

Yousefi Rad A, Ayhan H and Piskin E (1998) Adhesion of different bacterial strains to low-temperature plasma-treated sutures. *J Biomed Mater Res* **41**(3): 349–358.

Yuan HS, Finkel SE, Feng JA, Kaczor-Grzeskowiak M, Johnson RC and Dickerson RE (1991) The molecular structure of wild-type and a mutant Fis protein: relationship between mutational changes and recombinational enhancer function or DNA binding. *Proc Natl Acad Sci U S A* **88**(21): 9558–9562.

ACKNOWLEDGEMENTS

First of all I would like to thank my supervisor and mentor Riho for all the great years that I`ve worked and studied in his research group. I sincerely thank you for all the help, support and knowledge you`ve given me and especially for believing in me and motivating me with your clever tricks. You are a great teacher!

I am grateful to have been a part of Fis-research group, with all the great people over the years: Julia, Anna, Annika, Hanna, Jana, Pavel and Johana – thank you all for the help and team-work. Extra thanks go to Hanna, for taking some time from your busy schedule to read my thesis and give me greatly useful tips and comments. And Annika, believe me, if I can do it, you can!

I am also thankful to all the people in department of genetics for creating a friendly atmosphere. People from room 111: Julia, Tanja, Kärt, Tanel, Signe – thank you for the music! My fellow students from room 106: Andres, Hedvig, Kadi, Karl, Mari – thank you for the humour. I am grateful for all the advice from Maia, Rita, Heili and all the other brilliant scientists from genetics department. And special thanks to Julia and Hedvig for both of your theses, they have been a huge help for me.

I also truly appreciate the help from Dimitri Lubenets with FACS analysis and Annely Kukk, Ene Põldroos, Milvi Siniroht and Mart Roos for helping with other technical and administrative issues.

Margus Leppik, my gratitude to you for useful corrections and comments on my thesis. And also thank you for all the help in isotope measurements.

My greatest thanks also go to Hermann from UFZ Leipzig for temporarily taking me into your research group and giving me a great opportunity for collaboration and working in a lab abroad. Also many thanks to all the other people from UFZ lab, I really appreciate the help and advice with my experiments.

Last but not least, I want to thank my family. My parents and grandparents for all the support over the years. My big brother for all the awesome music that you`ve shared with me, which has helped me relax and reduce stress. And Kirsika, words can't describe how thankful I am to you for being there on my side. I couldn`t have done it without you!

PUBLICATIONS

CURRICULUM VITAE

Name: Andrio Lahesaare
Date of Birth: February 18, 1986
Citizenship: Estonian
Contact: Veerenni 53a, Tallinn, Estonia
andrio.lahesaare@gmail.com
+372 53982871

Education:

Since 2011	University of Tartu, PhD student (molecular and cell biology);
2009–2011	University of Tartu, MSc (gene technology);
2005–2009	University of Tartu, BSc (gene technology);
1993–2005	Pärnu Koidula Gymnasium

Language skills:
Estonian, English; Russian in intermediate level

Research interests:
Global transcription regulator Fis and its role in regulating the adaptation mechanisms of soil bacterium *Pseudomonas putida*.

List of publications:
1. Moor H, Teppo A, Lahesaare A, Kivisaar M, Teras R. Fis overexpression enhances *Pseudomonas putida* biofilm formation by regulating the ratio of LapA and LapF. Microbiology. 2014 Dec. 160(Pt 12): 2681–93.
2. Lahesaare A, Moor H, Kivisaar M, Teras R. *Pseudomonas putida* Fis binds to the *lapF* promoter *in vitro* and represses the expression of LapF. PloS ONE. 2014 Dec. 9(12):e115901.
3. Lahesaare A, Ainelo H, Teppo A, Kivisaar M, Heipieper HJ, Teras R. LapF and its regulation by Fis affect the cell surface hydrophobicity of *Pseudomonas putida*. PloS ONE. 2016 Nov. 11(11):e0166078.
4. Ainelo H, Lahesaare A, Teppo A, Kivisaar M, Teras R. The promoter region of *lapA* and its transcriptional regulation by Fis in *Pseudomonas putida*. PloS ONE. 2017 Sep. 12(9):e0185482.

Scholarships and research grants:
1. FEMS Research Grant, 2014 and 2015
2. Kristjan Jaak Scholarship, 2015
3. Olev and Talvi Maimets scholarship, 2016

Other organisational and professional activities:
1. Supervisor of Practical Course in Genetics, 2012–2014
2. Member of Estonian Society for Microbiologists, since 2013
3. Participated in several international conferences

ELULOOKIRJELDUS

Nimi: Andrio Lahesaare
Sünniaeg: 18. veebruar 1986
Kodakondsus: Eesti
Kontaktandmed: Veerenni 53a, Tallinn, Eesti
andrio.lahesaare@gmail.com
+372 53982871

Hariduskäik:

Alates 2011	Tartu Ülikool, doktorantuur (molekulaar- ja rakubioloogia);
2009–2011	Tartu Ülikool, MSc (geenitehnoloogia);
2005–2009	Tartu Ülikool, BSc (geenitehnoloogia);
1993–2005	Pärnu Koidula Gümnaasium

Keeleoskus:
Eesti ja inglise keel; vene keel kesktasemel

Teadustegevus:
Globaalse regulaatorvalgu Fis roll keskkonnatingimustega kohanemiseks vajalike mehhanismide reguleerimisel mullabakteris *Pseudomonas putida*.

Publikatsioonide loetelu:
1. Moor H, Teppo A, Lahesaare A, Kivisaar M, Teras R. Fis overexpression enhances *Pseudomonas putida* biofilm formation by regulating the ratio of LapA and LapF. Microbiology. 2014 Dec. 160(Pt 12): 2681–93.
2. Lahesaare A, Moor H, Kivisaar M, Teras R. *Pseudomonas putida* Fis binds to the *lapF* promoter *in vitro* and represses the expression of LapF. PloS ONE. 2014 Dec. 9(12):e115901.
3. Lahesaare A, Ainelo H, Teppo A, Kivisaar M, Heipieper HJ, Teras R. LapF and its regulation by Fis affect the cell surface hydrophobicity of *Pseudomonas putida*. PloS ONE. 2016 Nov. 11(11):e0166078.
4. Ainelo H, Lahesaare A, Teppo A, Kivisaar M, Teras R. The promoter region of *lapA* and its transcriptional regulation by Fis in *Pseudomonas putida*. PloS ONE. 2017 Sep. 12(9):e0185482.

Saadud uurimistoetused ja stipendiumid:
1. FEMS`i noore teadlase stipendium, 2014 ja 2015
2. Kristjan Jaagu välissõidu stipendium, 2015
3. Olev ja Talvi Maimetsa stipendium, 2016

Muu teaduslik organisatsiooniline ja erialane tegevus:
1. Geneetika praktikumi juhendamine, 2012–2014
2. Eesti Mikrobioloogide ühenduse (EMÜ) liige, alates 2013
3. Osalemine mitmetel rahvusvahelistel konverentsidel

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro.* Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous grassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan**. Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu**. Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.

42. **Veljo Kisand**. Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa**. Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa**. Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik**. Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo**. Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo**. Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots**. Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero**. Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees**. Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks**. Cholecystokinin (CCK) – induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and serotonin. Tartu, 1999, 123 p.
52. **Ebe Sild**. Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva**. Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna**. Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro**. Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane**. Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm**. Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg**. Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild**. The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.
60. **Niilo Kaldalu**. Studies of the TOL plasmid transcription factor XylS. Tartu, 2000, 88 p.
61. **Dina Lepik**. Modulation of viral DNA replication by tumor suppressor protein p53. Tartu, 2000, 106 p.

62. **Kai Vellak**. Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu, 2000, 122 p.
63. **Jonne Kotta**. Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu, 2000, 160 p.
64. **Georg Martin**. Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000, 139 p.
65. **Silvia Sepp**. Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira**. On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000, 96 p.
67. **Priit Zingel**. The role of planktonic ciliates in lake ecosystems. Tartu, 2001, 111 p.
68. **Tiit Teder**. Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu, 2001, 122 p.
69. **Hannes Kollist**. Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu, 2001, 80 p.
70. **Reet Marits**. Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu, 2001, 112 p.
71. **Vallo Tilgar**. Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002, 126 p.
72. **Rita Hõrak**. Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002, 108 p.
73. **Liina Eek-Piirsoo**. The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002, 74 p.
74. **Krõõt Aasamaa**. Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002, 110 p.
75. **Nele Ingerpuu**. Bryophyte diversity and vascular plants. Tartu, 2002, 112 p.
76. **Neeme Tõnisson**. Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002, 124 p.
77. **Margus Pensa**. Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003, 110 p.
78. **Asko Lõhmus**. Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003, 168 p.
79. **Viljar Jaks**. p53 – a switch in cellular circuit. Tartu, 2003, 160 p.
80. **Jaana Männik**. Characterization and genetic studies of four ATP-binding cassette (ABC) transporters. Tartu, 2003, 140 p.
81. **Marek Sammul**. Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003, 159 p
82. **Ivar Ilves**. Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003, 89 p.

83. **Andres Männik**. Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003, 109 p.
84. **Ivika Ostonen**. Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu, 2003, 158 p.
85. **Gudrun Veldre**. Somatic status of 12–15-year-old Tartu schoolchildren. Tartu, 2003, 199 p.
86. **Ülo Väli**. The greater spotted eagle *Aquila clanga* and the lesser spotted eagle *A. pomarina*: taxonomy, phylogeography and ecology. Tartu, 2004, 159 p.
87. **Aare Abroi**. The determinants for the native activities of the bovine papillomavirus type 1 E2 protein are separable. Tartu, 2004, 135 p.
88. **Tiina Kahre**. Cystic fibrosis in Estonia. Tartu, 2004, 116 p.
89. **Helen Orav-Kotta**. Habitat choice and feeding activity of benthic suspension feeders and mesograzers in the northern Baltic Sea. Tartu, 2004, 117 p.
90. **Maarja Öpik**. Diversity of arbuscular mycorrhizal fungi in the roots of perennial plants and their effect on plant performance. Tartu, 2004, 175 p.
91. **Kadri Tali**. Species structure of *Neotinea ustulata*. Tartu, 2004, 109 p.
92. **Kristiina Tambets**. Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: a phylogeographic approach. Tartu, 2004, 163 p.
93. **Arvi Jõers**. Regulation of p53-dependent transcription. Tartu, 2004, 103 p.
94. **Lilian Kadaja**. Studies on modulation of the activity of tumor suppressor protein p53. Tartu, 2004, 103 p.
95. **Jaak Truu**. Oil shale industry wastewater: impact on river microbial community and possibilities for bioremediation. Tartu, 2004, 128 p.
96. **Maire Peters**. Natural horizontal transfer of the *pheBA* operon. Tartu, 2004, 105 p.
97. **Ülo Maiväli**. Studies on the structure-function relationship of the bacterial ribosome. Tartu, 2004, 130 p.
98. **Merit Otsus**. Plant community regeneration and species diversity in dry calcareous grasslands. Tartu, 2004, 103 p.
99. **Mikk Heidemaa**. Systematic studies on sawflies of the genera *Dolerus, Empria,* and *Caliroa* (Hymenoptera: Tenthredinidae). Tartu, 2004, 167 p.
100. **Ilmar Tõnno**. The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N_2 fixation in some Estonian lakes. Tartu, 2004, 111 p.
101. **Lauri Saks**. Immune function, parasites, and carotenoid-based ornaments in greenfinches. Tartu, 2004, 144 p.
102. **Siiri Rootsi**. Human Y-chromosomal variation in European populations. Tartu, 2004, 142 p.
103. **Eve Vedler**. Structure of the 2,4-dichloro-phenoxyacetic acid-degradative plasmid pEST4011. Tartu, 2005. 106 p.

104. **Andres Tover**. Regulation of transcription of the phenol degradation *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 126 p.
105. **Helen Udras**. Hexose kinases and glucose transport in the yeast *Hansenula polymorpha*. Tartu, 2005, 100 p.
106. **Ave Suija**. Lichens and lichenicolous fungi in Estonia: diversity, distribution patterns, taxonomy. Tartu, 2005, 162 p.
107. **Piret Lõhmus**. Forest lichens and their substrata in Estonia. Tartu, 2005, 162 p.
108. **Inga Lips**. Abiotic factors controlling the cyanobacterial bloom occurrence in the Gulf of Finland. Tartu, 2005, 156 p.
109. **Kaasik, Krista**. Circadian clock genes in mammalian clockwork, metabolism and behaviour. Tartu, 2005, 121 p.
110. **Juhan Javoiš**. The effects of experience on host acceptance in ovipositing moths. Tartu, 2005, 112 p.
111. **Tiina Sedman**. Characterization of the yeast *Saccharomyces cerevisiae* mitochondrial DNA helicase Hmi1. Tartu, 2005, 103 p.
112. **Ruth Aguraiuja**. Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Tartu, 2005, 112 p.
113. **Riho Teras**. Regulation of transcription from the fusion promoters generated by transposition of Tn*4652* into the upstream region of *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 106 p.
114. **Mait Metspalu**. Through the course of prehistory in india: tracing the mtDNA trail. Tartu, 2005, 138 p.
115. **Elin Lõhmussaar**. The comparative patterns of linkage disequilibrium in European populations and its implication for genetic association studies. Tartu, 2006, 124 p.
116. **Priit Kupper**. Hydraulic and environmental limitations to leaf water relations in trees with respect to canopy position. Tartu, 2006, 126 p.
117. **Heili Ilves**. Stress-induced transposition of Tn*4652* in *Pseudomonas Putida.* Tartu, 2006, 120 p.
118. **Silja Kuusk**. Biochemical properties of Hmi1p, a DNA helicase from *Saccharomyces cerevisiae* mitochondria. Tartu, 2006, 126 p.
119. **Kersti Püssa**. Forest edges on medium resolution landsat thematic mapper satellite images. Tartu, 2006, 90 p.
120. **Lea Tummeleht**. Physiological condition and immune function in great tits (*Parus major* l.): Sources of variation and trade-offs in relation to growth. Tartu, 2006, 94 p.
121. **Toomas Esperk**. Larval instar as a key element of insect growth schedules. Tartu, 2006, 186 p.
122. **Harri Valdmann**. Lynx (*Lynx lynx*) and wolf (*Canis lupus*) in the Baltic region: Diets, helminth parasites and genetic variation. Tartu, 2006. 102 p.
123. **Priit Jõers**. Studies of the mitochondrial helicase Hmi1p in *Candida albicans* and *Saccharomyces cerevisia*. Tartu, 2006. 113 p.
124. **Kersti Lilleväli**. Gata3 and Gata2 in inner ear development. Tartu, 2007, 123 p.

125. **Kai Rünk**. Comparative ecology of three fern species: *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *D. expansa* (C. Presl) Fraser-Jenkins & Jermy and *D. dilatata* (Hoffm.) A. Gray (Dryopteridaceae). Tartu, 2007, 143 p.
126. **Aveliina Helm**. Formation and persistence of dry grassland diversity: role of human history and landscape structure. Tartu, 2007, 89 p.
127. **Leho Tedersoo**. Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Tartu, 2007, 233 p.
128. **Marko Mägi**. The habitat-related variation of reproductive performance of great tits in a deciduous-coniferous forest mosaic: looking for causes and consequences. Tartu, 2007, 135 p.
129. **Valeria Lulla**. Replication strategies and applications of Semliki Forest virus. Tartu, 2007, 109 p.
130. **Ülle Reier**. Estonian threatened vascular plant species: causes of rarity and conservation. Tartu, 2007, 79 p.
131. **Inga Jüriado**. Diversity of lichen species in Estonia: influence of regional and local factors. Tartu, 2007, 171 p.
132. **Tatjana Krama**. Mobbing behaviour in birds: costs and reciprocity based cooperation. Tartu, 2007, 112 p.
133. **Signe Saumaa**. The role of DNA mismatch repair and oxidative DNA damage defense systems in avoidance of stationary phase mutations in *Pseudomonas putida.* Tartu, 2007, 172 p.
134. **Reedik Mägi**. The linkage disequilibrium and the selection of genetic markers for association studies in european populations. Tartu, 2007, 96 p.
135. **Priit Kilgas**. Blood parameters as indicators of physiological condition and skeletal development in great tits (*Parus major*): natural variation and application in the reproductive ecology of birds. Tartu, 2007, 129 p.
136. **Anu Albert**. The role of water salinity in structuring eastern Baltic coastal fish communities. Tartu, 2007, 95 p.
137. **Kärt Padari**. Protein transduction mechanisms of transportans. Tartu, 2008, 128 p.
138. **Siiri-Lii Sandre**. Selective forces on larval colouration in a moth. Tartu, 2008, 125 p.
139. **Ülle Jõgar**. Conservation and restoration of semi-natural floodplain meadows and their rare plant species. Tartu, 2008, 99 p.
140. **Lauri Laanisto**. Macroecological approach in vegetation science: generality of ecological relationships at the global scale. Tartu, 2008, 133 p.
141. **Reidar Andreson**. Methods and software for predicting PCR failure rate in large genomes. Tartu, 2008, 105 p.
142. **Birgot Paavel**. Bio-optical properties of turbid lakes. Tartu, 2008, 175 p.
143. **Kaire Torn**. Distribution and ecology of charophytes in the Baltic Sea. Tartu, 2008, 98 p.
144. **Vladimir Vimberg**. Peptide mediated macrolide resistance. Tartu, 2008, 190 p.
145. **Daima Örd**. Studies on the stress-inducible pseudokinase TRB3, a novel inhibitor of transcription factor ATF4. Tartu, 2008, 108 p.

146. **Lauri Saag**. Taxonomic and ecologic problems in the genus *Lepraria* (*Stereocaulaceae*, lichenised *Ascomycota*). Tartu, 2008, 175 p.
147. **Ulvi Karu**. Antioxidant protection, carotenoids and coccidians in greenfinches – assessment of the costs of immune activation and mechanisms of parasite resistance in a passerine with carotenoid-based ornaments. Tartu, 2008, 124 p.
148. **Jaanus Remm**. Tree-cavities in forests: density, characteristics and occupancy by animals. Tartu, 2008, 128 p.
149. **Epp Moks**. Tapeworm parasites *Echinococcus multilocularis* and *E. granulosus* in Estonia: phylogenetic relationships and occurrence in wild carnivores and ungulates. Tartu, 2008, 82 p.
150. **Eve Eensalu**. Acclimation of stomatal structure and function in tree canopy: effect of light and CO_2 concentration. Tartu, 2008, 108 p.
151. **Janne Pullat**. Design, functionlization and application of an *in situ* synthesized oligonucleotide microarray. Tartu, 2008, 108 p.
152. **Marta Putrinš**. Responses of *Pseudomonas putida* to phenol-induced metabolic and stress signals. Tartu, 2008, 142 p.
153. **Marina Semtšenko**. Plant root behaviour: responses to neighbours and physical obstructions. Tartu, 2008, 106 p.
154. **Marge Starast**. Influence of cultivation techniques on productivity and fruit quality of some *Vaccinium* and *Rubus* taxa. Tartu, 2008, 154 p.
155. **Age Tats**. Sequence motifs influencing the efficiency of translation. Tartu, 2009, 104 p.
156. **Radi Tegova**. The role of specialized DNA polymerases in mutagenesis in *Pseudomonas putida.* Tartu, 2009, 124 p.
157. **Tsipe Aavik**. Plant species richness, composition and functional trait pattern in agricultural landscapes – the role of land use intensity and landscape structure. Tartu, 2009, 112 p.
158. **Kaja Kiiver**. Semliki forest virus based vectors and cell lines for studying the replication and interactions of alphaviruses and hepaciviruses. Tartu, 2009, 104 p.
159. **Meelis Kadaja**. Papillomavirus Replication Machinery Induces Genomic Instability in its Host Cell. Tartu, 2009, 126 p.
160. **Pille Hallast**. Human and chimpanzee Luteinizing hormone/Chorionic Gonadotropin beta (*LHB/CGB*) gene clusters: diversity and divergence of young duplicated genes. Tartu, 2009, 168 p.
161. **Ain Vellak**. Spatial and temporal aspects of plant species conservation. Tartu, 2009, 86 p.
162. **Triinu Remmel**. Body size evolution in insects with different colouration strategies: the role of predation risk. Tartu, 2009, 168 p.
163. **Jaana Salujõe**. Zooplankton as the indicator of ecological quality and fish predation in lake ecosystems. Tartu, 2009, 129 p.
164. **Ele Vahtmäe**. Mapping benthic habitat with remote sensing in optically complex coastal environments. Tartu, 2009, 109 p.

165. **Liisa Metsamaa**. Model-based assessment to improve the use of remote sensing in recognition and quantitative mapping of cyanobacteria. Tartu, 2009, 114 p.
166. **Pille Säälik**. The role of endocytosis in the protein transduction by cell-penetrating peptides. Tartu, 2009, 155 p.
167. **Lauri Peil**. Ribosome assembly factors in *Escherichia coli.* Tartu, 2009, 147 p.
168. **Lea Hallik**. Generality and specificity in light harvesting, carbon gain capacity and shade tolerance among plant functional groups. Tartu, 2009, 99 p.
169. **Mariliis Tark**. Mutagenic potential of DNA damage repair and tolerance mechanisms under starvation stress. Tartu, 2009, 191 p.
170. **Riinu Rannap**. Impacts of habitat loss and restoration on amphibian populations. Tartu, 2009, 117 p.
171. **Maarja Adojaan**. Molecular variation of HIV-1 and the use of this knowledge in vaccine development. Tartu, 2009, 95 p.
172. **Signe Altmäe**. Genomics and transcriptomics of human induced ovarian folliculogenesis. Tartu, 2010, 179 p.
173. **Triin Suvi**. Mycorrhizal fungi of native and introduced trees in the Seychelles Islands. Tartu, 2010, 107 p.
174. **Velda Lauringson**. Role of suspension feeding in a brackish-water coastal sea. Tartu, 2010, 123 p.
175. **Eero Talts**. Photosynthetic cyclic electron transport – measurement and variably proton-coupled mechanism. Tartu, 2010, 121 p.
176. **Mari Nelis**. Genetic structure of the Estonian population and genetic distance from other populations of European descent. Tartu, 2010, 97 p.
177. **Kaarel Krjutškov**. Arrayed Primer Extension-2 as a multiplex PCR-based method for nucleic acid variation analysis: method and applications. Tartu, 2010, 129 p.
178. **Egle Köster**. Morphological and genetical variation within species complexes: *Anthyllis vulneraria* s. l. and *Alchemilla vulgaris* (coll.). Tartu, 2010, 101 p.
179. **Erki Õunap**. Systematic studies on the subfamily Sterrhinae (Lepidoptera: Geometridae). Tartu, 2010, 111 p.
180. **Merike Jõesaar**. Diversity of key catabolic genes at degradation of phenol and *p*-cresol in pseudomonads. Tartu, 2010, 125 p.
181. **Kristjan Herkül**. Effects of physical disturbance and habitat-modifying species on sediment properties and benthic communities in the northern Baltic Sea. Tartu, 2010, 123 p.
182. **Arto Pulk**. Studies on bacterial ribosomes by chemical modification approaches. Tartu, 2010, 161 p.
183. **Maria Põllupüü**. Ecological relations of cladocerans in a brackish-water ecosystem. Tartu, 2010, 126 p.
184. **Toomas Silla**. Study of the segregation mechanism of the Bovine Papillomavirus Type 1. Tartu, 2010, 188 p.

185. **Gyaneshwer Chaubey**. The demographic history of India: A perspective based on genetic evidence. Tartu, 2010, 184 p.
186. **Katrin Kepp**. Genes involved in cardiovascular traits: detection of genetic variation in Estonian and Czech populations. Tartu, 2010, 164 p.
187. **Virve Sõber**. The role of biotic interactions in plant reproductive performance. Tartu, 2010, 92 p.
188. **Kersti Kangro**. The response of phytoplankton community to the changes in nutrient loading. Tartu, 2010, 144 p.
189. **Joachim M. Gerhold**. Replication and Recombination of mitochondrial DNA in Yeast. Tartu, 2010, 120 p.
190. **Helen Tammert**. Ecological role of physiological and phylogenetic diversity in aquatic bacterial communities. Tartu, 2010, 140 p.
191. **Elle Rajandu**. Factors determining plant and lichen species diversity and composition in Estonian *Calamagrostis* and *Hepatica* site type forests. Tartu, 2010, 123 p.
192. **Paula Ann Kivistik**. ColR-ColS signalling system and transposition of Tn*4652* in the adaptation of *Pseudomonas putida.* Tartu, 2010, 118 p.
193. **Siim Sõber**. Blood pressure genetics: from candidate genes to genome-wide association studies. Tartu, 2011, 120 p.
194. **Kalle Kipper**. Studies on the role of helix 69 of 23S rRNA in the factor-dependent stages of translation initiation, elongation, and termination. Tartu, 2011, 178 p.
195. **Triinu Siibak**. Effect of antibiotics on ribosome assembly is indirect. Tartu, 2011, 134 p.
196. **Tambet Tõnissoo**. Identification and molecular analysis of the role of guanine nucleotide exchange factor RIC-8 in mouse development and neural function. Tartu, 2011, 110 p.
197. **Helin Räägel**. Multiple faces of cell-penetrating peptides – their intracellular trafficking, stability and endosomal escape during protein transduction. Tartu, 2011, 161 p.
198. **Andres Jaanus**. Phytoplankton in Estonian coastal waters – variability, trends and response to environmental pressures. Tartu, 2011, 157 p.
199. **Tiit Nikopensius**. Genetic predisposition to nonsyndromic orofacial clefts. Tartu, 2011, 152 p.
200. **Signe Värv**. Studies on the mechanisms of RNA polymerase II-dependent transcription elongation. Tartu, 2011, 108 p.
201. **Kristjan Välk**. Gene expression profiling and genome-wide association studies of non-small cell lung cancer. Tartu, 2011, 98 p.
202. **Arno Põllumäe**. Spatio-temporal patterns of native and invasive zooplankton species under changing climate and eutrophication conditions. Tartu, 2011, 153 p.
203. **Egle Tammeleht**. Brown bear (*Ursus arctos*) population structure, demographic processes and variations in diet in northern Eurasia. Tartu, 2011, 143 p.

205. **Teele Jairus**. Species composition and host preference among ectomycorrhizal fungi in Australian and African ecosystems. Tartu, 2011, 106 p.
206. **Kessy Abarenkov**. PlutoF – cloud database and computing services supporting biological research. Tartu, 2011, 125 p.
207. **Marina Grigorova**. Fine-scale genetic variation of follicle-stimulating hormone beta-subunit coding gene (*FSHB*) and its association with reproductive health. Tartu, 2011, 184 p.
208. **Anu Tiitsaar**. The effects of predation risk and habitat history on butterfly communities. Tartu, 2011, 97 p.
209. **Elin Sild**. Oxidative defences in immunoecological context: validation and application of assays for nitric oxide production and oxidative burst in a wild passerine. Tartu, 2011, 105 p.
210. **Irja Saar**. The taxonomy and phylogeny of the genera *Cystoderma* and *Cystodermella* (Agaricales, Fungi). Tartu, 2012, 167 p.
211. **Pauli Saag**. Natural variation in plumage bacterial assemblages in two wild breeding passerines. Tartu, 2012, 113 p.
212. **Aleksei Lulla**. Alphaviral nonstructural protease and its polyprotein substrate: arrangements for the perfect marriage. Tartu, 2012, 143 p.
213. **Mari Järve**. Different genetic perspectives on human history in Europe and the Caucasus: the stories told by uniparental and autosomal markers. Tartu, 2012, 119 p.
214. **Ott Scheler**. The application of tmRNA as a marker molecule in bacterial diagnostics using microarray and biosensor technology. Tartu, 2012, 93 p.
215. **Anna Balikova**. Studies on the functions of tumor-associated mucin-like leukosialin (CD43) in human cancer cells. Tartu, 2012, 129 p.
216. **Triinu Kõressaar**. Improvement of PCR primer design for detection of prokaryotic species. Tartu, 2012, 83 p.
217. **Tuul Sepp**. Hematological health state indices of greenfinches: sources of individual variation and responses to immune system manipulation. Tartu, 2012, 117 p.
218. **Rya Ero**. Modifier view of the bacterial ribosome. Tartu, 2012, 146 p.
219. **Mohammad Bahram**. Biogeography of ectomycorrhizal fungi across different spatial scales. Tartu, 2012, 165 p.
220. **Annely Lorents**. Overcoming the plasma membrane barrier: uptake of amphipathic cell-penetrating peptides induces influx of calcium ions and downstream responses. Tartu, 2012, 113 p.
221. **Katrin Männik**. Exploring the genomics of cognitive impairment: whole-genome SNP genotyping experience in Estonian patients and general population. Tartu, 2012, 171 p.
222. **Marko Prous**. Taxonomy and phylogeny of the sawfly genus *Empria* (Hymenoptera, Tenthredinidae). Tartu, 2012, 192 p.
223. **Triinu Visnapuu**. Levansucrases encoded in the genome of *Pseudomonas syringae* pv. tomato DC3000: heterologous expression, biochemical characterization, mutational analysis and spectrum of polymerization products. Tartu, 2012, 160 p.

224. **Nele Tamberg**. Studies on Semliki Forest virus replication and pathogenesis. Tartu, 2012, 109 p.
225. **Tõnu Esko**. Novel applications of SNP array data in the analysis of the genetic structure of Europeans and in genetic association studies. Tartu, 2012, 149 p.
226. **Timo Arula**. Ecology of early life-history stages of herring *Clupea harengus membras* in the northeastern Baltic Sea. Tartu, 2012, 143 p.
227. **Inga Hiiesalu**. Belowground plant diversity and coexistence patterns in grassland ecosystems. Tartu, 2012, 130 p.
228. **Kadri Koorem**. The influence of abiotic and biotic factors on small-scale plant community patterns and regeneration in boreonemoral forest. Tartu, 2012, 114 p.
229. **Liis Andresen**. Regulation of virulence in plant-pathogenic pectobacteria. Tartu, 2012, 122 p.
230. **Kaupo Kohv**. The direct and indirect effects of management on boreal forest structure and field layer vegetation. Tartu, 2012, 124 p.
231. **Mart Jüssi**. Living on an edge: landlocked seals in changing climate. Tartu, 2012, 114 p.
232. **Riina Klais**. Phytoplankton trends in the Baltic Sea. Tartu, 2012, 136 p.
233. **Rauno Veeroja**. Effects of winter weather, population density and timing of reproduction on life-history traits and population dynamics of moose (*Alces alces*) in Estonia. Tartu, 2012, 92 p.
234. **Marju Keis**. Brown bear (*Ursus arctos*) phylogeography in northern Eurasia. Tartu, 2013, 142 p.
235. **Sergei Põlme**. Biogeography and ecology of *alnus*- associated ectomycorrhizal fungi – from regional to global scale. Tartu, 2013, 90 p.
236. **Liis Usküla**. Placental gene expression in normal and complicated pregnancy. Tartu, 2013, 173 p.
237. **Marko Lõoke**. Studies on DNA replication initiation in *Saccharomyces cerevisiae.* Tartu, 2013, 112 p.
238. **Anne Aan**. Light- and nitrogen-use and biomass allocation along productivity gradients in multilayer plant communities. Tartu, 2013, 127 p.
239. **Heidi Tamm**. Comprehending phylogenetic diversity – case studies in three groups of ascomycetes. Tartu, 2013, 136 p.
240. **Liina Kangur**. High-Pressure Spectroscopy Study of Chromophore-Binding Hydrogen Bonds in Light-Harvesting Complexes of Photosynthetic Bacteria. Tartu, 2013, 150 p.
241. **Margus Leppik**. Substrate specificity of the multisite specific pseudouridine synthase RluD. Tartu, 2013, 111 p.
242. **Lauris Kaplinski**. The application of oligonucleotide hybridization model for PCR and microarray optimization. Tartu, 2013, 103 p.
243. **Merli Pärnoja**. Patterns of macrophyte distribution and productivity in coastal ecosystems: effect of abiotic and biotic forcing. Tartu, 2013, 155 p.
244. **Tõnu Margus**. Distribution and phylogeny of the bacterial translational GTPases and the Mqsr/YgiT regulatory system. Tartu, 2013, 126 p.

245. **Pille Mänd**. Light use capacity and carbon and nitrogen budget of plants: remote assessment and physiological determinants. Tartu, 2013, 128 p.
246. **Mario Plaas**. Animal model of Wolfram Syndrome in mice: behavioural, biochemical and psychopharmacological characterization. Tartu, 2013, 144 p.
247. **Georgi Hudjašov**. Maps of mitochondrial DNA, Y-chromosome and tyrosinase variation in Eurasian and Oceanian populations. Tartu, 2013, 115 p.
248. **Mari Lepik**. Plasticity to light in herbaceous plants and its importance for community structure and diversity. Tartu, 2013, 102 p.
249. **Ede Leppik**. Diversity of lichens in semi-natural habitats of Estonia. Tartu, 2013, 151 p.
250. **Ülle Saks**. Arbuscular mycorrhizal fungal diversity patterns in boreonemoral forest ecosystems. Tartu, 2013, 151 p.
251. **Eneli Oitmaa**. Development of arrayed primer extension microarray assays for molecular diagnostic applications. Tartu, 2013, 147 p.
252. **Jekaterina Jutkina**. The horizontal gene pool for aromatics degradation: bacterial catabolic plasmids of the Baltic Sea aquatic system. Tartu, 2013, 121 p.
253. **Helen Vellau**. Reaction norms for size and age at maturity in insects: rules and exceptions. Tartu, 2014, 132 p.
254. **Randel Kreitsberg**. Using biomarkers in assessment of environmental contamination in fish – new perspectives. Tartu, 2014, 107 p.
255. **Krista Takkis**. Changes in plant species richness and population performance in response to habitat loss and fragmentation.Tartu, 2014, 141 p.
256. **Liina Nagirnaja**. Global and fine-scale genetic determinants of recurrent pregnancy loss. Tartu, 2014, 211 p.
257. **Triin Triisberg**. Factors influencing the re-vegetation of abandoned extracted peatlands in Estonia. Tartu, 2014, 133 p.
258. **Villu Soon**. A phylogenetic revision of the *Chrysis ignita* species group (Hymenoptera: Chrysididae) with emphasis on the northern European fauna. Tartu, 2014, 211 p.
259. **Andrei Nikonov**. RNA-Dependent RNA Polymerase Activity as a Basis for the Detection of Positive-Strand RNA Viruses by Vertebrate Host Cells. Tartu, 2014, 207 p.
260. **Eele Õunapuu-Pikas**. Spatio-temporal variability of leaf hydraulic conductance in woody plants: ecophysiological consequences. Tartu, 2014, 135 p.
261. **Marju Männiste**. Physiological ecology of greenfinches: information content of feathers in relation to immune function and behavior. Tartu, 2014, 121 p.
262. **Katre Kets**. Effects of elevated concentrations of CO_2 and O_3 on leaf photosynthetic parameters in *Populus tremuloides*: diurnal, seasonal and interannual patterns. Tartu, 2014, 115 p.

263. **Külli Lokko**. Seasonal and spatial variability of zoopsammon communities in relation to environmental parameters. Tartu, 2014, 129 p.
264. **Olga Žilina**. Chromosomal microarray analysis as diagnostic tool: Estonian experience. Tartu, 2014, 152 p.
265. **Kertu Lõhmus**. Colonisation ecology of forest-dwelling vascular plants and the conservation value of rural manor parks. Tartu, 2014, 111 p.
266. **Anu Aun**. Mitochondria as integral modulators of cellular signaling. Tartu, 2014, 167 p.
267. **Chandana Basu Mallick**. Genetics of adaptive traits and gender-specific demographic processes in South Asian populations. Tartu, 2014, 160 p.
268. **Riin Tamme**. The relationship between small-scale environmental heterogeneity and plant species diversity. Tartu, 2014, 130 p.
269. **Liina Remm**. Impacts of forest drainage on biodiversity and habitat quality: implications for sustainable management and conservation. Tartu, 2015, 126 p.
270. **Tiina Talve**. Genetic diversity and taxonomy within the genus *Rhinanthus.* Tartu, 2015, 106 p.
271. **Mehis Rohtla**. Otolith sclerochronological studies on migrations, spawning habitat preferences and age of freshwater fishes inhabiting the Baltic Sea. Tartu, 2015, 137 p.
272. **Alexey Reshchikov**. The world fauna of the genus *Lathrolestes* (Hymenoptera, Ichneumonidae). Tartu, 2015, 247 p.
273. **Martin Pook**. Studies on artificial and extracellular matrix protein-rich surfaces as regulators of cell growth and differentiation. Tartu, 2015, 142 p.
274. **Mai Kukumägi**. Factors affecting soil respiration and its components in silver birch and Norway spruce stands. Tartu, 2015, 155 p.
275. **Helen Karu**. Development of ecosystems under human activity in the North-East Estonian industrial region: forests on post-mining sites and bogs. Tartu, 2015, 152 p.
276. **Hedi Peterson**. Exploiting high-throughput data for establishing relationships between genes. Tartu, 2015, 186 p.
277. **Priit Adler**. Analysis and visualisation of large scale microarray data, Tartu, 2015, 126 p.
278. **Aigar Niglas**. Effects of environmental factors on gas exchange in deciduous trees: focus on photosynthetic water-use efficiency. Tartu, 2015, 152 p.
279. **Silja Laht**. Classification and identification of conopeptides using profile hidden Markov models and position-specific scoring matrices. Tartu, 2015, 100 p.
280. **Martin Kesler**. Biological characteristics and restoration of Atlantic salmon *Salmo salar* populations in the Rivers of Northern Estonia. Tartu, 2015, 97 p.
281. **Pratyush Kumar Das**. Biochemical perspective on alphaviral nonstructural protein 2: a tale from multiple domains to enzymatic profiling. Tartu, 2015, 205 p

282. **Priit Palta**. Computational methods for DNA copy number detection. Tartu, 2015, 130 p.
283. **Julia Sidorenko**. Combating DNA damage and maintenance of genome integrity in pseudomonads. Tartu, 2015, 174 p.
284. **Anastasiia Kovtun-Kante**. Charophytes of Estonian inland and coastal waters: distribution and environmental preferences. Tartu, 2015, 97 p.
285. **Ly Lindman**. The ecology of protected butterfly species in Estonia. Tartu, 2015, 171 p.
286. **Jaanis Lodjak**. Association of Insulin-like Growth Factor I and Corticosterone with Nestling Growth and Fledging Success in Wild Passerines. Tartu, 2016, 113 p.
287. **Ann Kraut**. Conservation of Wood-Inhabiting Biodiversity – Semi-Natural Forests as an Opportunity. Tartu, 2016, 141 p.
288. **Tiit Örd**. Functions and regulation of the mammalian pseudokinase TRIB3. Tartu, 2016, 182. p.
289. **Kairi Käiro**. Biological Quality According to Macroinvertebrates in Streams of Estonia (Baltic Ecoregion of Europe): Effects of Human-induced Hydromorphological Changes. Tartu, 2016, 126 p.
290. **Leidi Laurimaa**. *Echinococcus multilocularis* and other zoonotic parasites in Estonian canids. Tartu, 2016, 144 p.
291. **Helerin Margus**. Characterization of cell-penetrating peptide/nucleic acid nanocomplexes and their cell-entry mechanisms. Tartu, 2016, 173 p.
292. **Kadri Runnel**. Fungal targets and tools for forest conservation. Tartu, 2016, 157 p.
293. **Urmo Võsa**. MicroRNAs in disease and health: aberrant regulation in lung cancer and association with genomic variation. Tartu, 2016, 163 p.
294. **Kristina Mäemets-Allas**. Studies on cell growth promoting AKT signaling pathway – a promising anti-cancer drug target. Tartu, 2016, 146 p.
295. **Janeli Viil**. Studies on cellular and molecular mechanisms that drive normal and regenerative processes in the liver and pathological processes in Dupuytren's contracture. Tartu, 2016, 175 p.
296. **Ene Kook**. Genetic diversity and evolution of *Pulmonaria angustifolia* L. and *Myosotis laxa sensu lato* (Boraginaceae). Tartu, 2016, 106 p.
297. **Kadri Peil**. RNA polymerase II-dependent transcription elongation in *Saccharomyces cerevisiae*. Tartu, 2016, 113 p.
298. **Katrin Ruisu**. The role of RIC8A in mouse development and its function in cell-matrix adhesion and actin cytoskeletal organisation. Tartu, 2016, 129 p.
299. **Janely Pae**. Translocation of cell-penetrating peptides across biological membranes and interactions with plasma membrane constituents. Tartu, 2016, 126 p.
300. **Argo Ronk**. Plant diversity patterns across Europe: observed and dark diversity. Tartu, 2016, 153 p.

301. **Kristiina Mark**. Diversification and species delimitation of lichenized fungi in selected groups of the family Parmeliaceae (Ascomycota). Tartu, 2016, 181 p.
302. **Jaak-Albert Metsoja**. Vegetation dynamics in floodplain meadows: influence of mowing and sediment application. Tartu, 2016, 140 p.
303. **Hedvig Tamman**. The GraTA toxin-antitoxin system of *Pseudomonas putida*: regulation and role in stress tolerance. Tartu, 2016, 154 p.
304. **Kadri Pärtel**. Application of ultrastructural and molecular data in the taxonomy of helotialean fungi. Tartu, 2016, 183 p.
305. **Maris Hindrikson**. Grey wolf (*Canis lupus*) populations in Estonia and Europe: genetic diversity, population structure and -processes, and hybridization between wolves and dogs. Tartu, 2016, 121 p.
306. **Polina Degtjarenko**. Impacts of alkaline dust pollution on biodiversity of plants and lichens: from communities to genetic diversity. Tartu, 2016, 126 p.
307. **Liina Pajusalu**. The effect of CO_2 enrichment on net photosynthesis of macrophytes in a brackish water environment. Tartu, 2016, 126 p.
308. **Stoyan Tankov**. Random walks in the stringent response. Tartu, 2016, 94 p.
309. **Liis Leitsalu**. Communicating genomic research results to population-based biobank participants. Tartu, 2016, 158 p.
310. **Richard Meitern**. Redox physiology of wild birds: validation and application of techniques for detecting oxidative stress. Tartu, 2016, 134 p.
311. **Kaie Lokk**. Comparative genome-wide DNA methylation studies of healthy human tissues and non-small cell lung cancer tissue. Tartu, 2016, 127 p.
312. **Mihhail Kurašin**. Processivity of cellulases and chitinases. Tartu, 2017, 132 p.
313. **Carmen Tali**. Scavenger receptors as a target for nucleic acid delivery with peptide vectors. Tartu, 2017, 155 p.
314. **Katarina Oganjan**. Distribution, feeding and habitat of benthic suspension feeders in a shallow coastal sea. Tartu, 2017, 132 p.
315. **Taavi Paal**. Immigration limitation of forest plants into wooded landscape corridors. Tartu, 2017, 145 p.
316. **Kadri Õunap**. The Williams-Beuren syndrome chromosome region protein WBSCR22 is a ribosome biogenesis factor. Tartu, 2017, 135 p.
317. **Riin Tamm**. In-depth analysis of factors affecting variability in thiopurine methyltransferase activity. Tartu, 2017, 170 p.
318. **Keiu Kask**. The role of RIC8A in the development and regulation of mouse nervous system. Tartu, 2017, 184 p.
319. **Tiia Möller**. Mapping and modelling of the spatial distribution of benthic macrovegetation in the NE Baltic Sea with a special focus on the eelgrass *Zostera marina* Linnaeus, 1753. Tartu, 2017, 162 p.
320. **Silva Kasela**. Genetic regulation of gene expression: detection of tissue- and cell type-specific effects. Tartu, 2017, 150 p.

321. **Karmen Süld**. Food habits, parasites and space use of the raccoon dog *Nyctereutes procyonoides*: the role of an alien species as a predator and vector of zoonotic diseases in Estonia. Tartu, 2017, p.
322. **Ragne Oja**. Consequences of supplementary feeding of wild boar – concern for ground-nesting birds and endoparasite infection. Tartu, 2017, 141 p.
323. **Riin Kont**. The acquisition of cellulose chain by a processive cellobiohydrolase. Tartu, 2017, 117 p.
324. **Liis Kasari**. Plant diversity of semi-natural grasslands: drivers, current status and conservation challenges. Tartu, 2017, 141 p.
325. **Sirgi Saar**. Belowground interactions: the roles of plant genetic relatedness, root exudation and soil legacies. Tartu, 2017, 113 p.
326. **Sten Anslan**. Molecular identification of Collembola and their fungal associates. Tartu, 2017, 125 p.
327. **Imre Taal**. Causes of variation in littoral fish communities of the Eastern Baltic Sea: from community structure to individual life histories. Tartu, 2017, 118 p.
328. **Jürgen Jalak**. Dissecting the Mechanism of Enzymatic Degradation of Cellulose Using Low Molecular Weight Model Substrates. Tartu, 2017, 137 p.
329. **Kairi Kiik**. Reproduction and behaviour of the endangered European mink (*Mustela lutreola*) in captivity. Tartu, 2018, 112 p.
330. **Ivan Kuprijanov**. Habitat use and trophic interactions of native and invasive predatory macroinvertebrates in the northern Baltic Sea. Tartu, 2018, 117 p.
331. **Hendrik Meister**. Evolutionary ecology of insect growth: from geographic patterns to biochemical trade-offs. Tartu, 2018, 147 p.
332. **Ilja Gaidutšik.** Irc3 is a mitochondrial branch migration enzyme in *Saccharomyces cerevisiae.* Tartu, 2018, 161 p.
333. **Lena Neuenkamp**. The dynamics of plant and arbuscular mycorrhizal fungal communities in grasslands under changing land use. Tartu, 2018, 241 p.
334. **Laura Kasak.** Genome structural variation modulating the placenta and pregnancy maintenance. Tartu, 2018, 181 p.
335. **Kersti Riibak.** Importance of dispersal limitation in determining dark diversity of plants across spatial scales. Tartu, 2018, 133 p.
336. **Liina Saar.** Dynamics of grassland plant diversity in changing landscapes. Tartu, 2018, 206 p.
337. **Hanna Ainelo.** Fis regulates *Pseudomonas putida* biofilm formation by controlling the expression of *lapA*. Tartu, 2018, 143 p.
338. **Natalia Pervjakova.** Genomic imprinting in complex traits. Tartu, 2018, 176 p.