Supplementary Chapter

Physiological characterization of C. freundii so4 and S. multivorum w15

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Introduction

The genus *Citrobacter* was for first time assigned by Werkman and Gillen (1932). This is a polyphyletic genus that belong to the phylum *Proteobacteria*. They have been recovered from very different environment such as water, sewage and soil (Octavia and Lan 2014). By other hand, the genus *Sphingobacterium* belongs to the phylum *Bacteroidetes*, this phylum was created by Eiko Yabuuchi et al. (1983). *Sphingobacterium* group shows a particular biochemical profile, where the cell wall contains high quantities of sphingophospholipids. Currently, the *Sphingobacterium* genus includes up to 22 species. They have been isolated from several habitats such as diverse soil and compost (Lambiase 2014). *S. multivorum* comes from *multus*, many; and -*vorum*, devouring; *multivorum*, intended to mean “produces acid from many carbohydrates” (Taylor et al. 2012).

**Materiales and methods**

**Physiological characterization**

Staining Gram, transmission electron microscopy, motility assay, determination of optimal rage of temperature, pH and salinity

**Staining Gram**

The gram stained slides were prepared by taking a single colony and a drop of saline solution, the mix was spread on the slide and fixed with the flame. Gram Staining Kit (Sigma-Aldrich, Darmstadt, Germany) was used for the staining, the conventional method was applied, first the application and incubation of the solution and then rinsing with water the order was the following: 1) oxalate crystal violet solution, incubated 1 min; 2) iodine solution, incubated 1 min; 3) decolorizer solution (alcohol/acetona) 20s and 4) safranin solution, incubated 1 min. The slides were observed in an optical microscope 40x and the pictures were taken using 100x objective using immersion oil.

**Transmission electron microscopy**

Bacterial cells were incubated overnight in liquid media at 28 °C at 180 rpm and then 1 µL of the culture was deposited onto carbon copper grid and negatively stained with 2% (w/v) uranyl acetate for 1 min. Cell were observed under a Phillips CM120 electron microscopy (FEI Tecnai G2 Polara, Oregon, USA).
**Phenotypic test**

For identification and confirmation of the two gram negative strains, *C. freundii* so4 and *S. multivorum* w15, was used the GN2 MicroPlate (Gram negative identification). GN2 performs 95 discrete tests simultaneously and provides the “metabolic fingerprint”. The principle of the BIOLOG MicroPlate test is based on reduction of the redox dye tetrAzolium violet by metabolically-active bacterial cells. First, single colonies, of each strain, were chosen from the, sub-cultured on TSA plates, and incubated overnight at 28°C. A homogenous suspension of inoculum was made with GN-GP inoculation fluid (72101) and diluted to 0.001 OD at 590 nm. The inoculum was starved for 2 h at room temperature and then 150 μL of the suspension were added into each well of the GN2 MicroPlate. The microplates were incubated at 28°C and read at 0, 6, 24, 48, 72 and 168 hours with a microtiter plate reader at 590nm (Miller and Rhoden 1991; Holmes et al. 1994).

**Motility assay**

It was assessed by growing the bacteria strains on Motility Test Medium (10g/L pancreatin digest of casein, 3g/L NaCl, 4 g/L meat extract and 4 g/L agar) with triphenyltetrazolium chloride (TCC: 0.5 g/L, Sigma-Aldrich, Darmstadt, Germany). Tetrazolium salt is colourless; as the microorganism grows the dye is reduced to an insoluble red pigment. Motile organisms produce a pink colour that diffuses from the stab line. Organisms that are non-motile produce a red pigment that is confined to the stab line (Kelly and Fulton 1953).

**Determination of optimal rage of temperature, pH and salinity**

Optimal temperature of growth of *C. freundii* so4 and *S. multivorum* w15 was detected by growing the strains on Lennox media (Sigma-Aldrich, Darmstadt, Germany) and monitoring the growth at different temperature (4, 20, 30, 50 and 80 °C). The pH range was verified by growing the bacteria strains on Lennox medium at different pH (3, 4, 5, 7, 9 10), while salt tolerance was tested by growing bacteria strains on Lennox medium at 0, 0.25, 0.5, 1, 4.5, 5, 10 and 20% of NaCl (Sigma-Aldrich, Darmstadt, Germany).
Results of physiological characterization of *C. freundii* so4

*C. freundii* so4 presented gram negative staining and rod shape (Figure S1). The pictures taken of *C. freundii* so4 by electron microscope exhibited the presence of flagella; the results in the soft agar an extended range oxidation of TCC (indicating the displacement in the agar) confirmed the motility capacity of the strain (Figure S2). Strain so4 exhibited to be mesophilic, because it was able to grow in the range temperature from 20°C to 40°C and it did not present growth at 45°C, 60°C and 80°C (Figure S3), while the optimal temperature of growth was around 30°C. In the case of pH tolerance, *C. freundii* so4 was able to grow between a pH of four and nine, being the optimal pH at seven (Figure S4). In the salt tolerance resistance, strains so4 was able to grow from 0 to 1% of NaCl (Figure S5).

Results of physiological characterization of *S. multivorum* w15

*S. multivorum* w15 presented gram-negative with a bacilli shape (Figure S1). The images of *S. multivorum* w15 by electron microscope showed that the strains tended to stay aggregated, even growing in liquid medium. In soft agar presented no motility and oxidation of TCC was mainly done in the surface of the inoculation point (Figure S2). This strain showed mesophilic range of temperature, it was able to grow from 20°C to 30°C, being the optimal at 28 °C (Figure S3), while it did not present growth at 40°C, 45°C, 60°C and 80°C. In the case of pH tolerance, *C. freundii* so4 was able to grow between a pH of five and nine, being the optimal pH at seven (Figure S4). This strain could tolerate until 1% of NaCl (Figure S5).
Figures

A) *C. freundii* so4  

B) *S. multivorum* w15

**Figure S1** Tincion Gram.

(A) *C. freundii* so4  

(B) *S. multivorum* w15

**Figure S1** Electron, micrograph, of degrader bacteria strains in A) *C. freundii* so4, where is observed the production of flagella and B) *S. multivorum* w15, which did not present flagella. The picture were taken with FEI, Tecnai, G2, Polara, electronic, microscope.
**Figure S2 Motility assay.** (A) Image shows inoculation point time; (B) Bacterial strains were incubated aerobically for 24 hours at 28ºC. *S. multivorum w15* did not exhibited motility capacity, while *C. freundii so4* presented motility determined by the red coloration due to the oxidation of the triphenyltetrazolium chloride (TCC: 0.5 g/L).
Temperature range

**A) C. freundii so4**

**B) S. multivorum w15**

Figure S3 Growth response of (A) *C. freundii* so4 and (B) *S. multivorum* w15 at temperature range between 4 to 80°C.

pH range

**A) C. freundii so4**

**A) S. multivorum w15**

Figure S4 Growth response of (A) *C. freundii* so4 and (B) *S. multivorum* w15 at pH range between pH 3 and pH 10.

Salinity gradient

**A) C. freundii so4**

**B) S. multivorum w15**

Figure S5 Growth response of (A) *C. freundii* so4 and (B) *S. multivorum* w15 at salinity range between 0 and 5% of NaCl.
Supplementary Chapter. Physiologically characterization

References


Samenvatting

De uitputting van olie afgeleide energie en de opwarming van de aarde openen de deur naar nieuwe ecologische manieren om energie en producten te verkrijgen. Lignocellulosesubstraten (LCB), zoals tarwestro (WS1), schakelgras (SG), maïs (CS) en suikerriet, vertegenwoordigen uitstekende en goedkope bronnen van koolstof die in waardevolle (energie) verbindingen kunnen worden omgezet. Niettemin is hun toepassing op grote schaal nog steeds beperkt.

Dit proefschrift opent met een inleiding, waarin ik een overzicht geef van de complexiteit van de structuur en samenstelling van LCB en de behoefte aan zeer divers arsenaal aan enzymen dat nodig is voor volledige afbraak. Dit arsenaal zal niet alleen cellulases, hemicellulases en ligninases bevatten, maar ook hulpenzymen. Dit in een poging om de factoren die van invloed zijn op de LCB bioconversie en de grotere uitdagingen te begrijpen, alsmede de toepassing van LCB op industriële schaal. Daarnaast beschrijf ik het mogelijke gebruik van microbiële consortia, in een eco-biotechnologische benadering, voor de afbraak van het inherent recalcitrante LCB. Tenslotte beschrijf ik de relevantie van de interacties binnen geselecteerde afbraakconsortia. Microbiële interacties sturen de stabiliteit en functionaliteit van microbiële gemeenschappen. Het is fundamenteel om grip te hebben op de interacties in de geselecteerde microbiële consortia teneinde een beter ontwerp van LCB-degrader consortia en hun toepassing in de industrie mogelijk te maken.

Vervolgens wordt in hoofdstuk 2 het effect onderzocht van het gebruik van WS1, SG, CS, en tarwestro bij pH 9,0 (WS2), met bosbodem als enig inoculum, voor de selectie van LCB-afbrekende microbiële consortia in sequentiele batchcultures. De uiteindelijke consortia werden fylogenetisch en functioneel onderzocht. PCR-DGGE analyse gaf aan dat de bacteriegemeenschappen stabiliteit bereikten na overdracht 6 in WS1, SG en CS en na overdracht 4 in WS2. Voor schimmelgemeenschappen werd stabiliteit gevonden na overdracht 6 in WS1 en SG en na overdracht 4 voor WS2 en CS. Substraattype, naast pH, was een sleuteldriver - voor de behandelingen met WS – van de bacteriële gemeenschapsstructuren. We vonden een “kern” van stammen in de laatste vier microbiële consortia gevormd door *Sphingobacterium* kitahiroshimense, *Enterobacter amnigenus*, *Raoultella terrigena*, *Pseudomonas putida* en *Stenotrophomonas rhizophila*, evenals de schimmels *Coniochaeta ligniaria* en *Acremonium* sp. Alle stammen in de kern
vertoonden CMC-ase- en xylanase-activiteiten. Het LCB-degradatiepotentieel van de geselecteerde consortia werd bepaald met Fourier-transform infrared-spectroscopie (FT-IR). De resultaten lieten zien dat substraattypen de uiteindelijke samenstelling van de consortia bepaalt; diverse LCB-substraten veroorzaakten een combinatie van consortia, zelfs van een gewone inoculumbron.

In hoofdstuk 3 onderzocht ik het belang van de inoculumbron bij de selectie van microbiële afbraakconsortia door drie verschillende inocula uit bosbodem, kanaalbezinksel en rottend hout en het LCB-tarwestro als enige koolstofbron toe te passen bij de selectie van LCB-abrekkende microbiële consortia. De structuren van de bacteriële en schimmelgemeenschappen van de uiteindelijke geselecteerde consortia vertoonden een duidelijke clusterings langs de inoculumbron en significante verschillen tussen de consortia werden gevonden.

Van hout afkomstige consortia bereikten als eerste stabiliteit, gevolgd door van de bodem afgeleide organismen, waarvan sediment-consortia nooit een stabilisatie van meer dan 50% bereikte. Nauwkeuriger karakterisering van de uiteindelijke samenstelling van de bacteriële gemeenschap, door 16S rRNA gen amplicon sequencing, toonde aan dat de meest voorkomende leden van de gemeenschap een bacteriële kern vormden, gemeenschappelijk tussen de drie uiteindelijke consortia. Deze kern werd gevormd door de soorten *Sphingobacterium*, *Citrobacter*, *Acinetobacter* en *Flavobacterium* of *Chryseobacterium*. Ook schimmelstammen waren specifiek van het consortium en (hoofdstuk 2) *Coniochaeta ligniaria* en *Acremonium* sp. werden geïdentificeerd als sleutelorganismen hierin. Al deze organismen vertoonden hoge LCB transformerende activiteiten. De uiteindelijke consortia verbruikten hemicellulose-, cellulose- en ligninecomponentsubstraat op in grote lijnen vergelijkbare manier. Niettemin presenteerde elk van hen een uniek enzymatisch profiel. Samenvattend lieten de gegevens zien dat de uiteindelijke samenstelling en stabiliteit sterk werden beïnvloed door het initiële inoculum.

Vervolgens onderzocht ik in hoofdstuk 4 het potentieel van het gebruik van zoutmoerasbodem als inoculum voor de productie van microbiële consortia die in staat zijn om tarwestro te gebruiken onder zoute omstandigheden. Dit teneinde industriële condities na te bootsen. Verder heb ik onderzocht hoe het groeisubstraat van invloed was op de samenstelling van de microbiële consortia. Om dit te doen, in het eerste deel van de verrijking, voerde ik de consortia met vers substraat, terwijl ik in het tweede deel van de verrijking
voorverteerd substraat gebruikte. Het voorverteerde substraat veroorzaakte een dramatische verschuiving van de bacteriesamenstelling met een opvallend effect op de schimmelgemeenschappen. Het verse substraat had meer generalistische microbiële consortia, terwijl het voorverteerde meer gespecialiseerde microbiële consortia selecteerde die beter in staat waren om cellulose en lignine af te breken dan de eerdergenoemde. Ik identificeerde de lytische activiteit in de belangrijkste cultureerbare degrader bacteriën en schimmels, waarbij de meest dominante bacteriën in de consortia Joostella marina, Flavobacterium beibuense, Algoriphagus ratkowskyi, Pseudomonas putida en Halomonas meridiana waren. Deze consortia zijn een potentiële bron van hydrolytische enzymen die zijn gespecialiseerd op recalcitrante lignocellulose en die in staat zijn onder zoute omstandigheden te werken.

Daarna, in hoofdstuk 5, heb ik de samenwerkingscapaciteit van geselecteerde microbiële afbraakstammen onderzocht (die in hoofdstuk 3 zijn gevonden). Tevens heb ik onderzocht hoe deze positieve interactie afhankelijk was van de complexiteit van de koolstofbron. Van stammen die domineerden in de geselecteerde consortia werd het vermogen om op tarwestro te groeien getest, evenals de productie van hydrolytische enzymen. Vervolgens creëerde ik minimale synthetische consortia met geselecteerde stammen en onderzocht ik hun interactiviteit op tarwestro. Het meest synergistische paar werd gevormd door Citrobacter freundii so4 en Sphingobacterium multivorum w15. Voor het onderzoeken van stimulus op bidirectionele wijze gebeurde, paste ik een inductie-experiment toe, waaruit bleek dat de stammen wederzijdse invloeden op elkaar uitoefenen. De stimulus werd toegeschreven aan verbindingen die zich in de supernatants van elk van hen bevonden. Tot slot toonde ik aan dat de mate van interactie samenhangt met de complexiteit van de koolstofbron, aan de hand van in monoculturen en co-culturen in koolstofbronnen met verschillende niveaus van "recalcitrantie": glucose, synthetisch lignocellulose substraat (CMC, xylan, lignine) en WS. Recalcitrantie van het substraat gaf een synergistische groei en enzymatische activiteit van uitgescheiden lytische enzymen, en derhalve verhoogde recalcitrantie de coöperatieve relatie tussen de microbiële soorten.

In hoofdstuk 6 heb ik de genomen van Sphingobacterium multivorum w15 en Citrobacter freundii so4 gesequenced in een poging om de functionele complementen van de twee samenwerkende bacteriesoorten te begrijpen. De vergelijking tussen de genomen gaf aan dat de stammen complementaire afbraakcapaciteit vertoonden, evenals verschillende metabolismebehoeften,
waardoor ze waarschijnlijk verschillende soorten afbraakenzymen produceerden en niet konden concurreren om dezelfde voedingsbronnen. Derhalve poneer ik dat positieve samenwerking tussen de stammen berust op kruiselingse voeding of samenwerking op basis van uitwisseling van metaboliëten. Het genoom van *S. multivorum* w15 vertoonde 22 genen uit familie glycosylhydrolase 43, evenals 45 genen uit de familie van koolhydraatesterases; beide families zijn relevant geworden in afbraak van hemicellulose en recalcitrante bindingen in LCB.

Tenslotte, in hoofdstuk 7, heb ik de verschillende soorten consortia herbeschouwd. Samenvattend, ik heb speciaal aandacht besteed aan de geïdentificeerde microbiële stammen in de verrijkte consortia. Alle consortia verkregen onder niet-saline condities bevatten een kern van bacteriën gevormd uit leden van de families *Enterobacteriaceae*, *Xanthomonadaceae*, *Pseudomonadaceae* en *Sphingobacteriaceae*. Ondertussen was in de halotolerante consortia een kern aanwezig gevormd door *Flavobacteriaceae*, *Cyclobacteriaceae*, *Pseudomonadaceae* en *Halomonadaceae*. Opmerkelijk is dat alleen *Pseudomonas* sp. en *Flavobacterium* sp. alomtegenwoordig waren. Vervolgens, in een poging om de samenwerkingsrelatie tussen *S. multivorum* w15 en *C. freundii* so4, de meest synergistische stammen, te begrijpen, analyseerde ik de genomische en de fysiologische gegevens en gebruikte ik ze om een mogelijk mechanisme voor lignocelluloseafbraak voor te stellen voor dit samenwerkingspaar; in het model zou *S. multivorum* w15 kunnen werken als primaire afbreker en *C. freundii* so4 als secundaire afbreker. Daarnaast waren de productie en uitscheiding van secundaire metaboliëten en modulatie van een stressrespons mogelijk andere belangrijke mechanismen.
Summary

The depletion of oil reserves as well as global warming open the door for the possibility to develop ecologically-sustainable ways to obtain energy and products. Lignocellulose biomass (LCB) such as wheat straw (WS1), switch grass (SG), maize (corn stover - CS) and sugar cane represent excellent and cheap sources of carbon that can be transformed into valuable (energy) compounds. Nevertheless, their application at large scale is still limited to date.

This thesis opens with an introduction, where I gave an overview of the complexity of the structure and composition of LCBs and the need for very diverse enzymes to achieve their complete breakdown. This includes not only cellulases, hemicellulases and ligninases, but also the mandatory participation of auxiliary enzymes. There is a need to explain and understand the factors that affect LCB bioconversion in order to tackle larger challenges involved in the processing of LCB at industrial scale. In the Introduction, I stressed the potential of microbial consortia (in an eco-biotechnological approach) for decomposition of LCB so as to deal with its inherent recalcitrant nature. Finally, I described the relevance of key interactions within such degrader consortia. Microbial interactions drive and shape the structure, stability and functionality of microbial communities. It is fundamental to understand the interactions within selected microbial consortia to allow the design of optimized LCB-degrader consortia for application in industry.

The next Chapter, 2, explores the effect of the use of WS1, SG, CS, wheat straw at pH 9.0 (WS2), and forest soil as inocula for the selection of LCB-degrading microbial consortia in a sequential batch approach. The final enriched consortia were studied phylogenetically and functionally. PCR-DGGE analyses indicated that the bacterial communities reached stability after transfer 6 in WS1, SG and CS and after transfer 4 in WS2. For fungal communities, stability was reached after transfers 6 in WS1 and SG and after transfers 4 for WS2 and CS. We found substrate type, next to pH, to drive the bacterial community structures for the treatments using WS. A “core” set of strains was found in the final four microbial consortia; the core set was formed by Sphingobacterium kitahiroshimense, Enterobacter amnigenus, Raoultella terrigena, Pseudomonas putida and Stenotrophomonas rhizophila, next to the fungi Coniochaeta ligniaria and Acremonium sp. All strains in the core presented CMC-ase and xylanase activities. The LCB (wheat straw)
degradation potential of the selected consortia was determined by using Fourier-transform infrared spectroscopy (FT-IR). The data revealed that substrate type determines the final structure of the consortia, and so diverse LCB substrates drive consortia apart, even from a common inoculum source.

In Chapter 3 I explored the importance of the inoculum source in the selection of LCB degrader consortia by applying three different inocula (from forest soil, canal sediment and decaying wood) and wheat straw as the sole carbon source in the selection of LCB degrading microbial consortia. The bacterial and fungal community structures in the final consortia clustered along inoculum source, with significant differences between the different consortia. Wood-derived consortia were the first to reach stability, followed by the soil-derived ones, with sediment-derived ones never reaching stabilization above 50% similarity. More precise characterization of the final bacterial community structures, by 16S rRNA gene amplicon sequencing, showed that the most abundant members of the community formed a bacterial core, which was common between the three final consortia. This core was formed by the genera *Sphingobacterium*, *Citrobacter*, *Acinetobacter* and *Flavobacterium* or *Chryseobacterium*. The fungal genera were consortium-specific; as in Chapter 2, *Coniochaeta ligniaria* and *Acremonium* sp. were found. All organisms presented high LCB transforming activities. The final consortia consumed hemicellulose, cellulose and lignin components to grossly similar extents. Nevertheless, each consortium revealed a unique enzymatic profile. Thus, the final consortium structure and stability were strongly influenced by the initial inoculum source.

In subsequent work (chapter 4), I explored the potential of salt-marsh soil to serve as the inoculum for the production of microbial consortia capable of using wheat straw under highly saline conditions; this mimicked realistic industrial conditions. Furthermore, I studied how an increase of the recalcitrance of the substrate affects the consortial structures. To do this, in the first part of the enrichment, I fed the consortia with fresh substrate whereas in the second part of the enrichment, I replaced fresh by pre-digested substrate. Pre-digested substrate caused a dramatic shift in the bacterial community structures, and also had a striking effect on the fungal communities. The fresh substrate selected a more generalist microbial community, while the predigested WS selected a more specialized microbial community that was better capable to degrade cellulose and lignin than the former one. I identified, and tested for lytic activity, key cultivable bacteria and fungi; the most dominant degrader bacteria in the consortia were
Joostella marina, Flavobacterium beibuense, Algoriphagus ratkowskyi, Pseudomonas putida and Halomonas meridiana. The final consortia constitute a potential source of hydrolytic enzymes specialized on recalcitrant lignocellulose substrate and capable to work under saline conditions.

In a next research effort, I explored the collaborative capacity of microbial degrader strains isolated from the consortia grown under non-saline conditions in relation to the complexity of the carbon source. First, I selected the abundant strains in the consortia, and screened these for their ability to grow singly on wheat straw as well as produce LCB hydrolytic enzymes. Then, I created minimal synthetic consortia with selected degrader strains and examined their interactivity on wheat straw. The most synergistic pair was formed by Citrobacter freundii so4 and Sphingobacterium multivorum w15. To assess the directionality of the synergism, I applied a reciprocal induction experiment, and showed that the two strains exert mutual influences on each other. The stimulus was attributed to compounds contained in the respective strain supernatants. Finally, I demonstrated that the positive interaction was triggered by the complexity of the carbon source, as it was largely absent from cultures grown in glucose and synthetic lignocellulose substrate (CMC, xylan, lignin), versus strongly present in those in WS. The WS substrate probably triggered synergistic growth and activity of secreted lytic enzymes. Overall, I concluded that recalcitrance increases the cooperative relationship between the microbial species.

In subsequent work (chapter 6), I sequenced the genomes of Sphingobacterium multivorum w15 and Citrobacter freundii so4 so as to understand the functional complements of the two collaborating bacterial species. Comparison of the two genomes indicated that the strains had complementary LCB degradative capacity as well as different metabolic needs, which probably allowed them to contribute with different types of degradation enzymes and to not compete for the same nutritional resources. Then, I posited that the positive cooperation between the strains came about as a result of cross-feeding or cooperation based on metabolite exchanges. Interestingly, I noticed that the genome of S. multivorum w15 exhibits 22 genes from glycosyl hydrolase family 43, as well as 45 genes from a family of carbohydrate esterases; both families have become relevant in the degradation of hemicellulose and recalcitrant bonds in the LCB, respectively.

Finally, the findings of the previous chapters were summarized and placed in a broader perspective, placing special emphasis on the microbial degrader
strains found over all enriched consortia (chapter 7). All consortia at regular salt concentration revealed the presence of a core set of bacteria, consisting of members of the families Enterobacteriaceae, Xanthomonadaceae, Pseudomonadaceae and Sphingobacteriaceae. In contrast, the halotolerant consortia were formed by Flavobacteriaceae, Cyclobacteriaceae, Pseudomonadaceae and Halomonadaceae. Remarkably, only Pseudomonas sp. and Flavobacterium sp. were present in all selected consortia. Then, in an effort to understand the cooperative relationship between S. multivorum w15 and C. freundii so4 (the most synergistic strains), I joined the genome analyses and the physiological data and used them to propose a possible mechanism for lignocellulose degradation within this collaborative pair. Briefly, it is possible that S. multivorum w15 is acting as the primary degrader and C. freundii so4 as a secondary degrader. In addition to the production and excretion of secondary metabolites and a contribution with stress response modulation.
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Larisa Cortes Tonalpa was born on the 19th of April 1985 in the small Mexico City and grew up in the quartier of Iztapalapa. At the age of 16 she was accepted in CECyT 6 “Miguel Othon de Mendizabal” aka “Voca 6”, a specialized high school in biological sciences from National Polytechnique Institute (IPN). In this place she had her first encounter with the world of biology, chemistry and physics, feeling in love of laboratories science. In spite of undergoing two major spine surgeries, she concluded her high school on time and graduated as a chemical laboratory technician. In 2003, she started her bachelor studies in UPIBI-IPN, but she had to stop due to her third and final spine surgery. One year later she returned to study the Biotechnology Engineering major. She finished her studies in 2008, specialized in bioprocess, and for obtaining her final degree she designed a new pilot plant for the pharmaceutical company FERMIC. In 2009 she is accepted in the program of master in Biochemical Science in the Biotechnology Institute of UNAM – Cuernavaca. She worked in the lab of Dr. Bolivar Zapata, where the main research line is the metabolic engineering pathways of Escherichia coli. In this group, she worked in the transcriptomic analysis of the production of shikimic acid in 1L fermentor, specializing in biochemistry and microbial physiology. In September 2013, Larisa arrived in the Netherlands to start her PhD studies in the cluster of Microbial Ecology at the University of Groningen. She worked in a project base in eco-biotechnology, focused in the development and study of microbial consortia for the degradation of lignocellulose substrates for the production of valuable compounds. She is currently looking for new projects and she expects to contribute with her beloved Mexico with all the things she has learnt living abroad.
List of publications


