

University of Groningen

## That gut instinct! Novel insights in the interplay between the microbiota, serotonin metabolism and gut function

Waclawiková, Barbora

DOI:  
[10.33612/diss.215906461](https://doi.org/10.33612/diss.215906461)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2022

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Waclawiková, B. (2022). *That gut instinct! Novel insights in the interplay between the microbiota, serotonin metabolism and gut function*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.215906461>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



## **APPENDIX**

**PUBLIC SUMMARY**

**SAMENVATTING VOOR HET BREDE PUBLIEK**

**SHRNUTÍ**

**ACKNOWLEDGMENTS**

**ABOUT THE AUTHOR**

**LIST OF PUBLICATIONS**

## Public summary

Bacteria are all around us and they even inhabit our **gastrointestinal tracts**, where they help us digest the food we eat. In recent years, the research into **human-microbe interactions** is getting increasingly noticeable, allowing us to better understand this amazing interactive environment.

**Gut bacteria** are living organisms, just as humans or animals, and they need nutrients for their survival. In our gut, these bacteria get the necessary nutrients from the food we eat. A portion of the nutrients gut bacteria consume are transformed into **messenger molecules**, which in turn, induce **chemically-mediated interactions** between gut microbiota and the **gut**. For example, the beneficial members of this community can produce valuable products that can keep our intestinal **immune system** healthy, so that no bad bacteria can spread around and cause unwanted side effects, such as diarrhea or other intestinal disorders.

In this thesis research, we studied how gut bacteria interact with **5-hydroxytryptophan**, which is the natural **precursor** of the hormone of happiness, **serotonin**. We showed how 5-hydroxytryptophan can be metabolized by the gut bacteria to an **indole derivative, 5-hydroxyindole**. This bacterial product affected **gut motility**, and had a marginal effect on the **microbiota composition**. In parallel, we uncovered that specific gut bacteria can metabolize serotonin to **5-hydroxyindoleacetic acid**. Our research also suggested that the metabolization process (serotonin → 5-hydroxyindoleacetic acid) might have an impact on both the **bacterial fitness** and on the enhancement of the **intestinal epithelial barrier** integrity.

## Glossary

**5-hydroxyindole:** a molecule which was identified (in this thesis research) to be produced by gut bacteria from 5-hydroxytryptophan

**5-hydroxyindoleacetic acid:** a molecule which was identified (in this thesis research) to be produced by gut bacteria from serotonin

**5-hydroxytryptophan:** an amino acid, which is used as a dietary supplement for enhancement of serotonin in the body, and as a treatment for depression

**Bacterial fitness:** an ability of bacteria to adjust to the changing environment, in order to survive and grow

**Chemically-mediated interactions:** communications which are induced by chemical signals

**Gastrointestinal tract:** the series of joined hollow organs that consist of the mouth, esophagus, stomach, small intestine, large intestine, and anus

**Gut:** mostly used to refer to the small and large intestine

**Gut bacteria:** the bacteria living inside the gastrointestinal tract

**Gut motility:** a movement of the gastrointestinal tract which helps with absorption and digestion of food

**Human-microbe interactions:** a mutualistic interaction between the human gastrointestinal tract and its inhabitants, the gut bacteria

**Immune system:** the network of biological processes that protects our bodies from diseases

**Indole:** a molecule which is widely distributed in the natural environment and is produced by gut bacteria

**Indole derivative:** a molecule which is derived from indole

**Intestinal epithelial barrier:** a part of our intestine, which allows to keep the bacteria in the gastrointestinal tract at distance from the intestinal walls, in order to avoid immune responses like inflammation

**Microbes:** microorganisms which include bacteria, yeast, and other single-celled organisms

**Microbiota composition:** the structure of gut bacteria in the gut

**Messenger molecules:** molecules which are used for communication in, for example human body

**Precursor:** a molecule from which another substance is formed

**Serotonin:** a molecule of well-being and happiness, which is produced in large quantities (>90%) in the gastrointestinal tract, it regulates many physiological process, for example, it modulates intestinal motility, regulates the immune system as well as our mood and behaviour

## Samenvatting voor het brede publiek

Bacteriën zijn overal om ons heen en ze bewonen zelfs onze **maagdarmkanaal**, waar ze ons helpen het voedsel dat we eten te verteren. De laatste jaren valt het onderzoek naar interacties tussen mens en microbe steeds meer op, waardoor we deze verbazingwekkende interactieve omgeving beter kunnen begrijpen.

**Darmbacteriën** zijn levende organismen, net als mensen en dieren, en ze hebben voedingsstoffen nodig om te overleven. In onze darmen halen deze bacteriën de nodige voedingsstoffen uit het voedsel dat we eten. Een deel van de voedingsstoffen die darmbacteriën consumeren, wordt omgezet in **boodschappermoleculen**, die op hun beurt **chemisch gemedieerde interacties** tussen de darmmicrobiota en de **darmen** veroorzaken. De heilzame leden van deze gemeenschap kunnen bijvoorbeeld waardevolle producten produceren die ons **darmimmuunsysteem** gezond kunnen houden, zodat slechte bacteriën zich niet kunnen verspreiden en deze geen ongewenste bijwerkingen kunnen veroorzaken, zoals diarree of andere darmziekten.

In dit proefschrift hebben we onderzocht hoe darmbacteriën interageren met **5-hydroxytryptofaan**, de natuurlijke voorloper van het gelukshormoon **serotonine**. We hebben laten zien hoe 5-hydroxytryptofaan door de darmbacteriën kan worden gemetaboliseerd tot een **indoolderivaat**, **5-hydroxyindool**. Dit bacteriële product tastte de **darmbeweging** aan en had een marginaal effect op de **microbiota samenstelling**. Tegelijkertijd hebben we ontdekt dat specifieke darmbacteriën serotonine kunnen metaboliseren tot **5-hydroxyindoolazijnzuur**. Ons onderzoek suggereerde ook dat het metabolisatieproces (serotonine -> 5-hydroxyindolazijnzuur) een impact zou kunnen hebben op zowel de **bacteriële fitheid** als op de verbetering van de integriteit van de **darmepitheelbarrière**.

## Woordenlijst

**5-hydroxyindool:** een molecuul waarvan (in dit proefschrift) werd ontdekt dat het door darmbacteriën wordt geproduceerd uit 5-hydroxytryptofaan

**5-hydroxyindoolazijnzuur:** een molecuul waarvan (in dit proefschrift) werd ontdekt dat het door darmbacteriën wordt geproduceerd uit serotonine

**5-hydroxytryptofaan:** een aminozuur, het kan worden gebruikt als voedingssupplement om de serotonine in het lichaam te versterken en als een behandeling voor depressie

**Bacteriële fitheid:** het vermogen van bacteriën om zich aan te passen aan de veranderende omgeving, om te overleven en te groeien

**Boodschappermoleculen:** moleculen die gebruikt worden voor communicatie in b.v. menselijk lichaam

**Chemisch gemedieerde interacties:** communicatie die wordt veroorzaakt door chemische signalen

**Darmen:** meestal gebruikt om te verwijzen naar de dunne en de dikke darm

**Darmbacteriën:** de bacteriën die in het maagdarmkanaal leven

**Darmepitheelbarrière:** een deel van onze darm, dat het mogelijk maakt om de bacteriën in het maagdarmkanaal op afstand van de darmwanden te houden, om immunoreacties zoals ontstekingen te voorkomen

**Darmbeweging:** een beweging van het maagdarmkanaal die helpt bij de opname en vertering van voedsel

**Immuunsysteem:** het netwerk van biologische processen dat ons lichaam beschermt tegen ziekten

**Indool:** een molecuul dat wijd verspreid is in de natuurlijke omgeving en wordt geproduceerd door darmbacteriën

**Indoolderivaat:** een molecuul dat is afgeleid van indool

**Maagdarmkanaal:** de reeks verbonden holle organen die bestaan uit de mond, slokdarm, maag, dunne darm, dikke darm en anus

**Mens-microbe interacties:** een mutualistische interactie tussen het menselijke maagdarmkanaal en zijn bewoners, de darmbacteriën

**Microben:** micro-organismen waaronder bacteriën, gisten en andere eencellige organismen

**Microbiota samenstelling:** de structuur van darmbacteriën in de darm

**Precursor:** een molecuul waaruit een andere stof wordt gevormd

**Serotonine:** een molecuul van welzijn en geluk, dat in grote hoeveelheden (> 90%) in het maagdarmkanaal wordt geproduceerd, het reguleert veel fysiologische processen, het moduleert bijvoorbeeld de darmbeweging, reguleert het immuunsysteem en onze stemming en gedrag

## Shrnutí

Bakterie jsou všudypřítomné organismy a dokonce obývají i náš zažívací trakt, kde mimo jiné pomáhají trávit jídlo, které jíme. V posledních letech se studium **interakcí (vzájemných vztahů) mezi lidmi a mikroby** dostává stále více do popředí zájmu. Každý nový objev v tomto odvětví nás přibližuje pochopení tohoto úžasného prostředí.

**Střevní bakterie** jsou živé organismy, stejně jako lidé nebo zvířata, a stejně tak ke svému přežití potřebují živiny. Vzhledem k tomu, že tyto bakterie žijí v našich střevech, získávají potřebné živiny z potravy, kterou jíme. Živiny, které střevní bakterie zkonzumují jsou jejich aktivitou následně přeměněny na **chemické signály**, které **zprostředkují interakce** mezi střevní mikrobiotou a střevem. Některé druhy bakterií mohou produkovat molekuly (působky), které pomáhají udržet náš střevní **imunitní systém** zdravý, tak aby se žádné „špatné“ bakterie nemohly přemnožit a způsobit tak různá střevní onemocnění projevující se např. průjmami.

Předmětem této disertační práce byla snaha zjistit jakým způsobem střevní bakterie interagují s **5-hydroxytryptofanem**, což je přirozený prekurzor hormonu štěstí **serotoninu**. Zjistili jsme jakým způsobem je 5-hydroxytryptofan metabolizován střevními bakteriemi na **5-hydroxyindol**. Tento bakteriální produkt výrazně ovlivnil střevní motilitu a měl jen zanedbatelný vliv na složení střevní mikrobioty. Kromě toho jsme také zjistili, že některé typy střevních bakterií mohou metabolizovat serotonin na **kyselinu 5-hydroxyindolactovou**. Náš výzkum také naznačil, že tento proces, kde serotonin je metabolizován na kyselinu 5-hydroxyindolactovou, by mohl mít dopad jak na **bakteriální zdatnost**, tak na zlepšení funkce **střevní epitelální bariéry**.



## Slovník pojmů

**5-hydroxyindol:** molekula, která byla objevena (během našeho výzkumu), a kterou produkují střevní bakterie z 5-hydroxytryptofanu

**5-hydroxytryptofan:** aminokyselina, kterou lze použít jako doplněk stravy pro zvýšení hladiny serotoninu v těle a použít jej tak pro léčbu deprese

**Bakteriální zdatnost:** schopnost bakterií přizpůsobit se měnícímu se prostředí, tak aby mohly v nových podmínkách přežít a množit se

**Chemické signály:** molekuly, které se slouží pro komunikaci např. v lidském těle

**Chemicky zprostředkované interakce:** komunikace zprostředkována chemickými signály

**Gastrointestinální trakt:** je soustava dutých na sebe navazujících orgánů sestávající se z dutiny ústní, jícnu žaludku, tenkého střeva, tlustého střeva a konečníku

**Imunitní systém:** systém, který chrání organismus před nemocí

**Indol:** molekula, která je široce distribuována v přirozeném prostředí a je produkována střevními bakteriemi

**Indolový derivát:** molekula, která je odvozena od indolu

**Interakce mezi člověkem a střevním mikrobiomem:** symbiotická (vzájemně prospěšná) komunikace mezi lidským

gastrointestinálním traktem a střevními bakteriemi (mikroflórou resp. mikrobiomem). !!ale pozor ne vždycky je vzájemně prospěšná tudíž ne vždy je to symbióza.

**Kyselina 5-hydroxyindolactová:** molekula, která byla objevena (v tomto výzkumu práce), a kterou produkují střevní bakterie ze serotoninu

**Mikroby:** mikroorganismy, které zahrnují bakterie, kvasinky a další jednobuněčné organismy

**Střevní bakterie:** bakterie žijící v trávicím traktu

**Střevní epitelální bariéra:** bariéra tvořená sliznicí (buňkami (epitelem), střevní sliznice - výstelky) ve střevě, která neumožňuje přímý kontakt mezi antigeny (látky vyvolávající imunitní reakci), bakteriemi a toxiny a vnitřním prostředím

**Střevní motilita (hybnost):** pohyb gastrointestinálního (zažívacího) traktu, který napomáhá správnému vstřebávání a trávení potravy

**Prekurzor:** původní molekula (látky), ze které se její úpravou tvoří další látka

**Serotonin:** molekula „pohody a štěstí“, která je ve velkém množství (> 90 %) produkována v zažívacím traktu, mimo to reguluje i mnoho fyziologických procesů v lidském těle, například ovlivňuje střevní motilitu a uplatňuje se i v řízení imunitního systému, nálady a chování.

## Acknowledgments

I would like to start this section with a small story how I actually ended up in Groningen, where I now live almost 8 years. This story essentially starts with my father moving to the Netherlands and starting to work in Appingedam (a small town 24 km northeast from Groningen) in 2013. During the summer of 2013, I spent two months working in the company (where my father worked) and during that time, me and my family visited Groningen a few times. After the summer, I had a last year of my bachelor's programme in Czech republic in front of me, and was deciding what to do after that. One day, I started to look into the study programmes at the University of Groningen and liked a few. Few weeks later, I presented the idea to study in Groningen to my family, and my father immediately said: "Yes, do it!". So I applied and I got in. My journey in Groningen thus began in September 2014, when I started my master's programme in Molecular Biology and Biotechnology at the University of Groningen.

During my master's programme, I got to love the city of Groningen, the people and the international environment and I got enthusiastic about science and working in the lab. I started my master's programme, maybe a bit unconventionally, almost immediately with a research project in the Membrane Enzymology group. I would like to thank **Dirk Slotboom** for giving me the opportunity to work in his lab. I am happy that I did my first internship in such a nice group. At some point during this research project, somebody asked me what I would like to do after my masters and I automatically replied a PhD. So from that point onwards I was decided to do a PhD. Of course, before I had to finish my masters. Second research project I did in Jan-Willem Veening's group, at that time, embedded in Molecular Genetics group. I would also like to thank **Jan-Willem Veening** for the opportunity to work in his lab, it was a very memorable internship, with a great working environment and I learned so much during that time. Few months, after graduating my masters, in April 2017, I got an opportunity to work as a research assistant in Molecular Genetics group. I would really like to thank **Oscar Kuipers** for this chance. Maybe without this chance, I would not have come in contact with **Sahar El Aidy**, who I met once in the corridor and who told me that there might be an opportunity in her lab to do a PhD. I did my colloquium with **Sahar El Aidy** during my masters, so I knew about what her lab's research is about and when she told me about what the PhD project should be about, it sounded really interesting and exciting.

So that is how my PhD journey started (in September 2017).

Here we are in April 2022, when I am writing this acknowledgments part, reminiscing about all the ups and downs on the PhD journey. In the following paragraphs, I would like to thank all who took part in it, hope I will not forget anybody.

First of all, I would like to give my large thanks to **Sahar**. You gave me the chance to become a doctor and showed me the bumpy road which I have to take to get it. Even if it was a bumpy road, it was an amazing journey. You taught me so much, how to write scientific articles, how to present and communicate my results, how to be more confident about myself, how to be more critical, how to take a lead in discussion and the list can go on. Really, I am amazed how much effort you put into your work, how you are always so enthusiastic about discussing results and how you always have time for a discussion. Also, I don't think, I would be able to write my thesis in such a short time, without your super quick revisions. So thank you again, for being a remarkable supervisor and I am looking forward to keep our journey going (I am staying in Sahar's lab as a postdoctoral researcher).

Secondly, I would like to thank my copromotor, **Siewert-Jan Marrink**. Siewert-Jan, thank you for helping us with the development of Chapter 4 in this thesis, for your help with Dutch translations and for agreeing to be my copromotor.

I would like to also give my special thanks to **Michiel Kleerebezem** for his critical evaluation of research projects which I was working on followed by very useful feedbacks. Thank you :)

Further, I would like to thank all our collaborators who participated in this thesis research: **Ad Nelemans, Gertjan van Dijk, Agnese Codutti, Karen Alim, Paulo Cesar Telles de Souza, Constantinos Neochoritis (Dinos), Anne de Jong, Tina Tran** and **Stefania Senger**

**Ad**, you helped me a lot with the planning and the setup of the organ bath experiments. Thank you very much for always being available for discussion about how to plan the organ bath experiments and how to conduct them. I would like to thank you for helping me fix any organ bath which was not working. I will remember that day, when we were setting up and connecting the six organ baths, it took us I think one whole day, maybe even two days, but in the end we managed. It was worth the effort.

**Gertjan**, thank you for always being available for any question about an animal experiment, or about something related to your field of expertise, or pointing me



to somebody who can provide me with some rat tissue for my organ bath experiments. Also thank you for helping us with the animal experiment.

**Agnese and Karen**, thank you for agreeing on writing a review article with us, I think we wrote a very interesting review article together and hope we can collaborate on something again in the future.

**Paulo**, thank you for collaborating with us on the Chapter 4 of this thesis and contributing with a set of very exciting results, looking forward to publishing the Chapter 4 in a good journal.

**Dinos**, thank you for helping us setting up the screening experiment of the Chapter 4 and thank you for the helpful discussions and for your feedback on the Chapter 4.

**Anne**, thank you very much for all the bioinformatic analysis you helped me with, starting with the bacterial genome sequencing to the RNA-seq analysis.

**Tina and Stefania**, thank you for agreeing to collaborate with us on the Chapter 5. Thank you for contributing with very interesting sets of results and looking forward to publishing the Chapter 5 in a good journal.

I also want to thank **Sietse de Boer, Christa Reitzema, Martijn Salomons** and **Linda Pals-Robben**, who helped me a lot when I needed to collect rat intestinal tissue for organ bath experiments.

Next, I would like to thank all my students who I had the pleasure to supervise during my PhD, **Semih Toptas, Jack Jansma, Hussam Abbas, Akke Visser, Amber Bullock, Marta Cardoso**.

**Semih**, thank you for initiating with me the *Pseudomonas* project (Chapter 5), you were also very helpful with the initial screening of the gut bacterial strains in the first year of my PhD. And I couldn't wish for more easy-going first student in my PhD. Thank you.

**Jack**, you were always so independent during your master research project, I will never forget that. Since you also became one of my great colleagues, my thanks to you will be given a bit later in this acknowledgment section.

**Hussam**, I really enjoyed working with you, you were always so calm, motivated and full of new ideas, thank you for your hard efforts in the lab to find the enzyme



which is involved in the serotonin degradation. I wish you successful finish of your masters.

**Akke**, your stories about a farm life during our lunches were always very interesting and thank you for contributing to the *Pseudomonas* project.

**Amber**, I will always remember our great and long talks during the organ bath experiments, also quite memorable, for you (and also for me), will be the animal experiment, when we had to spent hours collecting rat poop to found out whether their gut transit time was affected after administration of 5-hydroxyindole (Chapter 2), thank you for always being so enthusiastic and motivated. I hope to see you again someday.

**Marta**, you were my student only for a short while, however you were a great help nevertheless. I will remember your super tiny and systematic writing style, you were always critical and asking lot of questions, you gave me several new ideas for my project and it was great working with you. I wish you all the best and good luck for your future.

I am also very grateful to all my friends/acquaintances who I met during my times in Groningen: **Warner Hoornenborg, Marten Exterkate, Nitin Nair, Aishwarya Ravi, Markus Böger, Luis Suarez, Diego García de la Morena, Ruben Cebrían Castillo, Renske van Raaphorst, Raj Singh, Vicky Fernández Cantos, Patricia Arias Orozco, Willem Dijkman, Chrysovalantou Chatziioannou.**

**Warner**, I cannot thank you enough for all the things you helped me with. I think we were really “good bussy” throughout the several sessions extracting some rat intestinal tissue in the animal facility. I always had so much fun during these sessions, thank you for being always up to help me with that task, which I couldn’t do myself. I will always remember our good talks, jokes and sometimes some smelly things ending on the lab coats. I wish you also successful finish of your PhD and of course don’t forget to be “good bussy” until the end.

**Marten**, so many long and good talks we had when we met in the 7<sup>th</sup> floor of Linnæusborg. I cannot thank you enough for all the help with mainly the LC-MS, but also other things when I needed something. I will not forget how many good parties (e.g. “Tonight we drink” some Czech beers) we had together with also other guys from my group, Molgen and the 7<sup>th</sup> floor. It was great to meet you and now I know a junior group leader in Düsseldorf, which is amazing, once again congrats on getting that position :)



**Nitin**, it was great to meet you, many congratulations with finding a position outside of academia and hope to see you soon one day.

**Ash**, I will always remember you as a very energetic and always smiling/laughing person, so many good days we had in the lab (during my Molgen days). The crazy dances you and Fleur did, I never forget that. Hope you are doing good in Norway and hope to see you soon again!

**Markus (Böger)**, you were my office mate for several years, and I was also honored to be your paronymph. I will not forget our dinners in Kapteynborg when I had long experiments and you joined me for some substantial food and our good talks about everything during those evenings. You were a great colleague, and I am so happy that I met you during my PhD. Hope to see you soon again!

**Luis**, you are an amazing person, your energy and enthusiasm is contagious. We met during iGEM competition and both of us we continued our PhD journeys at the University of Groningen. It was great to go with you to all the Pathé Film Festivals. I am just really sad that corona pandemic ruined our days out spent in Pathé watching movies all day. I am really happy for you that you found a lecturer position in Amsterdam, I think you are the right person to be a lecturer. I hope we can soon go to some Pathé Film Festival again or any other movie festival!

**Diego**, I am happy that I could meet you, thank you for all the good talks we had, unfortunately corona happened, otherwise I think we would have more amazing parties and gatherings together than we had, I hope we can still catch up with that a bit until you are in Groningen!

**Ruben**, it was a great pleasure to meet you, you are a great scientist, great father and great person to have around when there is some gathering happening. I won't forget all the fun we had with a few glasses of red wine :P. I wish you all the best in Granada in your new job and for you and for your family!

**Renske**, I am happy that I could have been your student during my second research project, the experience I got during the internship with you helped me greatly during my PhD. I will never forget all the great talks, parties, things we did together also with other people from Jan-Willem's group. Hope to see you soon again!

**Raj**, I am happy that I could have been your student during my first research project, the things you taught me helped me a lot during my PhD. You were always



so helpful and patient with me, explaining all the things in the lab in detail. Thank you for all the amazing food you made, I think you also taught me to like (a lot) Indian food. I wish you all the best in Sweden or any other country you decide to go next! And hope to see you soon!

**Vicky**, I am happy that I met you, you are a great and fun person to be around. It is a pity, that because of corona we could not have had more parties and gatherings, because all the things we did together were/are very memorable. I hope we can catch up still until we are both in Groningen.

**Paty**, I am so happy that we could meet. You are such a caring and amazing person. It was great to be able to experience all the great and crazy things with you, and I hope that we will still experience some while we are still both in Groningen! I wish you all the good luck in the world in the finishing phase of your PhD.

**Willem**, thank you for all the help with the protein purification and characterization. You had always great ideas and were always so willing to help. It was great to have you around in the lab (also if it was just for a bit). I wish you all the best for you and your family!

**Valantou**, thank you very much for all the help with the chromatography and the analytical techniques. I won't forget your working attitude, when you were finishing your PhD, sleeping three to four hours in the night, and the next day looking like you slept like a baby the whole night, I was really amazed by that. It was great to have you around as a colleague and a friend, always so helpful, friendly and positive. Also when we were fixing (I think maybe multiple times) HPLC until late in the night, I will always remember that!

I also want to thank my dear classmates from my masters, **Nina Vesel, Bart Bruininks, Neus Mestre Farras, Dian Spakman, Sebastiano Pontalti, Fleur Ruijne**. I am not sure if I would have chosen to do a PhD, if it was not for you guys (because all of you planned to do a PhD, a bit of a group pressure :P). I won't forget our awesome trips to Heidelberg, Slovenia, Italy and Czech republic. We had so much fun during those days and during our masters. Almost all of us are now living in a different country, Switzerland, Spain, Finland/Netherlands, Italy and Netherlands, but we try after all the years keep in touch and I know we will meet again and go on a memorable trip! Of course, I cannot forget **Clement Gallay**, you were not my classmate, but you actually kind of became one after



you got together with Nina :) I cannot forget how energetic person you are and your crazy ideas, like trying some glycerol and glucose in the lab :P

I want to additionally highlight our Groningen PhD crew. **Bart** and **Fleur**, we should meet again and drink a few Dropshots! **Bart**, your philosophical thinking and ideas are beyond my understanding sometimes, however I learned so much from you, and I am so happy that I got to know you. Last (of my classmates), but not least, **Fleur**, I am so happy that you decided to do a PhD in Groningen and that we could stay in touch for the past years and become such good friends. Thank you for all the very long talks in your or my office about everything, for all the exceptional parties we had, like our 80' birthday party, or when you always invited me for "one" beer, or our recent cocktail workshop :P I wish you all the good luck in finishing of your PhD as well! Then we will be both **Phinally Done!**

My large thanks also go to our former secretary team, **Anmara** and **Bea**. You were always so helpful with everything and there was no question which you could not answer or at least point me where to ask for the right answer. Thank you for everything!

My acknowledgments are already quite long, and still so many people I have to thank!

I want to thank my former colleagues, from the Molecular Immunology group, **Frans Bianchi, Sjors Maassen, Femmy Stempels, Pieter Grijpstra, Maxim Baranov, Deepti Dabral, Harry Warner, Alexine de Wit, Melina Ioannidis, Myrthe Frans, Geert van der Bogaart**. Thank you all for creating a great atmosphere during our Friday borrels (before corona) and we had some memorable lab outings together.

**Frans**, thank you for all the good talks we had either when we were still office mates or after. Your enthusiasm for building things never stopped/stops surprising me, like when you once came to the office and started to explain the idea how you want to build a very complex child's bed for Eva.

**Sjors (Sjo, Sjo)**, I think you and me were a great team organizing the awesome labuitje with the graffiti workshop and lablympics. Thank you also for always being very helpful and I hope you will have smooth finishing phase of your PhD.





**Femmy**, I am happy that I could meet you, you are a great person, who is always willing to help and who is fun to be around. I wish you all the best in finishing of your PhD and when you will be also Phinally Phinished!

**Pieter**, not sure what would many of us do without you. You know probably everything in the lab. Thank you for always being very helpful with everything!

Also want to thank my former colleagues from Microbial Physiology group that helped me somewhere along my PhD, **Hien Pham, Huifang Yin, Sander van Leeuwen, Geralt ten Kate, Laura Fernández De Las Heras, Joana Gangoiti Muñecas, Evelien te Poele, Rivca Valk-Weeber, Cecile Deelman-Driessen.**

Of course, I have to thank my amazing team of (former) colleagues and friends from a newly formed HMI group! **Ahmed Osama El-Gendy, Gwen Ruitenbeek, Rogier van Essen, Daan Bunt, Sebastiaan van Kessel, Jack Jansma, Markus Schwalbe, Panagiotis Kelefiotis Stratidakis, Carmen Aranzamendi, Pamela González Dávila, Julia Aresti Sanz.** The journey of my PhD would be way more difficult if I would not have such a great people around me, working with me and supporting me. You are all such a great people and I could not wish for better colleagues!

**Osama**, you were a great and always very helpful colleague, even though you joined us only for a short while. I will never forget how hard you worked, spending hours and hours on your experiments. You were so dedicated and always so enthusiastic. Thank you for all your help and all you contributions to our group.

**Gwen**, I am very happy that I could meet you, even if we are not too much in contact now, I really enjoyed working with you in the lab, I hope you are doing well and would be great if we can meet each other again soon.

**Rogier(os)**, I will never forget your pink hoodie :P it was great to have you around in the lab, you were always really easy-going, and also when you were sitting in your office (“cave”) without windows you didn’t really complain, hope to see you soon!

**Daan**, we have not seen each other that much until now, but looking forward for working together in the future :)

**Sebas**, you taught me so much during the PhD, starting with the HPLC (still running nicely :)), ending with how to write, and which programme to use for the thesis. I don’t know how many times I came to your office and asked you something about



the HPLC and how many times we were fixing it together. I won't forget your enthusiasm about everything, asking what am I doing, how is it going, how are my results and then discussing with me all the things and giving your input. You are a great person, and I hope that we keep in touch! I wish you all the best for you and your family!

**Jack(ie/os)**, your dry sense of humor and pragmatic attitude will be remembered. Thank you for listening to all my complaining at some point. Thank you for making us exercise in the office, "making" us do some squads, and of course thank you for taking me for the excellent 26 km long walk somewhere in Drenthe crossing electric fences etc. :D Also for the beers and nasi afterwards! Unfortunately, I was not able to find an answer to the question: "What does it all mean?", but I am sure you will find it one day!

**Markus(ito)**, I have to thank you again for all the help with the "computer stuff". It is amazing to have such a knowledgeable office mate, I think I can ask you anything and you will find an answer or you will "programme" the answer. Again, I have to tell you that it is amazing how you can speak the computer language. I think we are a good office team, with our Lodewijk monster (we should not forget to water him though :P). Let's keep the good office vibes in the future as well, I am looking forward to learn more from you and looking forward to many more of our good talks.

**Panos(ito)**, you are always so helpful with everything! It is great to have you around to deepen my knowledge about hip hop, sneakers, basketball and (Greek) politics. Thanks a lot for being your Greek you, always on time (it is getting better I know :P), always talkative, always full of energy! Hope we will manage to go to Crete together also with the others at some point! Would be great! I am looking forward to all the upcoming great conversations during Friday borrels.

**Carmen**, thank you for taking so good care of ordering materials and chemicals for the lab. Thank you for teaching me how to culture mammalian cells, it is very useful skills to have and thank you for all the experiments you did for me or together with me. Looking forward to working with you also in the future, and to all the good conversations we had and we will have.

**Pamelita & Julianita**, first of all I would like to thank you for accepting to be my paranympths. It will be great to have you both around during my big day, during my defense. I am sure you will make me less nervous and will be very supportive.

A

You are both amazing and great people. And both of you are the best cooks I've ever met! I loved every girls night we had together and the amazing food you both made! **Pamelita**, you are always full of energy, always loving and so positive. I am amazed how you could do all the stuff besides your PhD, being a part of the PhD council, taking care of all the "thousands" of plants in your house, keeping in touch with all your friends, travelling, visiting festivals, and all the other things you did and do. You are really enjoying your life to the fullest and try to teach everybody else to do the same. We have so many stories together which would take another several pages to write, so I won't write them, but I am sure that we both remember them and I am sure that we will still have many more to tell in the future. Thank you for everything you taught me! **Julianita**, you have very rational view of life, but you are also experiencing everything with so much passion, thank you for recommending all the crime shows on Netflix, we are both weird in that way that we like to watch these shows so much, but it is great to have a reliable partner in crime :P. Thank you for being for several years a great office mate, even though in last years you were mostly at Eriba. I really enjoyed all the conversations we had about everything. I am sure that we will keep touch also in the future and we will visit you in Rome. Looking forward to eat Italian pizza :) And don't forget, "Nescafé, what else?" :D

I also would like to thank our current students in the HMI group, **Paula Meth, Linsey Bhiexharie** and **Maria José Guerraty Olavarrieta**. **Paula**, thank you for being such an enthusiastic student, we know each other just for a bit, but I really like and enjoy working with you and I hope we will have some exciting results from the projects you are currently working on. **Linsey**, I really like your strict black dress code every day! **Maria José**, thank you for being part of the group, you fitted-in perfectly among us, I think :)

I would also like to thank to the following people who helped me in anyway during my PhD: **Nicola Thome, Gilles Wezel, Ioannis Pavlidis, Saniye Kaga, Marco Fraaije, Annick Mercenier, Karin Wolters, Jonas Cremer, Jan Bruggink, Wanda Douwenga** and also to people who I met during my times in Groningen: **Morten Kjos, Rieza Aprianto, Robin Sorg, Jelle Slager, Dimitra Synefiaridou, Xue Liu, Arnau Domenech, Lance Keller, Stefano Sanselicio, Luiza Morawska, Barbara Marcelli, Amanda Y. van Tilburg, Jhonatan Hernandez-Valdes, Fleur Brinkman, Pinar Onat, Maria Castejón Mariscal de Gante, Hiltje de Jong, Thom**



**Weitenberg, Aldert de Jong, Simon Winkel, Stephanie Roethig, Maartje Wolf, Bindert Algra, Maja Stevanoska, Willemijn Brouwer.**

Finally, I would like to thank,

**My friends** that stayed close even though we live far away. **Wendulko** a **Kesínku**, je super, že i když se pár měsíců nevidíme a úplně si i nepíšeme každý den, když se nakonec vidíme, máme si pořád co říct a je to jako za starých časů. Jsem moc ráda, že se pokoušíme vidět pokaždé když dojedu do Česka, a doufám, že to i tak zůstane. Moc ráda bych vám někdy splnila váš opakovaný návrh, že se mám přestěhovat zpátky do Česka, uvidíme jak se další roky vyvinou, třeba se to někdy splní :)

**My family in law, Meint, Maria, Lisette, Henk (Jonathan & Gabriëlle)**, bedankt voor jullie steun tijdens mijn PhD periode, bedankt voor alle geweldige bijeenkomsten die we samen hebben gehad en bedankt dat jullie altijd in mij hebben geloofd.

**My family, mami, tati**, děkuji za všechno co jste pro mě za uplynulých 30 let udělali, za vaši podporu ve všem co jsem se rozhodla studovat a dělat, bez vás bych toho všeho určitě nedosáhla. Děkuji, že jste mi vždycky věřili, že to dokážu nebo jste si aspoň moc přáli ať to všechno klapne. **Vili**, děkuji za to že jsi nejlepší brácha na světě. Doufám, že dosáhneš všeho co si umaneš, určitě na to všechno máš! Mám vás všechny moc ráda!

**My Erik**, I cannot thank you enough for all the support, help and motivation during my PhD. You were present during all the ups and downs. Thank you for always making me feel better during the down periods and enjoying with me the up moments. Thank you for being so patient and supportive during the times when I had to work until late in the night or when I told you that I will be at home at certain time, but in the end it was sometimes couple of hours later because something did not go according to the plan in the lab. Thank you for enduring all these moments. I love you so much! Without you it won't be possible for me to become Dr. Bara :)



## About the author

### EN

Barbora Waclawiková was born on January 12, 1992 in Šternberk, Czechoslovakia. In 2011 she graduated from a secondary school, Gymnázium Hejčín in Olomouc in the Czech Republic and then started her bachelor's program Biochemistry at the Masaryk University in Brno in the Czech Republic. In 2014, she completed her bachelor's exams and bachelor's thesis named The HPLC analysis and tyrosinase inhibition studies of some *Agrimonia* and *Plectranthus* plants. After obtaining her bachelor's degree in Biochemistry, she decided to move to Groningen, The Netherlands to study the master's program Molecular Biology and Biotechnology at the University of Groningen. During her master's, she did two research internships, first at the Membrane Enzymology department and the second at the Molecular Genetics department. During her master's program she also participated in the International Genetically Engineered Machine (iGEM) competition 2016 in Boston (MA, US), where together with her team (CryptoGEM: Encode it, keep it) she won the Information Processing track and was nominated for Best Software Tool, Best Education and Public Engagement and Best Wiki page. In January 2017, she obtained her master's degree and then from April 2017 worked for 4 months as a research assistant at the Molecular Genetics department. In September 2017, she started his PhD research in the department of Microbial Physiology (later subdivided into the department of Host-Microbe Interactions), as described in this thesis. Currently, she is employed as a postdoc researcher at the department of Host-Microbe Interactions at the University of Groningen.

### NL

Barbora Waclawiková werd op 12 januari 1992 geboren te Šternberk, Tsjecho-Slowakije. In 2011 studeerde ze af aan de middelbare school, Gymnázium Hejčín in Olomouc in Tsjechië en begon daarna aan haar bacheloropleiding Biochemie aan de Masaryk Universiteit in Brno in Tsjechië. In 2014 sloot ze haar bachelorexamen af met een bachelorscriptie genaamd The HPLC-analyse en tyrosinaseremmingsstudies van enkele *Agrimonia*- en *Plectranthus*-planten. Na het behalen van haar bachelor Biochemie besloot ze naar Groningen te verhuizen om de master Molecular Biology and Biotechnology te gaan studeren aan de Rijksuniversiteit Groningen. Tijdens haar master heeft ze twee onderzoekstages



gelopen, eerst bij de afdeling Membrane Enzymology en de tweede bij de afdeling Molecular Genetics. Tijdens haar masteropleiding nam ze ook deel aan de International Genetically Engineered Machine (iGEM) competitie 2016 in Boston (MA, US), waar ze samen met haar team (CryptoGERM: Encode it, keep it) de Information Processing track won en genomineerd werd voor Best Software Tool, Best Education en Publieke betrokkenheid en beste Wiki-pagina. In januari 2017 behaalde ze haar masterdiploma en werkte in april 2017 voor 4 maanden als onderzoeksassistent bij de afdeling Moleculaire Genetica. In september 2017 begon zij aan haar promotieonderzoek bij de afdeling Microbiële Fysiologie (later onderverdeeld in de afdeling Gastheer-Microbe Interacties), zoals beschreven in dit proefschrift. Momenteel is zij werkzaam als postdoc onderzoeker bij de afdeling Host-Microbe Interactions van de Rijksuniversiteit Groningen.

## **CZ**

Barbora Waclawiková se narodila 12. ledna 1992 ve Šternberku v Československu. V roce 2011 dokončila střední školu gymnázium Hejčín v Olomouci a poté nastoupila do bakalářského programu biochemie na Masarykově univerzitě v Brně. V roce 2014 ukončila bakalářské zkoušky a bakalářskou práci s názvem HPLC analýza a antityrosinázová aktivita vybraných druhů rodu *Agrimonia* a *Plectranthus*. Po získání bakalářského titulu v oboru biochemie se rozhodla studovat magisterský program molekulární biologie a biotechnologie na univerzitě v Groningenu. Během magisterského studia absolvovala dva výzkumné programy, nejprve na katedře membránové enzymologie a druhou na katedře molekulární genetiky. Během magisterského programu se také zúčastnila soutěže International Genetically Engineered Machine (iGEM) 2016 v Bostonu (MA, USA), kde společně se svým týmem (CryptoGERM: Encode it, keep it) vyhrála v zadaném tématu Information Processing a byla nominována na nejlepší softwarový nástroj, nejlepší vzdělávání a veřejné zapojení a nejlepší stránka Wiki. V lednu 2017 získala magisterský titul a poté od dubna 2017 pracovala 4 měsíce jako odborný asistent na katedře molekulární genetiky. V září 2017 zahájila doktorské studium na katedře mikrobiální fyziologie (později známé jako oddělení mikrobiálních interakcí (Host-Microbe Interactions)), jak je popsáno v této práci. V současné době je zaměstnána jako výzkumný pracovník na univerzitě v Groningenu.

## List of publications

El Aidy, S., & **Waclawiková, B.** (2022). 5-hydroxyindole and analogs thereof as stimulants of gut motility. (Patent No. WO2022010352).

**Waclawiková B\***, Codutti A\*, Alim K & El Aidy S. Gut microbiota-motility inter-regulation: insights from *in vivo*, *ex vivo* and *in silico* studies. *Gut Microbes.* **2022** Jan 3;14(1):1997296. doi: 10.1080/19490976.2021.1997296

\*shared first authorship

**Waclawiková B**, Bullock A, Schwalbe M, Aranzamendi C, et al. Gut bacteria-derived 5-hydroxyindole is a potent stimulant of intestinal motility via its action on L-type calcium channels. *PLoS Biol.* **2021** Jan 22;19(1):e3001070. doi: 10.1371/journal.pbio.3001070.

**Waclawiková B**, El Aidy S. Role of Microbiota and Tryptophan Metabolites in the Remote Effect of Intestinal Inflammation on Brain and Depression. *Pharmaceuticals (Basel).* **2018** Jun 25;11(3):63. doi: 10.3390/ph11030063.



