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

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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 darm**immuunsysteem** 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 immuunreacties 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 mikroflórou 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. průjmy.

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 mikroflóry. Kromě toho jsme také zjistili, že některé typy střevních bakterií mohou metabolizovat serotonin na **kyselinu 5-hydroxyindoloctovou**. Náš výzkum také naznačil, že tento proces, kde serotonin je metabolizován na kyselinu 5-hydroxyindoloctovou, by mohl mít dopad jak na **bakteriální zdatnost**, tak na zlepšení funkce **střevní epiteliální 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 přežívat 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 orgánu sestávající se z dutiny ústní, jícnu žaludku, tenkého střeva, tlustého střeva a konečníku

Imunitní systém: systém, který chrání organismus před nemocemi

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-hydroxyindoloctová: 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 epiteliální bariéra: bariéra tvořená sliznicí (buňkami (epitelem), střevní sliznice - výstelky) ve střevě, která neumožňuje přímý kontakt mezi antigeny (látka 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 (látka), 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.

A

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.

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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 (CryptoGERM: 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 onderzoeksstages 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).

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