Nuoret Tutkijat/ Young Scientists

2024

Bioteknologian laitos/ Department of Life Technologies

UNIVERSITY OF TURKU

NUORET TUTKIJAT / YOUNG SCIENTISTS 2024

Aleksi Kauro	Linda Laiholahti
Alvar Rosenqvist	Linnea Korhonen
Anton Maunu	Mahsa Jafari
Beda Anttila	Marleena Kangas
Cecilia Österlund	Marta Stachnik
Dilakshi Naotunna Palliya Guruge	Meeri Santikko
Dzmitry Paturemski	Megan Maher
Eemeli Vahteristo	Michaela Kotsiou
Ella Suistala	Miisa Litmanen
Elsa Haloila	Niklas Nurminen
Emilia Hemmilä	Niloufar Kazemi
Emma Relander	Nuwandi Jayasenthu Kankanamge
Emmi-Lotta Virta	Osagie Iboi
Frans Uusipaavalniemi	Paola Moyano Gomez
Heli Merenheimo	Paulus Nyman
Ida Bäckström	Pete Tarvonen
Iida Karhu	Ronja Mehtonen
Iida Valta	Roope Korkea-Aho
Iida Raaska	Roosa Pelkonen
Irene Callus	Sambavi Amirthalingam
Israt Esti	Samuel Svärd
Jaakko Hakakari	Sayma Farabi
Jesper Mickos	Shahnawaz Ami
Joonas Silmu	Sofia Antin
Juho Kotikoski	Sonja Tuominen
Julia Pyysalo	Susanna Kallio
Julia Känkänen	Tayyab Saleem
Juuso Triipponen	Teemu Ranta
Katariina Rauhanen	Teemu Lintunen
Katja Niittysalo	Temitope Oyedokun
Katja Salonen	Tuisku Immonen
Klinton Ali	Ville Levola
Lakshmi Karath	Vilma Tupala
Laura Kauppi	Vilma Ryynänen
Laura Mikkola	Zakir Hossain
Liisa Hokkanen	

SEMINAARISESSIOT/ SEMINAR SESSIONS

Mon 08.04.2024 – Fri 12.04.2024; 8:30 onwards
Arje Scheinin auditorium, Dentalia, 2nd floor, Lemminkäisenkatu 2, 20520, Turku

POSTERITAPAHTUMA/ POSTER SESSION

Wed 10.04.2024; 12:15- 14:05
Dentalia, 2nd floor lobby, Lemminkäisenkatu 2, 20520, Turku

MAANANTAI / MONDAY 08.04.2024

8:30-8:35		Professor Jyrki Heino	Nuoret Tutkijat 2024 tapahtuman avaus/Opening of Young Scientists 2024
Session 1		*Chair: Salli Talvi*	
1	8:35-8:55	Beda Anttila	RNA-Decoder - uusi metodi RNA-proteiinikompleksien etsimiseen
2	8:55-9:15	Iida Karhu	Korkean glukoosin vaikutus luuytimen stroomasolujen erilaistumiseen osteoblasteiksi tai adiposyyteiksi
3	9:15-9:35	Vilma Tupala	3D-tulostetun nikkelivapaan metallin vaikutukset osteoblasteihin *in vitro*
4	9:35-9:55	Susanna Kallio	Fibronektiinireseptori embigiini ihmisalkion munuaissolujen soluadheesiossa ja metaboliassa
	9:55-10:15	Tauko/Break	
Session 2		*Chair: Heli Tirkkonen*	
5	10:15-10:35	Marleena Kangas	Hypoksian ja SUMOlaation yhteisvaikutukset munuaissyövässä
6	10:35-10:55	Ronja Mehtonen	PIM-kinaasit estrogeenireseptori α:n säätelijöinä rintasyöpäsoluissa
7	10:55-11:15	Teemu Lintunen	Silikapohjaisten lääkevalmisteiden valmistusprosessin toistettavuuden tutkiminen
8	11:15-11:35	Pete Tarvonen	Aktinomykeettien kehittäminen entsyymien tuottajiksi yksittäisten solujen mutanttien valinnan avulla
	11:35-12:40	Lounastauko/Lunch break	
Session 3		*Chair: Aleksi Nuutila*	
9	12:40-13:00	Ville Levola	Functional analysis of spirochetal promoters using a reconstituted transcription system
10	13:00-13:20	Katariina Rauhanen	From auramycinone to resomycin: Elucidation of the chartreusin biosynthetic pathway
11	13:20-13:40	Julia Känkänen	Purification of ligand-free *Borrelia burgdorferi* Basic membrane protein D
12	13:40-14:00	Sofia Antin	The biosynthesis of the antibiotic lugdunomycin and production of 8-*O*-methyltetrangomycin in *Streptomyces ceolicolor*
13	14:00-14:20	Anton Maunu	Serum complement activity in differentiating between bacterial and viral infections
	14:20-14:40	Tauko/Break	
Session 4		*Chair: Janne Mäkinen*	
14	14:40-15:00	Jesper Mickos	Targeting Clever-1 to overcome therapy resistance in acute myeloid leukemia

15	15:00-15:20	Julia Pyysalo	Purification, Characterization, and Crystallization of a Deoxynivalenol Detoxifying Glutathione Transferase from *Trichoderma reesei*
16	15:20-15:40	Tuisku Immonen	Enzyme activity screening methods for fungal solid-state fermentation
	15:40-16:00	Alumni talk: Aleksi Nyqvist, 3PBIOVIAN	

TIISTAI / TUESDAY 09.04.2024

Session 5		*Chair: Anastasiia Kushnarova-Vakal*	
17	8:30-8:50	Paulus Nyman	Selection and characterization of DARPins against cytoplasmic proteins
18	8:50-9:10	Eemeli Vahteristo	Physical parameters of 3D *in vitro* models of bone directing collagen orientation
19	9:10-9:30	Emilia Hemmilä	Studies on the processing of plasma and patient samples
20	9:30-9:50	Niloufar Kazemi	Characterization of a novel disulfide-stabilized scFv library
	9:50-10:10	Tauko/Break	
Session 6		*Chair: Aapo Knuutila*	
21	10:10-10:30	Miisa Litmanen	An acid-free glyoxal as a fixative for fresh histological tissue samples
22	10:30-10:50	Heli Merenheimo	Spectrally multiplexed quantitative lateral flow immunoassay for *Bordetella pertussis* antibodies
23	10:50-11:10	Ida Bäckström	Development of anti-immunocomplex antibodies for sensitive detection of testosterone
	11:10-12:30	Lounastauko/Lunch break	
Session 7		*Chair: Sami Oksanen*	
24	12:30-12:50	Irene Callus	Development of antibodies for cyanobacterial neurotoxin: anatoxin-a
25	12:50-13:10	Emma Relander	Evaluation of CA19-9 glycovariant immunoassays for the detection of clinically significant prostate cancer
26	13:10-13:30	Iida Raaska	Immunoturbidimetric assay for in-process determination of polyclonal antibody functionality
27	13:30-13:50	Klinton Ali	High sensitive rapid detection of urinary EVs with up converting nanoparticle based lateral flow immunoassay
	13:50-14:10	Tauko/Break	
Session 8		*Chair: Oskar Haavisto*	

28	14:10-14:30	Roope Korkea-Aho	Development of antibody fragments for targeted PET-imaging and single EV-imaging
29	14:30-14:50	Samuel Svärd	Unraveling the spatial dynamics of neurovascular coupling in retinopathy of a mutant MITF mouse model
30	14:50-15:10	Vilma Ryynänen	96-well plate compatible assay format for screening ankyrin repeat proteins on filter paper
31	15:10-15:30	Linnea Korhonen	Mammalian display screening for increased antibody developability
	15:30-15:50	Alumni talk: M.Sc. Mika Saramäki, co-founder and CEO, Anidiagnostics Ltd	

KESKIVIIKKO / WEDNESDAY 10.04.2024

	8:00-8:30	Poster hanging in Dentalia, 2nd floor lobby	
Session 9		*Chair: Selma Salonen*	
32	8:30-8:50	Linda Laiholahti	RT-PCR-kemian optimointi uudelle infektiotestijärjestelmälle
33	8:50-9:10	Katja Niittysalo	Anti-hAFP / anti-hCGβ kuoppalevyn tuotantoprosessin kehitys
34	9:10-9:30	Teemu Ranta	Integroidun sylkinäytteenoton kehittäminen kotitestaukseen
35	9:30-9:50	Alvar Rosenqvist	Troponiini T:n eri muotojen samanaikainen tunnistus sydäninfarktin diagnosoinnissa
	9:50-11:10	Posterinlukuaika/Poster Viewing	
	11:10-12:15	Lunch break	
	12:15-14:05	Posteritapahtuma/Poster Session	
	14:05-14:25	Tauko/Break	
Session 10		*Chair: Helea Junes*	
36	14:25-14:45	Laura Kauppi	Hydrofobiseen vuorovaikutuskromatografiaan perustuvan karakterisointimenetelmän kehittäminen Eu-kelaateilla leimatuille vasta-aineille
37	14:45-15:05	Ella Suistala	Lääkeaineiden kuljetukseen tarkoitettujen polymeerimikropartikkeleiden valmistusmenetelmät
38	15:05-15:25	Joonas Silmu	RNA-polymeraasin ω-alayksikön rooli *Synechocystis* syanobakteerin sopeutuessa typen puutostilaan
	15:25-16:00	Removal of posters from Dentalia, 2nd floor lobby	

TORSTAI / THURSDAY 11.04.2024

Session 11		*Chair: Henri Avela*	
39	8:30-8:50	Roosa Pelkonen	Kaurapohjaisten maidonkorvikkeiden tuotannon CIP-pesujen optimointi ennen lämpökäsittelyä
40	8:50-9:10	Elsa Haloila	Lipidiluokat ja rasvahapot valituissa suomalaisissa järvimikrolevissä
41	9:10-9:30	Liisa Hokkanen	A2- ja hydrolysoidun laktoosittoman A1A2-maidon vaikutukset tulehdukseen ja suolisto-oireisiin
42	9:30-9:50	Emmi-Lotta Virta	Ravitsemusinterventio kaupallisilla kasviproteiinivalmisteilla: Terveysvaikutukset glukoosi- ja lipidiaineenvaihduntaan terveillä koehenkilöillä
43	9:50-10:10	Laura Mikkola	Determination of serum inflammatory markers in response to a plant-based protein dietary intervention
	10:10-10:30	Tauko/Break	
Session 12		*Chair: Shania Saini*	
44	10:30-10:50	Nuwandi Jayasenthu Kankanamge	Development of a novel food product by fermentation of faba bean flour
45	10:50-11:10	Lakshmi Karath	Analysis of alkaloids in fermented and nonfermented blue lupin (*L. angustifolius*) products
46	11:10-11:30	Dzmitry Paturemski	Development, metabolite profile, and sensory qualities of water kefirs with sucrose and non-sucrose sweeteners
47	11:30-11:50	Mahsa Sadat Jafari	Volatile compounds in juices and ciders made from Finnish apple cultivars
	11:50-12:50	Lounastauko/Lunch break	
Session 13		*Chair: Priscilla Ollennu-Chuasam*	
48	12:50-13:10	Sambavi Amirthalingam	The variation of protein, sugars, and organic acids in oat hulls after bioprocessing with enzymes
49	13:10-13:30	Zakir Hossain	Phenolic compounds in selected oat and rice products
50	13:30-13:50	Osagie Iboi	Impact of Latitude and Environmental Conditions on the Tocopherol and Phenolic Content of Sea Buckthorn Leaves
51	13:50-14:10	Meeri Santikko	Identification of carotenoids and phenolic compounds in bacteria-based protein using LC and MS techniques
	14:10-14:30	Tauko/Break	
Session 14		*Chair: Timo Seitz*	

52	14:30-14:50	Iida Valta	Behaviour of n-3 polyunsaturated fatty acid ethyl esters in INFOGEST *in vitro* model
53	14:50-15:10	Michaela Kotsiou	Variability in fatty acid concentration in hemp (*Cannabis Sativa* L.) seeds depending on soil conditions
54	15:10-15:30	Dilakshi Naotunna Palliya Guruge	Analysis of enantiomers and regioisomers of triacylglycerols using supercritical fluid chromatography
15:30-16:00		Alumni talk: M.Sc. Kristiina Aarnio, Product developer at Apetit Oyj	

PERJANTAI / FRIDAY 12.04.2024

Session 15		*Chair: Carla Vecenancio da Silva*	
55	8:10-8:30	Temitope Oyedokun	Fermentation of bladderwrack (*Fucus vesiculosus*) and its impact on composition
56	8:30-8:50	Megan Maher	Fermentation of *Fucus vesiculosus*: Sensory evaluation and product innovations
57	8:50-9:10	Niklas Nurminen	Lactic acid fermentation of crowberry juice: Effects on phenolic compounds
58	9:10-9:30	Jaakko Hakakari	Enzymatic and microbial solubilization of brewers' spent grain for sugar and phenolic compound extraction
59	9:30-9:50	Marta Stachnik	Utilization of brewer's spent grain and other selected by-products to create edible cutlery
9:50-10:10		Tauko/Break	
Session 16		*Chair: Minna Konert*	
60	10:10-10:30	Aleksi Kauro	Kilpailevien mikrobien vaikutus Streptomykeettien geenien ilmenemiseen
61	10:30-10:50	Juho Kotikoski	Transcriptome analysis unveils regulatory landscape of *Spirochaeta africana*
62	10:50-11:10	Tayyab Saleem	Transcriptome of the SigB overexpression strain of cyanobacterium *Synechocystis* sp. PCC6803
63	11:10-11:30	Shahnawaz Ami	Physiological roles of the chloroplast acetyltransferase GNAT1 and GNAT2 in *Arabidopsis thaliana*
11:30-12:30		Lounastauko/Lunch break	
Session 17		*Chair: Roland Ndeh*	
64	12:30-12:50	Israt Esti	New nanoparticle aided glycovariant biomarker tools to detect extracellular vesicles as a liquid biopsy for early diagnosis of bladder cancer

65	12:50-13:10	Sayma Farabi	Tunneling nanotubes enabling enterovirus cell-to-cell transmission – a novel way of spreading
66	13:10-13:30	Paola Moyano Gomez	Using Shape-Focused Pharmacophore Modeling to Improve Docking Screening with Acetylcholinesterase
67	13:30-13:50	Katja Salonen	Development of an LC-IM-MS method for profiling novel N-acyl amides in fecal samples
	13:50-14:10	Tauko/Break	
Session 18		*Chair: Lauri Kakko*	
68	14:10-14:30	Cecilia Österlund	A comparative analysis of qualification process for pharmaceutical process equipment
69	14:30-14:50	Sonja Tuominen	Methane potential of oat husk waste streams in anaerobic digestion
70	14:50-15:10	Juuso Triipponen	Fotoautotrofinen etyleenin tuotanto korkean solutiheyden syanobakteerikasvatuksessa
71	15.10-15:30	Frans Uusipaavalniemi	Fotoautotrofinen 3-hydroksibutyraatin jatkuvatoiminen tuotanto ja optimointi muokatuissa *Synechocystis sp.* PCC 6803 -kannoissa
	15:30-15:50	Alumni talk: PhD Olli Virtanen, Research Associate, Free University of Amsterdam	
	15:50	Professor Eevi Rintamäki	Päätössanat/Closing words

PRACTICAL INFORMATION FOR THE EVENT

- Register to each session using an information sheet circulating in the room.
- For the poster session on April 10th at 12:15-14:05, the authors must be present next to their poster stands. Similar to the presentation ballot voting, there will be a voting for the Best Poster along with refreshments.
- Kindly use the tag @studyinturku for social media engagement posts about the event.

EVENT'S ORGANIZING COMMITTEE

- Coordinators: Jukka-Pekka Suomela, Annelie Damerau, and Marika Kalpio
- Volunteers: Shania Saini (Booklet; Cover art) and Carla Vecenancio da Silva (Website; Press Release; Poster compilation)

RNA-Decoder — Uusi menetelmä RNA-proteiinikompleksien tutkimiseen

Beda Anttila
Ohjaajat: FT Matti Turtola, FT Desire Garcia Pichardo, Prof. Sebastian Marquardt
MOLEKYYLIBIOTIETEET, BIOKEMIA

Pitkät ei-koodaavat RNA-molekyylit (long non-coding RNA, lncRNA) ovat laaja joukko RNA-molekyylejä, jotka osallistuvat esimerkiksi solujen metabolian säätelyyn. Usein lncRNA:t eivät toimi soluissa yksin, vaan ne muodostavat yhdessä RNA:ta sitovien proteiinien kanssa funktionaalisia ribonukleoproteiinikomplekseja. Yhdessä ne vaikuttavat geenien ilmenemiseen esimerkiksi tekemällä epigeneettisiä muutoksia kromatiiniin tai säädellen transkriptiota ja translaatiota.

Tutkimuksen tavoitteena oli kehittää uusi menetelmä, RNA-Decoder, jonka avulla olisi mahdollista tunnistaa uusia RNA-proteiinikomplekseja *Saccharomyces serevisiae* -hiivasta ja saada lisätietoa lncRNA:iden vaikutusmekanismeista.

Tutkimuksessa vertailtiin RNA-proteiinikomplekseja kahdesta, lähekkäin olevasta lokuksesta, joista toinen tuottaa proteiinia koodaavaa lähetti-RNA:ta (messenger RNA, mRNA) ja toinen lncRNA:ta. Tutkimuksessa käytettiin tuhansia hiivakantoja sisältävää kirjastoa, jossa tutkittavissa lokuksissa oli kiinnitettynä spesifinen DNA-viivakoodi ja hiivan proteiineissa TAP-tag (tandem immunopurification tag) DNA-viivakoodit erosivat hiivakirjaston yksittäisissä TAP-tag -kannoissa, sisältäen tiedon mihin solun proteiiniin TAP-tag oli kiinnitetty kyseisessä solussa. Menetelmässä hiivoissa muodostuneet RNA-proteiinikompleksit eristettiin TAP-tag immunosaostuksella ja RNA käännettiin komplementaariseksi DNA:ksi (cDNA). cDNA monistettiin polymeraasiketjureaktiolla (PCR), jotta viivakoodialueet voitaisiin sekvensoida. Sekvensointitulokset mahdollistavat mRNA:han ja lncRNA:han kiinnittyvien proteiinien vertailun.

Alustavat tulokset osoittavat, että menetelmä toimii. Western blot ja Silver staining -määritykset osoittavat, että RNA-proteiinikompleksien immunosaostus oli onnistunut. cDNA:sta monistettujen PCR-tuotteiden koko vastasi positiivisen markkerin kokoa, viitaten siihen, että myös RNA:n kääntäminen cDNA:ksi ja cDNA:n monistaminen ovat onnistuneet. Tulosten varmistamiseksi ja muodostuvien mRNA-proteiini- ja lncRNA-proteiinikompleksien erojen havaitsemiseksi cDNA:n sisältämät viivakoodialueet pitää vielä sekvensoida.

Asiasanat: pitkä ei-koodaava RNA, lncRNA, RNA-proteiinikompleksi, immunopresipitaatio, DNA-viivakoodaus, Saccharomyces serevisiae

Korkean glukoosin vaikutus luuytimen stroomasolujen erilaistumiseen osteoblasteiksi tai adiposyyteiksi

Iida Karhu
Ohjaajat: PhD. Dos. Kaisa Ivaska-Papaioannou, M.Sc. Niki Jalava
BIOKEMIA

Luu on elävää kudosta, jota rakennetaan ja hajotetaan jatkuvasti elämän aikana. Luuytimestä löytyvät mesenkymaaliset stroomasolut, (MSC), voivat erilaistua mm. luuta muodostaviksi osteoblasteiksi ja rasvasoluiksi, eli adiposyyteiksi. Tyypin 2 diabetes (T2D) on metabolinen sairaus, jossa tyypillistä on pitkittynyt korkea verensokeri, eli hyperglykemia, insuliiniresistenssi sekä luustokomplikaatiot, kuten luumateriaalin hauraus ja luuytimen rasvoittuminen.
Aiemmissa tutkimuksissa havaittiin, että glukoosin kuljettajaproteiini 4 (GLUT4) säätelee MSC:n erilaistumista ja insuliinin säätelemä GLUT4:n toiminta heikkenee tyypin 2 diabeteksessa insuliiniresistenssin myötä. GLUT4 säätelee tioredoksiinin kanssa vuorovaikuttavan proteiinin (TXNIP) ilmentymistä. TXNIP:n ilmentyminen kasvaa hyperglykemiassa ja vaikuttaa solun oksidatiivisen stressin lisääntymiseen. Työn tavoitteena oli tutkia GLUT4 ja TXNIP hiljentämisen vaikutusta MSC-solujen erilaistumiseen, fysiologisessa (5.5 mM glukoosi), sekä hyperglykemisessä ympäristössä (25 mM glukoosi).
Rotan luuytimestä eristetyistä MSC-soluista hiljennettiin GLUT4 tai TXNIP siRNA-menetelmällä, minkä jälkeen solujen erilaistumista osteoblasteiksi tai adiposyyteiksi analysoitiin mittaamalla osteoblasteille ja adiposyyteille tyypillisten geenien ilmentymistä qPCR:llä. Lisäksi solujen elinkykyä mitattiin AlamarBlue määrityksellä.
GLUT4-hiljennettyjen solujen erilaistuminen ohjautui enemmän rasvasoluiksi heikentäen samalla osteoblastien erilaistumista. Vaikutus oli voimakkaampi hyperglykemisessä kasvuympäristössä. TXNIP-hiljennettyjen solujen erilaistuminen rasvasoluiksi puolestaan heikkeni, mutta GLUT4 ilmentyminen nousi, mikä viittaa, että TXNIP vaikuttaa GLUT4:n toimintaan. GLUT4-hiljennettyjen solujen elinkyky laski, mutta TXNIP:n hiljentämisellä ei näyttäisi olevan vaikutusta solujen elinkykyyn.
GLUT4 toiminta on välttämätöntä osteoblastien erilaistumiselle ja sen puute edistää erilaistumista adiposyyteiksi, sekä nostaa TXNIP:n ilmentymistä. Jatkotutkimuksissa olisi kiinnostavaa selvittää syvällisemmin TXNIP:n ja GLUT4:n vuorovaikutusta ja sen merkitystä osteoblastien erilaistumiseen.

Avainsanat: MSC, Osteoblasti, Adiposyytti, T2D, hyperglykemia, GLUT4, TXNIP

3D-tulostetun nikkelivapaan metallin vaikutukset osteoblasteihin in vitro

Vilma Tupala
Ohjaajat: FT, Dos. Terhi Heino, FM Karoliina Kajander
SOLUBIOLOGIA

Osteoblastit ovat mesenkymaalisista kantasoluista peräisin olevia luuta muodostavia soluja. Luiden kasvun ja luun uudelleenmuodostumisen aikana ne vastaavat luukudoksen muodostumisesta ja mineralisaatiosta. Ikääntyessä kantasolujen määrä laskee ja niiden erilaistumiskapasiteetti heikkenee, minkä seurauksena luun uusiutumiskyky alenee. Tällöin luumassa vähenee, eivätkä esimerkiksi murtumat parane yhtä tehokkaasti.
Biomateriaalit ovat synteettisiä tai biologista alkuperää olevia materiaaleja, joilla pyritään korjaamaan vaurioitunutta kudosta ja palauttamaan sen toiminta ennalleen. Biomateriaaleja, kuten erilaisia metalleja, voidaan käyttää proteesien valmistamisessa, kun tavoitteena on palauttaa kudoksen tai elimen normaali anatominen rakenne. Metallin 3D-tulostaminen on uusi menetelmä, joka mahdollistaa potilaskohtaisten implanttien valmistamisen. Metalliseoksissa usein käytetty nikkeli aiheuttaa kuitenkin ongelmia, sillä se on yleinen kosketusallergian aiheuttaja ja voi myös mahdollisesti aiheuttaa syöpää.
Tässä tutkimuksessa selvitettiin 3D-tulostetun nikkelivapaan ruostumattoman teräksen, PANACEAn, vaikutusta osteoblasteihin. Tavoitteena oli arvioida sen mahdollista käyttöä tulevaisuudessa implanttimateriaalina. Tutkimuksessa materiaaleina käytettiin nikkelivapaata ruostumatonta terästä (PANACEA) ja kontrollimateriaalina nikkeliä sisältävää ruostumatonta terästä 316ss. Tutkimuksessa tarkasteltiin sitä, miten koemateriaalit vaikuttavat osteoblastien elinkelpoisuuteen ja erilaistumiseen. Tämän selvittämiseksi hiirestä eristetyn osteoblastisen solulinjan soluja viljeltiin 3D-tulostettujen teräskappaleiden päällä. Solujen elinkelpoisuutta mitattiin AlamarBlue reagenssilla ja metallien vaikutusta osteoblastien erilaistumiseen tutkittiin värjäämällä solut niiden erilaistumisesta kertovan alkaalisen fosfataasin suhteen.
Tutkimuksessa havaittiin, että solujen kasvu metallien päällä oli heikkoa, verrattuna solujen kasvuun soluviljelymuovilla. Nikkelivapaan PANACEAn päällä solut kasvoivat paremmin verrattuna metalliin 316ss, mutta metallien välinen ero oli pieni. Näin selkeitä eroja ei nähty mitattaessa solujen erilaistumista. Havaittiin, että ne solut, jotka olivat saaneet kasvaa ilman metalleja, erilaistuivat paremmin, mutta erilaistumista oli selkeästi havaittavissa myös metallien päällä kasvaneilla soluilla.

Avainsanat: osteoblasti, biomateriaali, PANACEA, 3D-tulostus, viabiliteetti

Fibronektiinireseptori embigiini ihmisalkion munuaissolujen soluadheesiossa ja metaboliassa

Susanna Kallio
Ohjaajat: FT Johanna Jokinen, FM Salli Talvi, Prof. Jyrki Heino
SOLUBIOLOGIA

Embigiini (EMB, GP70) on fibronektiinireseptori, joka kuuluu immunoglobuliini superperheeseen. Se on soluadheesioon osallistuva solukalvoproteiini, mutta se osallistuu myös solun metaboliaan toimimalla avustavana proteiinina monokarboksylaattikuljettimille (monocarboxylate transporter, MCT). Monokarboksylaattikuljettimet katalysoivat monokarboksylaattien, kuten L-laktaatin ja pyruvaatin, kuljettamista solukalvon läpi. Embigiini avustaa monokarboksylaattikuljettimia asettumaan solukalvolle ja muodostaa niiden kanssa kompleksin solukalvolla. Embigiinin tiedetään avustavan erityisesti MCT2 lokalisaatiota, mutta myös MCT1, -3, -4 ja -7.
Erikoistyössä käytettiin ihmisalkion munuaissolujen (human embryonic kidney, HEK) 293T-kloonia. Työn tavoitteena oli selvittää, että vaikuttaako embigiinin ilmentymisen hiljentäminen solun adheesioon tai monokarboksylaattikuljettimien ilmentymiseen. Tutkimusta tehtiin solu-, proteiini- ja mRNA-tasolla. Embigiinin ilmentyminen soluissa karakterisoitiin immunoblottauksella ja monokarboksylaattikuljettimien puolestaan PCR-menetelmällä. Embigiinin ilmentymistä vähennettiin RNA-häirinnällä (RNA interference (RNAi)) transfektoimalla solun sisään embigiiniin kohdennettuja pieniä häiritseviä RNA-molekyylejä (small interfering RNA, siRNA). Hiljentymisen onnistuminen varmistettiin immunoblottauksella. Negatiivisen kontrollin ja hiljennettyjen solulinjojen kiinnittymistä fibronektiiniin, poly-L-lysiiniin ja naudan seerumin albumiiniin tutkittiin mikroskopialla ja xCELLigence-laitteella. Solulinjojen embigiinitasoa ja MCT1:n ilmentymistä tarkasteltiin konfokaalimikroskoopilla.
HEK293T-solujen havaittiin ilmentävän embigiiniä ja monokarboksylaattikuljettimia 1, 2 ja 7-10. Embigiini pystytään hiljentämään onnistuneesti kahdella eri siRNA:lla (84 ± 0 %, 88 ± 6 % (48 h); 93 ± 1 %, 90 ± 1 % (72 h)). Embigiinin hiljentäminen heikentää solujen adheesiota fibronektiiniin ja poly-L-lysiiniin. Hiljentäminen saattaa myös vaikuttaa MCT1:n ilmentymiseen, mutta tämä vaatii lisätutkimuksia.

Asiasanat: embigiini, fibronektiini, monokarboksylaattikuljetin, RNA-häirintä, soluadheesio

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Hypoksian ja SUMOlaation yhteisvaikutukset munuaissyövässä

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Ohjaajat: Dos. Maria Sundvall, Prof. Panu Jaakkola, FM Antti Kukkula
SOLUBIOLOGIA

SUMOlaatio on translaation jälkeinen proteiinimodifikaatio, jossa pienet ubikitiinin kaltaiset SUMO-proteiinit säätelevät kohdeproteiinien toimintaa. Sen on havaittu muuttuvan vähähappisissa olosuhteissa ja vaikuttavan muiden proteiinien säätelyyn syövässä. SUMOlaatiota estävien lääkkeiden vaikutuksia on tutkittu eri syöpätyyppien hoidossa.
Hypoksia eli alhainen happipitoisuus saa aikaan hypoksian indusoituvan tekijän (HIF) pitoisuuden kasvun soluissa. Useimmissa munuaissolukarsinooma-tapauksissa (engl. *Renal cell carcinoma,* RCC) hypoksiassa aktivoituva transkriptiotekijä HIF, on jatkuvasti aktiivinen von-Hippel-Lindau geenin (VHL) mutaation seurauksena. HIF-tekijät lisäävät kasvaimen kasvua edistävien geenien ilmentymistä. SUMOlaation on näytetty säätelevän HIF:n toimintaa, mutta kokonaisuutena hypoksian vaikutuksesta SUMOlaatioon tai SUMOlaation eston vaikutuksista munuaissyöpäsoluissa ei ole vielä paljoa tietoa.
Tavoitteena oli tutkia kolmessa munuaissolukarsinoomasolulinjassa tapahtuvaa SUMOlaatiota sekä tavallisessa, että vähähappisissa olosuhteissa. Kokeissa tutkittiin SUMOlaatioon ja hypoksiaan liittyvien erilaisten proteiinitasojen ekspressiovaihteluita eri menetelmiä käyttäen. Lisäksi tutkimuksessa tarkasteltiin globaalia SUMOlaatiota vähentävän lääkeaineen vaikutusta solujen kasvuun ja proteiinien ekspressioon hypoksiassa. Työssä käytettiin muun muassa 3D-soluviljelyä mallintamaan lääkkeen vaikutusta solujen kasvuun. Tutkimuksessa oltiin kiinnostuneita siitä, vaikuttaako SUMOlaation esto munuaissyöpäsolujen kasvuun ja vaikuttaako hypoksiareitin aktivaatio globaaliin SUMOlaatioon.
Tutkimuksen tulokset viittasivat siihen, että hypoksia vaikuttaa globaaliin SUMOlaatioon ja SUMOlaation esto solujen kasvuun eri munuaissyöpäsolulinjoissa.

Asiasanat: hypoksia, hypoksian indusoiva tekijä (HIF), munuaissolukarsinooma, proteiinien post-translationaalinen muokkaus, SUMOlaatio

PIM-kinaasit estrogeenireseptori α:n säätelijöinä rintasyöpäsoluissa

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SOLUBIOLOGIA

Estrogeenireseptori α (ERα) on transkriptiofaktorina toimiva hormonireseptori. ERα-signalointi on tärkeä solujen jakautumisen säätelijä normaalissa fysiologiassa, mutta myös ERα:aa ekspressoivissa rintasyövissä. Noin 70 % rintasyövistä ekspressoi ERα:aa. ERα aktivoituu perinteisesti sen ligandin, estrogeenin, sitouduttua siihen, mikä mahdollistaa ERα-dimeerin muodostumisen. Dimeeri toimii transkriptiofaktorina ja joko inhiboi tai aktivoi kohdegeeniensä transkriptiota. Dimeeri säätelee suoraan geenejä, jotka sisältävät estrogeenin vaste-elementin (ERE) promoottorialueellaan. ERα-signalointia säätelee ligandin sitoutumisen lisäksi muun muassa ERα:n fosforylaatio. Fosforylaatio voi myös aktivoida ERα:n ilman ligandia.
PIM-kinaasit ovat proto-onkogeenejä ja niitä yliekspressoidaan monissa syövissä. Tutkimusryhmässämme on havaittu, että PIM-kinaasit voivat fosforyloida ERα:n seriini 167 (S167) -kohdasta. Tässä tutkimuksessa haluttiin tutkia S167-kohdan vaikutusta rintasyöpäsolujen fenotyyppiin poistamalla se soluista CRISPR-Cas9-pohjaisella prime-editointitekniikalla. Tutkimuksessa käytettiin T47D-soluja, jotka ovat endogeenisesti PIM-kinaaseja ja ERα:aa ekspressoivia rintasyöpäsoluja. S167-aminohappo saatiin muutettua alaniiniksi (S167A) T47D-soluissa heterotsygoottisesti, eli toinen *ERα*-alleeleista saatiin muokattua. Fenotyyppisissä kokeissa tämän S167A-mutaation ei havaittu vaikuttavan solujen jakautumis- tai elinkykyyn, mutta se lisäsi ERE-promoottorialueen säätelemän lusiferaasireportterigeenin ekspressiota. Sen sijaan yhden tunnetuimman estrogeenin säätelemän geenin, *TFF1*:n, ekspressio väheni selvästi proteiinitasolla. Tulokset viittaavat siihen, että S167-kohdan fosforylaatiolla on merkitystä rintasyöpäsolujen ERα-välitteisessä geenien ekspression säätelyssä. Aiheesta tarvitaan kuitenkin vielä lisätutkimuksia soluilla, joissa S167A-mutaatio on saatu tehtyä homotsygoottisesti eli kumpaankin *ERα*-alleeliin.

Asiasanat: estrogeenireseptori α, fosforylaatio, PIM-kinaasit, prime-editointi, rintasyöpä

Silikapohjaisten lääkevalmisteiden valmistusprosessin toistettavuuden tutkiminen

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BIOTEKNIIKKA

Edistys lääkekehityksessä on johtanut aiempaa monimutkaisempien lääkemolekyylien löytämiseen. Muun muassa biotekniikan edistysaskelet ovat mahdollistaneet entistä parempien menetelmien kehittämisen ja käyttöönoton, jolloin myös kehitettävät biomolekyylilääkkeet voivat olla rakenteeltaan monimutkaisempia. Mahdollisuus annostella lääke siten, että vaikuttava aine saadaan toimintakykyisenä sekä suunnitelluissa määrin elimistön puolustujärjestelmien ohi kohteeseen, on tärkeä osa valmistusprosessia. Koska lääkemolekyylin monimutkaisuus kulkee käsi kädessä rakenteen hienovaraisuuden kanssa, on monen lupaavan biomolekyylin käytettävyys lääkkeenä vaatinut ja tulee vaatimaan innovatiivisia ratkaisuja erityisesti lääkekuljetuksen saralla.
DelSiTech valmistaa lääkevalmisteita silikamikropartikkeli-silikahydrogeeli-komposiittiin perustuvalla menetelmällä. Tuotetut geelimäiset lääkevalmisteet, joiden rakenteessa on kapseloituna aktiivinen lääkeaine, voidaan annostella esimerkiksi potilaan silmään tippana, injektiona silmän sisään tai ihonalaisena injektiona, josta geelivalmisteen biohajoamisen säätelemänä lääkeaine vapautuu potilaan elimistöön. Kyseisellä teknologialla valmistetut lääkevalmisteet ovat lupaavia muun muassa pitkäaikaisten lääkitysten annostelussa sekä herkkien lääkeainemolekyylien toimittamisessa toimintakykyisinä perille.
Tässä työssä tutkitaan yrityksen lääkevalmisteiden valmistusprosessia kiinnittäen huomiota erityisesti prosessin toistettavuuteen. Toistettavuuden tutkimisessa perehdytään siihen, mikä vaikuttaa väli- ja lopputuotteiden ominaisuuksiin ja edelleen jos prosessi tehdään samalla tavalla useita kertoja, ovatko tuotteet samanlaisia. Tutkimus toteutetaan koesuunnittelua hyödyntäen toistamalla valmistusprosessia pienin muutoksin, jotta pystytään arvioimaan syy-seuraus-suhteita. Olennaisia menetelmiä valmistusprosessissa ovat sooli-geeli-tekniikka sekä sumukuivaus. Väli- ja lopputuotteita analysoidaan niiden viskositeetin, partikkelikokojakauman, geelautumisajan, reaktiolämmön, hapettavuuden sekä biohajoamisnopeuden perusteella.

Avainsanat: Lääkeformulointi, biomateriaalit, sooli-geeli-tekniikka, reologia, koesuunnittelu, sumukuivaus

Aktinomykeettien kehittäminen entsyymien tuottajiksi yksittäisten solujen mutanttien valinnan avulla

Pete Tarvonen
Ohjaajat: Tutkijatohtori Benjamin Nji Wandi, Prof. Mikko Metsä-Ketelä
BIOMOLEKYYLIEN TUOTANTO (DI)

Aktinomykeetit ovat grampositiivisia maaperän bakteereja, joilla on merkittävä rooli maaperän ekologiassa. Aktinomykeettejä hyödynnetään useissa eri bioteknisissä sovelluksissa ja yli puolet antibiooteista tuotetaankin aktinomykeetteihin kuuluvissa bakteereissa. Grampositiivisina bakteereina aktinomykeetit pystyvät tehokkaasti erittämään tuottamiaan yhdisteitä, kuten natiiveja proteiineja, solun ulkopuolelle. Aktinomykeeteillä on luonnostaan laaja kirjo entsyymejä erilaisten monimutkaisten kasvi- tai äyriäispohjaisten polymeerien pilkkomiseen. Nämä entsyymit ovat tärkeitä mm. paperi-, sellu- ja pesuaineteollisuudessa ja aktinomykeettejä käytetäänkin monien teollisuusentsyymien tuottajina.

Kannankehitys on tärkein, työläin ja hitain osa teollisuusproteiinien tuottoprosessissa. Proteiinien tuotto tapahtuu yleisesti bioreaktoreissa, joten proteiinin tuottotaso on suurimpia vaikuttajia tuottoprosessin kaupallisessa kannattavuudessa. Kannankehityksessä hyödynnetään perinteisesti sattumanvaraista mutageneesiä, jonka tuottamia mutanttikantoja kasvatetaan tuottotasojen ja -profiilin selvittämiseksi. Teollisen mittakaavan tuottokannan löytäminen voi vaatia kymmeniätuhansia pullokasvatuksia, joka vie etenkin aktinomykeettejä käytettäessä huomattavan määrän aikaa johtuen näiden bakteerien verrattain pitkästä 4–6 tunnin kaksiintumisajasta. Aktinomykeetit kasvavat rihmastona, mikä osaltaan myös sulkee pois yksittäisten solujen tasolla käytettävät perinteisemmät menetelmät kannankehityksessä.

Aktinomykeettien kannankehityksen helpottamiseksi kehitettiin menetelmä, joka mahdollistaa aktinomykeettien kannankehityksen yksittäisten solujen tasolla. Metodologia perustuu kaksitasoiseen selektioon, jonka avulla sattumanvaraisen mutageneesin tuottamat mutantit voidaan kasvattaa mutanttikirjastona, josta todennäköisesti parhaan tuoton omaavat yksilöt voidaan valita miljoonista mutanteista hyödyntäen virtaussytometriaa. Tässä työssä kyseistä metodologiaa testattiin kehittämällä viiden eri entsyymituottokannan tuottotasoja ja tutkimalla niiden kannankehitystä.

Avainsanat: aktinomykeetti, kannankehitys, entsyymi, virtaussytometria, sattumanvarainen mutageneesi

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Functional analysis of spirochetal promoters using a reconstituted transcription system

Ville Levola
Supervisors: M.Sc. Vilma Trapp, Assoc. Prof. Georgy Belogurov
BIOCHEMISTRY

Transcription is the first and most regulated step in gene expression. Transcription is catalyzed by RNA polymerase (RNAP), a complex multisubunit enzyme. RNAP can read regulatory signals encoded in the genomic DNA and respond to the changes in the concentration of substrate NTPs. However, most of the regulatory inputs are delivered to RNAP via accessory protein factors and regulatory RNAs. The transcription systems of model organisms such as *Escherichia coli* and *Bacillus subtilis* have been studied in great detail. In contrast, transcription systems of spirochetes are poorly understood. To study spirochetal transcription, we selected *Spirochaeta africana* as a model organism. We expressed and purified components, reconstituted the core transcription system of *S. africana in vitro* and measured the activities of selected promoters and transcription factors.

We measured the *in vitro* activity of *S. africana* and *E. coli* RNAPs at nine selected spirochetal promoters and a control *E. coli* promoter at pH 7.5, which is close to intracellular pH in *E. coli*. Our transcription templates encoded fluorogenic RNA aptamer called Broccoli downstream the studied promoters. We monitored the transcription output by following the fluorescence levels of the RNA aptamer bound to a fluorogen. The activity of spirochetal RNAP was several folds lower than that of *E. coli* RNAP at most promoters. We then measured the transcription activities of spirochetal RNAP at pH 9, which is close to the optimal growth pH for these bacteria. Increasing pH significantly increased the activity of *S. africana* RNAP at several promoters bringing the overall activity profile closer to that of *E. coli* RNAP. Prior studies in our laboratory indicate that activities of some *S. africana* promoters are modulated by transcription factor CarD. We reproduced previously observed effects of CarD at two promoters and discovered an additional CarD activatable promoter. Overall, the activity profile of spirochetal RNAP matched the *E. coli* RNAP profile best when the former enzyme was assayed at pH 9 in the presence of CarD. As a part of the investigation, we also used primer extension technique to map the transcription start sites for a subset of promoters to confirm their identities.

Overall, our studies on spirochetes aim to enhance the understanding of their physiological mechanisms. This information can be used in the development of treatments for diseases propagated by pathogenic spirochetes, such as borreliosis.

Keywords: fluorescence, primer extension, promoter, start sites, transcription

From auramycinone to resomycin: Elucidation of the chartreusin biosynthetic pathway

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BIOCHEMISTRY

Anthracyclines are aromatic polyketide antibiotics produced in bacteria genus *Streptomyces*. Anthracyclines possess many medically interesting bioactivities, and some are among the most effective cancer drugs. One important anthracycline, chartreusin has been researched for its antibacterial activity and many steps of its biosynthetic pathway have been elucidated. Chartreusin is structurally very similar to a potent cancer drug elsamicin A. Detailed knowledge of the biosynthetic pathway of chartreusin is essential for production of new compounds by genetic engineering and synthetic biology to gain improved drugs.

Anthracyclines are biosynthesized by type II polyketide synthases (PKSs), which first form a highly reactive polyketide carbon chain. Stable polycyclic anthracycline intermediates are made by accessory PKSs for example aromatases and cyclases. Tailoring PKSs for example oxidases and glycosylases form the final bioactive products. Tailoring steps are rather complex and make anthracyclines a chemically diverse group. The aim of this study was to find out which tailoring enzymes in the chartreusin pathway catalyse the conversion from auramycinone to resomycin, a process where two dehydration reactions occur.

The products from enzymatic assays were assessed with ultra-performance liquid chromatography (UPLC) and LC-MS (liquid chromatography-mass spectrometry). The results suggest a conversion from auramycinone to resomycin. The structure of resomycin needs to be verified by NMR (nuclear magnetic resonance) in the future.

Interestingly, the observed dehydration is a novel reaction type for the protein family, increasing its catalytic abilities. Proteins in the same family catalysing very different chemical reactions is probably a result of mutations in catalytic amino acids. For this reason, crystallization experiments have been initiated to solve crystal structures and to know how a switch in their chemistry has happened.

Keywords: natural products, biosynthesis, chartreusin, anthracyclines, dehydration, streptomyces

Purification of ligand-free *Borrelia burgdorferi* Basic membrane protein D

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BIOCHEMISTRY

Lyme borreliosis (LB) is an inflammatory disease caused by *Borrelia burgdorferi* transmitted by ticks. The symptoms vary depending on the bacterial species, but the infection causes flu-like symptoms, and inflammation in skin, joints, and central nervous system. LB is treated with broad-spectrum antibiotics. There is demand for a new specific antibiotic, since the incidence of the disease is growing, the health care costs are relatively high, and the currently used broad-spectrum antibiotics can cause adverse effects and antibiotic resistance.

Ribosome-inhibiting antibiotic hygromycin A has been found to be specifically imported into *B. burgdorferi* cells by Basic membrane protein D (BmpD), a purine nucleoside transporter. *B. burgdorferi* cannot synthesize purines *de novo* but needs to import them from the environment. This essential function makes BmpD a good target for antibiotic development.

The crystal structure of BmpD in complex with endogenous adenosine has been determined. Recombinant BmpD has been purified using metal affinity chromatography with an extensive wash step including 1 M urea to remove ligands, but the resulting protein still has bound adenosine. Ligands can be removed more thoroughly with on-column denaturation and refolding, but for an unknown reason, the current His-tagged protein is released from the metal affinity resin in denaturing conditions (8 M urea). The aim of this project is to optimize the construct of a tagged BmpD such that it could be denatured, washed, and refolded on-column, yielding a fully ligand-free protein in sufficient amounts for crystallization with novel ligands.

Keywords: antibiotics, BmpD, crystallization, Lyme borreliosis, protein purification

The biosynthesis of the antibiotic lugdunomycin and production of 8-*O*-methyltetrangomycin in *Streptomyces ceolicolor*

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CELL BIOLOGY

Antimicrobial resistance is a quickly growing threat with a huge global impact. Majority of antibiotics have been sourced from soil-dwelling bacteria of *Streptomyces* genus, which are naturally tremendous at producing broad-spectrum antibiotics, such as kanamycin and streptomycin. Still, their potential as antimicrobial compound producing organisms is to be fully harnessed. According to genomic studies, various species of *Streptomyces* are known to have the ability to produce numerous unknown secondary metabolites with potential bioactivity.
Polyketides are the largest group of secondary metabolites produced by *Streptomyces*. Several polyketides, such as tetracycline and erythromycin, are in wide clinical use as, for example, antibacterials, antitumor agents, and antifungals. Angucyclines are a large and diverse group of aromatic polyketides distinguished by their unique bent four-ring benz[*a*]anthraquinone scaffold. Lugdunomycin is an angucycline derived secondary metabolite which has been of great interest due to its complex and atypical structure and elusive biosynthetic patwhay. Lugdunomycin represents a novel subclass of aromatic polyketides which have undergone cleavage of the C-ring, leading to drastic and complex structural arrangements.
The lugdunomycin pathway is still to be fully characterized, as the biosynthesis requires multiple complex steps. The aim is to construct the pathway step by step using the BioBricks method for heterologous expression in *S. coelicolor*. With this method, a library consisting of single gene building blocks can be established. Producing the pathway intermediates for enzymatic assays provides essential information about the mechanism of the biosynthesis. Understanding the pathway is of great importance as it would greatly benefit designing novel synthetic antibiotic structures.

Keywords: antibiotic, *Streptomyces*, polyketides, angucyclines, lugdunomycin

Serum complement activity in differentiating between bacterial and viral infections

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CELL BIOLOGY

The serum complement system is humoral part of the innate immune system that helps kill pathogens by opsonizing them, recruits more phagocytes to the site of infection or uses its proteins to lyse the pathogens on their own. Complement system has three separate initiation pathways, classical-; lectin- and alternative-pathways, all of which lead to the activation of a common lytic reaction pathway and the formation of the membrane-attack complex (MAC). When MAC is formed on the target pathogens lipid layer, it makes a hole in it and lyses the cell.
Assembly of MAC is initiated by proteolytic cleavage of C5 component by C5 convertases formed in the initiation pathways into C5a and C5b, of which the latter starts the formation by recruiting C6 and C7 to form C5b67 complex. C5b67 anchors itself to the lipid layer hydrophobically when formed and after binding and anchoring C8 to the layer as well, the C8α-γ subunit recruits 10-16 C9 that form the ring-shaped structure that makes the hole on the layer and leads to the lysing of the target cell. This lysing function does work only on some pathogens for example *Escherichia Coli* which was used in this research.
The aim of this study was to see if the patient samples that had diagnosed bacterial infections when collected, differ from those that had diagnosed viral infections, in complement activity measured by exposing the Luciferase modified *Escherichia Coli (E. coli-lux)* to the diluted serum. We monitored this by measuring the bioluminescence levels, which act as proof of life, of the *E. coli-lux* for three and half hours after exposing it to different concentrations of the serum samples. Of the 166 bacterial infections and 168 viral infections measured there was significant difference in the complement activity in the data from 0,5% serum concentration levels.

Keywords: blood serum, patient samples, bacteria, virus, diagnostics

Targeting Clever-1 to overcome therapy resistance in acute myeloid leukemia

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CELL BIOLOGY

Acute myeloid leukaemia (AML) is a haematological malignancy which results from the accumulation of undifferentiated and hyperproliferative cells of the myeloid lineage. Current frontline treatment strategies often achieve remission and recent advances such as venetoclax have markedly improved patient outcomes. However, relapse and resistance are still common in previously responsive patients, indicating an unmet clinical need for effective drugs to overcome therapy resistance.

Relapse is caused by a subpopulation of therapy-resistant cells with a stem-like phenotype, termed leukemic stem cells. Accordingly, the expression of stemness markers in AML patients is a poor prognostic factor. Similarly, the expression of the Clever-1 scavenger receptor is indicative of a worse prognosis and its suppression of decreased viability in vitro. Myeloid malignancies, including AML, are the only known tumor types known to express Clever-1, indicating a possibility for direct anti-leukemic effects in addition to the immunomodulatory effects observed in solid tumors. Clever-1 blockade for myeloid malignancies is currently under clinical investigation, where it has been shown to overcome resistance to hypomethylating agents. However, the anti-leukemic mechanism of Clever-1 inhibition on AML cells is not understood. Therefore, this work aimed to investigate whether acquired therapy resistance or Clever-1 blockade modulates the stem-like phenotype of the AML cells in an effort to overcome therapy resistance.

Flow cytometry indicated that stem-like phenotype and Clever-1 expression were observed in the least differentiated cell line. Additionally, acquired resistance to venetoclax altered the phenotype of the cell lines. Metabolite analysis showed that cell lines that do not respond to Clever-1 blockade have a lower capacity for mitigating oxidative stress. Together with previous data, this indicates that Clever-1 blockade alters the metabolism in AML cells and is most effective in cell lines reliant on oxidative phosphorylation. Altogether, these data suggest that Clever-1 blockade alters the energy metabolism of AML cells and importantly, could target the metabolically inflexible leukemic stem cells.

Keywords: acute myeloid leukemia, Clever-1, glycolysis, metabolism, oxidative phosphorylation, stemness, therapy resistance

Purification, Characterization, and Crystallization of a Deoxynivalenol Detoxifying Glutathione Transferase from *Trichoderma reesei*

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BIOMOLECULAR PRODUCTION (TECH)

Fusarium gramianerum is one of the most threatening fungal pathogens in agriculture. It causes devastating annual yield losses, and the number of reported cases has increased in recent years owing to climate change and the rise of improper farming practices. The fungus produces a plethora of trichothecene mycotoxins, the most important of which is deoxynivalenol (DON). It is a virulence factor in wheat that inhibits protein synthesis, thus causing severe side effects when infected wheat is digested in large amounts and chronically. Fungicides have been used to combat *F. gramianerum* infection however their effectiveness is tied to timing of application. Moreover, the rise of multiherbicide-resistant fungi has increased the need for new methods, such as biocontrol agents, to combat these infections.

Glutathione transferases (GSTs) are superfamily of enzymes that detoxify a wide range of hydrophobic compounds. They are an integral part of the cell's defense system. The catalytic mechanism of GSTs involves conjugation of glutathione (GSH) to hydrophobic compounds, making them water-soluble. Fungal GSTs are not well characterized but there have been some reports of their different detoxifying abilities compared to GSTs of mammalian and plant origin. GSTs from wood-degrading fungi are a good example. These fungi contain many GSTs that bind and detoxify various compounds. A GST from *Trichoderma reesei* (*Tr*GST) has been shown to bind DON with high affinity using the 1-chloro-2,4-dinitrobenzene (CDNB) assay and to detoxify it demonstrated by LC/MS.

The aim of this thesis was to further characterize *Tr*GST. Enzyme stability and binding kinetics were studied using mass photometry and isothermal titration calorimetry. Crystallization with DON and its adduct DON-13-GSH were performed to produce crystals for X-ray structure determination.

The protein was stable and naturally formed a dimeric structure. It was also found to bind DON with and without GSH with a binding affinity of approximately 2.5 x 10^{-7} M and 3.6 x 10^{-6} M, respectively. The enzyme produced microneedles with DON and DON-13-GSH during crystallization trials.

Keywords: *Fusarium gramianerum*, trichothecenes, deoxynivalenol, glutathione transferase, *Trichoderma reesei*, mass photometry, isothermal titration calorimetry, crystallization

Enzyme activity screening methods for fungal solid-state fermentation

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BIOMOLECULAR PRODUCTION (TECH.)

Solid-state fermentation refers to a fermentation technology that takes place in a moist particle without being submerged into a liquid. Enzymes can be easier to produce in higher quantities in solid-state fermentation because of a more natural-like environment. Enzymes are commercially used in many processes. They have value in food industry applications among others. Glutaminase is one the enzymes that are used for flavor applications in the food industry. Glutaminase affects flavor compounds in food and beverage solid-state fermentation applications. Glutaminase is an enzyme that hydrolyzes L-glutamine to L-glutamate. Protease productivity is also a desired characteristic in food and beverage solid-state fermentation applications.

The aim of the study is to establish screening methods to differentiate enzyme activity and form a big picture of how enzyme activities change and develop in a fungal solid-state fermentation. Screening is done with four different strains of filamentous fungi. The aim is to be able to also establish differences between the four strains and their effects on enzyme activity.

Solid-state fermentations are done with two different substrate mixes with four different strains. General differences in enzyme activities in different strains are determined with on/off enzyme assay. More specific enzyme activities are analyzed with spectrophotometric methods as a function of time. The general idea is that the activity of a sample is determined by the rate of substrate hydrolysis that is detected by a specific kit. Methods are first modified in practice. Thereafter, these methods are used to continue the comprehensive activity screening.

Protease activity screening has required development. Casein as a substrate is suspected to cause interference and a new substrate is to be tested. Glutaminase method has been proven to function. Some differences in the glutaminase activity between strains have been established already. Glutaminase activity levels differ between strains. All strains follow a similar pattern with glutaminase progression. The highest activities are detected in the final parts of the cultivation. Also, different growth habits reflect on the glutaminase activity.

Keywords: filamentous fungi, enzymes, glutaminase, protease, solid-state fermentation, screening

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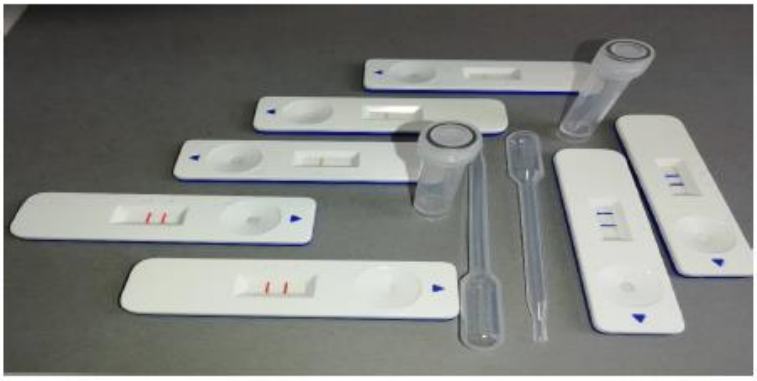

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Selection and characterization of DARPins against cytoplasmic proteins

Paulus Nyman
Supervisors: Doc. Tuomas Huovinen, Doc. Bryce Nelson, Doc. Ralf Paul
BIOMOLECULAR PRODUCTION (TECH.)

DARPins (designed ankyrin repeat proteins) are small non-antibody binders. Their stability, high expression in prokaryotic cells, and lack of disulfide bridges make them interesting research topic for therapeutic and diagnostic use.
In this study, a previously constructed DARPin library was screened against two intracellular target proteins (undisclosed, referred as X and Y) using phage display biopanning for isolating binders for functional screening experiments. The enrichment of binders was confirmed with phage immunoassay from total panning output and from individual clones. With four panning rounds, >70 % hit rate against X and >15 % against Y were achieved when signal to background ratio over three was counted as hit. The best binders against X and Y showed S/B ratios over 400 and 30, respectively. That confirmed the success of selections.
In order to create even stronger binders against target Y, DARPins from fourth round were combined with linkers of multiple lengths. The new bivalent library was screened with off-rate panning to select only the strongest binders.
As next steps, the binders from the last rounds will be sequenced. Individual DARPins and combinations showing the highest binding in immunoassay will be produced and further characterized for important properties such as affinity, thermostability and hydrophobicity. After this study, binders will be screened for functional properties against the target proteins. Validated binders could be used as drug candidates in further development.
This study has already proved the effectiveness of used pipeline for binder discovery, even against potentially challenging proteins. In the future, the pipeline could be used as a platform for early-stage drug development for various targets.

Keywords: binder, biopanning, DARPin, drug development, phage display

Physical parameters of 3D *in vitro* models of bone directing collagen orientation

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

The high strength of bone is primarily a result of its sub-structure, the orientation of the main components, intertwined layers of collagen and hydroxyapatite. Collagen, along with other proteins as well as cells living within the bone comprise the osteoid, or the organic part of the bone while hydroxyapatite and other calcium phosphates comprise the inorganic mineral component of bone. The osteoid forms first and its main component, collagen, primarily determines the bone organisation. Collagen can be oriented randomly or in layers with perpendicular collagen orientation in respect to each other. Layered bone is considered stronger and denser than randomly ordered bone and so it is the desired form of bone in medicine. Hydroxyapatite is crystallized onto the collagen scaffold and typically aligns with collagen fibres. Understanding the factors influencing bone organisation and thus it's strength and durability can be an asset in medical research. Optimizing in vitro bone growth parameters can be beneficial to bone prosthetic development and helping fracture healing.

Bone is porous and filled with canals less than 0,5 μm wide in which liquid moves quickly during exercise as the bone bends. The cells within and surrounding the bone are thus subjected to higher flow speeds than normal blood flow. To mimic the natural environment of bone cells, MC3T3-E1 mouse pre-osteocytes are grown in microfluidic chips as 2D and 3D models. Defined flow is run through the chips to simulate physiological flow experienced by the cells during exercise. These chips are imaged intermittently with Leica TCS SP5 while they are growing. Collagen orientation is determined by analysing the images with ImageJ and AI-based techniques. Samples are fixed with formaldehyde and stained with either Sirius red or alizarin red to stain collagen and hydroxyapatite, respectively. This allows the imaging of osteoid and mineral components and study their directionality in respect to flow direction. Future aims are to measure secreted alkaline phosphatase, a biomarker for bone mineralisation at different points of bone growth under flow with varying speed and direction.

Keywords: Bone remodelling, collagen, laminar flow, microfluidics, osteocytes

Studies on the processing of plasma and patient samples

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Radiometer Turku Oy manufactures semi-products used for quality control purposes for the AQT90 Flex products. These semi-products consist of plasma matrix and either patient sample antigen or recombinant antigen. Plasma matrices and patient sample pools are filtered before use to prevent the AQT90 Flex sample needle blocking. This process is challenging due to filtration rigidness and slow sample flow through, resulting in sample loss and in higher manufacturing cost. Therefore, the aim of this study was to investigate the patient sample pool filtration to remove clots without significant sample loss. Plasma used as a matrix is processed for 7 days in different temperatures before use, and this long protocol is inconvenient. In addition, at least one alternative plasma vendor is needed for the plasma. Another aim of the study was to test shorter plasma thawing protocols and test plasma from an alternative vendor as a semi-product plasma matrix.

In this study, filters from different manufacturers were tested for patient sample pool filtration. In addition, the necessity of the patient sample pool filtration was studied by comparing plasma matrix spiked with either centrifuged or filtered patient sample pool. In case of plasma processing, plasma thawing for 24 hours at +4 °C or for an hour at +37 °C were tested and the results were compared to the current plasma thawing protocol. Additionally, the stabilities of two antigens were tested in three different plasma lots from an alternative plasma vendor.

In filter testing, it was discovered that larger disc filter diameter and effective filtration area allow better sample flow through. In addition, it seemed that there are differences in filter materials from different manufacturers. Furthermore, in the patient sample pool filtration experiment, ≥9 % patient sample pool volume ratio to total plasma volume resulted in the clotting of the plasma matrix despite of the filtration of the patient sample pool. In plasma thawing experiment, plasma thawed for 24 hours at +4 °C did not clot during storage at +4 °C, whereas plasma thawed at +37 °C did clot. Additionally, antigens preserved their stability in plasma thawed for 24 hours at +4 °C or for an hour at +37 °C. In alternative plasma vendor testing, both used antigens were stable in all three plasma lots. However, further testing is needed with different plasma lots and lot combinations.

This study provided further information on plasma and patient sample pool processing. However, further testing is still needed on the patient sample pool filtration or to introduce a new plasma thawing protocol or an alternative plasma vendor.

Keywords: AQT90 Flex, patient sample pool filtration, plasma coagulation, plasma thawing

Characterization of a novel disulfide-stabilized scFv library

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Single chain variable fragment (scFv) composed only of the variable light and heavy chain domains is one of the smallest antibody fragments that can bind to an antigen. ScFv is easily produced in *E.coli* and well suited for phage display, but it is less stable than larger antibody fragments or intact antibodies. An extra disulfide bond introduced between the light and heavy complementary determining regions (CDR-L1 and CDR-H3) serving as an interdomain connection, enhances the stability and enables development of superstable scFvs. However, one of the intradomain disulfide links has to be removed to establish phage display. Five sublibraries using the dsScFv as a framework have been built with CDR-H3 region loop lengths varying from 13 to 19 amino acids. The aim of this project was to characterize these dsScFv libraries by selecting binders against different targets.
One panning round against protein L was carried out separately for each of the five sublibraries to eliminate clones with frameshifts and improper folding. To isolate binders for specific targets, three rounds of panning for pooled sublibraries were done against three antigens, HER2, hTROP2, and gremlin. Enriched libraries were characterized by phage immunoassays. Single clones were picked up, produced, and characterized by screening immunoassays and sequencing.
There was good enrichment after panning rounds based on panning results and phage immunoassay. Out of 90 clones that were screened from each antigen reaction, 56% of HER2, 48% of hTROP2, and 66% of Gremlin showed good binding to the target (signal-to-background ratio >10). Based on the sequencing, 6 unique binders for HER2 and 4 unique binders for Gremlin were found and selected for the next characterization phase. However, for hTROP2, the selected two clones were not part of our designed dsScFv library.
The unique dsScFv library with an additional disulfide bridge can be considered as a potential source of superstable scFv fragments due to the good panning enrichment and good activity of our binders against the selected antigens. The next step involves determining whether replacing the missing intradomain disulfide bridge can maintain the binding characteristics of particular binders.

Keywords: characterization, antibody library, disulfide-stabilized scFv, CDR, disulfide bond

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An acid-free glyoxal as a fixative for fresh histological tissue samples

Miisa Litmanen
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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Formalin fixation and paraffin embedding (FFPE) remains the standard method for tissue processing worldwide. However, some of the tissue specimens must be dissected and examined fresh, and the use of formalin for transport and preservation is not optimal due to formalin exposure, prolonged fixation and limitation of DNA and RNA quality. Therefore, novel approaches in the preanalytical phases of tissue handling are needed.
In this study, the suitability of an acid-free glyoxal (GAF) as a fixative for fresh histological tissues was analyzed because of its good chemical properties and ability of safe use. Specimens from human normal control tissue material were fixed in GAF over three time periods (24 – 72 hours, 1 – 2 weeks, and >1 month). The morphology was assessed with Hematoxylin-eosin (H&E) staining, antigenicity with immunohistochemistry (IHC) and the quality of nucleic acids with molecular tests. All samples were compared to standard FFPE-processed tissues.
The morphological quality in GAF-fixed tissues ranged from moderate to high, depending on tissue type. The quality remained relatively stable even after longer duration of fixation. Immunohistochemistry revealed good preservation of cytoplasmic antigens, but poor preservation of plasma membrane and nuclear antigens. The molecular analyses were promising, showing enrichment in DNA fragment size and less RNA degradation compared to FFPE-processed samples. These results suggest the possible role of acid-free glyoxal in molecular pathology. Since the histological procedures in pathology laboratory are optimized to formalin-fixed tissues, all procedures should be optimized for glyoxal-fixed tissues and further studied.

Keywords: fresh tissue, glyoxal, histology, molecular pathology, tissue diagnostics, tissue fixation

Spectrally multiplexed quantitative lateral flow immunoassay for *Bordetella pertussis* antibodies

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Pertussis, also known as whooping cough, is a bacterial infection caused by *Bordetella pertussis*. Despite a high vaccination coverage, pertussis remains endemic worldwide, with an increasing reported incident rate. Pertussis causes paroxysmal cough with a characteristic whooping, but with adolescents and adults the symptoms can often be atypical, leading to under and misdiagnosing. Serological testing for pertussis is based on measuring antibodies against pertussis toxin (PT) since it is solely produced by *Bordetella pertussis*. However, PT is also included in all acellular pertussis vaccines, which makes separating infection from vaccine induced responses challenging.

The aim of this research is a proof-of-concept for a spectrally multiplexed quantitative lateral flow immunoassay (LFIA) for anti-PT antibodies. The novel assay utilizes two upconverting labels with separate emissions, to measure anti-PT IgG and IgA from a single test line. Measuring both anti-PT IgG as well as anti-PT IgA, could aid in identifying a pertussis infection, since childhood vaccinations are shown not to cause anti-PT IgA responses.

Assay performance was evaluated with a patient sample panel (n=52) against a standardized enzyme-linked immunosorbent assay (ELISA). Spectral multiplexing showed great promise, as the combining of the two labels had minimal effect on the measured signals. However, quantification needs further optimization, as anti-PT IgA quantification was unsuccessful and anti-PT IgG showed only moderate correlation with ELISA.

Keywords: lateral flow, pertussis, spectral multiplexing, upconverting nanoparticles

Development of anti-immunocomplex antibodies for sensitive detection of testosterone

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Initially, steroid hormones were thought to solely regulate reproductive physiology, metabolism, and immune function. However, their broader significance now extends to various diseases and chronic conditions. Testosterone is a steroid hormone that plays a key part in these conditions, and even minor hormonal abnormalities can significantly impact health and disease risk. Thus, precise detection and measurement, even at low concentration levels, is essential. For this purpose, immunoassays offer a rapid, straightforward, and high-throughput method, in which the performance is influenced by assay format and antibody selection. While non-competitive sandwich enzyme-linked immunosorbent assay (ELISA) is effective for larger molecules, testosterone lacks sufficient epitopes for this method. Consequently, the competitive ELISA, although standard, does not offer comparable sensitivity and reproducibility for measuring testosterone. Given the demand for high-performance assays, novel non-competitive immunoassay concepts are needed. Anti-immunocomplex antibodies (anti-IC antibodies), which bind to the immune complex (IC) between a primary antibody and testosterone, can be used to construct an immunoassay that resembles the sandwich ELISA.
The aim of this work was to generate anti-IC antibodies for testosterone ICs, with the ultimate goal of utilizing them in future assays. A synthetic fragment antigen binding (Fab) library was screened for anti-IC Fabs using phage display panning. After several rounds of enrichment followed by screening, specific Fabs were found for the testosterone IC. The best binders were produced, and after further analysis with different concentrations of testosterone, some Fabs demonstrated superior performance. The EC50 for the clones were in the picomolar range, which lies within the reference range for testosterone. The successful development of the anti-IC Fab opens possibilities for novel immunoassay applications for enhanced testosterone detection.

Keywords: testosterone, anti-immunocomplex, antibody library, phage display, diagnostics

Development of antibodies for cyanobacterial neurotoxin: anatoxin-a

Irene Callus
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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Anatoxin-a, a secondary amine alkaloid, is a neurotoxin produced by certain species of cyanobacteria present in harmful algal blooms in water bodies worldwide. It may cause neurological symptoms in animals and humans when ingested and there have been several reports of deaths in animals and livestock due to anatoxin-a intoxication. Tests for detection of anatoxin-a are limited. This is mostly due to its small molecular mass of around 165 Da, making development of antibodies against it challenging because of limited binding sites. Other testing methods, such as by HPLC, are also available, however these are time-consuming and laborious methods. Thus, there is a need to produce more sensitive tests with better efficacy that could be used to test and monitor anatoxin-a levels in water.
The aim of this thesis was to find antibodies for anatoxin-a that could then be used in an immunoassay, with a more straightforward testing methodology. Previously, a single-chain variable fragment (scFv) library was interrogated by phage display against a synthetic biotin-anatoxin conjugate to find anatoxin-a binders. The enriched scFv pool was then converted to an antigen-binding fragment (Fab) library, during which three different variable light chains were incorporated, giving three separate Fab libraries.
In this master thesis work, Fab libraries were enriched against the biotinylated-anatoxin by three panning rounds of phage display. Fab genes were cloned into *Escherichia coli* to produce soluble Fab in microtiter plate-based cultures. The Fab clones were then screened for their capacity to recognize free anatoxin using a competitive immunoassay with free- and biotinylated-anatoxin. From a total of 1330 clones screened, 30 clones were showing possible binding to free anatoxin. However, this could not be confirmed in the further tests. Thus, the panning was repeated using HRP-anatoxin conjugate to enrich anatoxin-a binders for two rounds. The three original Fab libraries and the Fab libraries that were panned one time against biotinylated-anatoxin were used. The next step is to continue with screening using the competitive immunoassay, to see if there are any Fab antibodies that bind to anatoxin-a.

Keywords: anatoxin-a, immunoassay, panning, screening, small molecule compound

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Evaluation of CA19-9 glycovariant immunoassays for the detection of clinically significant prostate cancer

Emma Relander
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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Prostate cancer (PCa) is the second most common cancer in men and the second leading cause of cancer-related mortality in men who are over 50 years old. The current diagnosis of PCa relies on the prostate-specific antigen (PSA) blood test. However, the PSA test lacks sensitivity and specificity and is unable to distinguish between metastatic tumors and those that remain indolent. Therefore, there is a crucial need for new diagnostic biomarkers for differentiating between clinically significant PCa and benign conditions.

The aim of this study was to evaluate the glycovariant of cancer antigen 19-9 (CA19-9) as a putative biomarker for PCa using a highly sensitive nanoparticle-aided time-resolved fluorescence immunoassay (NP-TRFIA). Additionally, the study aimed to identify the protein expression patterns in prostate cancer and find the localization of the CA19-9 glycan epitope on the proteins expressed in PCa by using affinity pull-down and mass spectrometry.

To evaluate the CA19-9 as a biomarker, a cohort of around 600 plasma samples was used. The altered, cancer-dependent, glycosylation patterns of CA19-9 were measured with an NP-TRFIA using europium-doped nanoparticles to detect the binding. Europium nanoparticles were coated with either CA19-9 antibodies or with lectins including MBL and MGL. CA19-9-MBL assay enabled discrimination between benign and PCa (Gleason score 7-9) samples. In addition to evaluation of the CA19-9 as a biomarker, protein expression patterns were identified. To discover what proteins are expressed in the benign and prostate cancer samples, biotinylated CA19-9-binding Fab2 fragments and biotinylated MBL were used to bind proteins in benign and prostate cancer samples. The data showed the presence of specific proteins that were unique to prostate cancer samples.

In summary, CA19-9 is a potential biomarker for the identification of patients with well-differentiated PCa. In addition, further investigations of the proteins expressed in benign and prostate cancer samples should be conducted.

Keywords: cancer antigen 19-9, europium nanoparticles, lectin, prostate cancer, prostate-specific antigen, time-resolved fluorescence immunoassay

Immunoturbidimetric assay for in-process determination of polyclonal antibody functionality

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Polyclonal antibodies are used in diagnostic tests because of their multi-epitope binding abilities, higher sensitivity ranges than monoclonal antibodies in some assays, high avidity binding, abilities to form lattices with antigens, biophysical diversity that makes them more resistant to environmental changes such as pH and temperature, and cost-effectiveness. However, the use of polyclonal antibodies presents challenges such as limited specificity and variability between batches. To tackle the challenges, monitoring the functionality of polyclonal antibodies facilitates the production of more uniform batches.

In this study, bio-layer interferometry and immunoturbidimetric methods were developed and compared to determine, which would be more suitable for monitoring the production process. Very early in the study, it was noticed that the immunoturbidimetric alternative was more suitable for the purpose of use, and it was developed further. In the development of the method, the number of measurement points, the incubation time, the form of haemoglobin used as an analyte, and the azide possibly reacting with haemoglobin were examined. In the final method, 20 measuring points were used at equal concentration intervals so that the titer could be determined as precisely as possible, and a pseudo-endpoint of 1000 seconds was chosen as the incubation time. In the comparison of haemoglobin forms, methaemoglobin increased signals and gave 80 % higher titers than the comparative oxyhaemoglobin. Based on the results, the azide did not affect the titer but increased the response signal.

Keywords: polyclonal antibodies, haemoglobin, bio-layer interferometry, immunoturbidimetry, titer determination assay

High sensitive rapid detection of urinary EVs with up converting nanoparticle based lateral flow immunoassay

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Bladder cancer (BlCa) poses a substantial health burden globally, demanding advancements in diagnostic methodologies. Presently existing approaches, while effective, are invasive and cost-intensive. Therefore, this study has aimed to develop a rapid, cheap, and non-invasive diagnostic tool utilizing an upconverting nanoparticle (UCNP) based-lateral flow immunoassay (LFIA) to directly detect extracellular vesicles (EVs) from cancer cell line and urine samples.
Quantitative LFIAs were performed utilizing anti-tetraspanin antibody specifically, CD63 as a capture, while RAM (Rabbit Anti-Mouse Antibody) served as a control. This anti-CD63 antibody and RAM were printed onto a nitrocellulose membrane. The tracer, labeled with upconverting nanoparticles, was subsequently assessed for its detecting capacity. Assay standards were established using EVs-isolated from cancer cell line. Validation of CD63-positive EVs in urine specimens was carried out using pooled of urinary EVs (uEVs). Finally, uEVs from individual patients of BlCa (n=20), benign prostate hyperplasia (BPH) (n=20), and healthy (n=30) samples were captured using anti-CD63 antibody. Subsequently, the same CD63 antibody as a tracer labeled with UCNPs were used to detect these uEVs within microtitration wells. Following absorption from the mixture of sample and reporter solution onto the lateral flow strip, the strips were read with an Upcon reader device, resulting in up-converted luminescent signals after 1.2 hours.
So far, The results from this study demonstrates its high sensitivity in detecting EVs derived from cancer sources, with a detection limit of $1.9 \times 10^5/\mu L$. Specifically, CD63-CD63-UCNP assay was able to distinguish significantly between BlCa patients and individuals with benign conditions (p=0.002), as well as healthy individuals (p= 0.0001). However, more samples are required to further validate this study. In future, based on our developed UCNP-LFIA, cancer associated biomarkers in combination with lectins will be evaluated to detect bladder cancer more specifically.

Keywords: extracellular vesicles (EVs), urine, lateral flow immunoassay (LFIA), upconverting nanoparticles (UCNPs)

Development of antibody fragments for targeted PET-imaging and single EV-imaging

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Extracellular vesicles (EVs) are a diverse group of lipid structures that almost all cells secrete. They are found in many bodily fluids and are involved in cargo transportation, signaling and are also nowadays used as a biomarker. EVs are an emerging field in research, but the high diversity of the group has been a challenge in studying the communication between cells. That is why single EV-imaging techniques have been developed which can be enabled by using binders that bind to the membrane proteins of EVs. Tetraspanins are one of the membrane proteins that EVs express on their surface. Vesicle binders can then be labeled with a fluorescent label, which can be used to image and study these single EVs.
PET-imaging is an imaging technique that utilizes radioactive labels to achieve high resolution images of wanted targets. Some fields where PET-imaging can be used is in the diagnostics of cardiovascular diseases. Fatty acid binding protein 3 (FABP3) is one biomarker for cardiovascular diseases as the presence of FABP3 can indicate tissue damage.
Phage display technology is an excellent tool in the search of novel binders due to its potentially high diversity of binders but also for its ease of changing conditions in the pursuit of the best binders. Imaging techniques often require small binders so antigen-binding fragments (FABs) or nanobodies for example are used.
The aim of the project was to find antibody fragments that could be used for targeted PET-imaging and single EV-imaging. An in-house FabE-phage library was used to find suitable binders for FABP3 and tetraspanins CD9, CD63 and CD81. Multiple panning rounds were deployed and several potential binders for FABP3 were found during screening. Next steps in the project would include further characterization of selected FABP3 binders, labeling and sequencing. The characterization could include affinity measurements and also flow cytometry analysis by using fluorescent labels. After characterization and labeling they could be used in PET-imaging.

Keywords: antibody engineering, antigen binding fragment (FAB), antibody characterization, extracellular vesicles (EVs), fatty acid binding protein 3 (FABP3), imaging, PET-imaging, phage library, labeling, tetraspanin

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Unraveling the spatial dynamics of neurovascular coupling in retinopathy of a mutant MITF mouse model

Samuel Svärd
Supervisor: Doc. Gennady Yegutkin
MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Mice with single nucleotide mutations in the lineage-specific regulatory gene microphthalmia-associated transcription factor (MITF) have altered melanocyte development and pigmentation. Typically, MITF function is partially sustained even when mutated, but many mutational variants give rise to severe retinal pigmented epithelium dysfunction. In humans, pathogenic variants of MITF have been implicated in melanoma and rare genetic disorders, most notably in Waardenburg syndrome and Tietz syndrome.
$MITF^{mi\text{-}enu22(398)}$ is a largely unexplored mouse variant with a mutationally introduced stop codon in exon 2A, effectively resulting in MITF lacking exons 2A, 2B, and 3. Surprisingly, fundus photography of these eyes shows no distinct signs of pathology. However, we aimed to properly characterize the model by studying the spatial dynamics of the retina using three-dimensional multiplexed immunofluorescence, electron microscopy and enzyme histochemistry for the detection of ectonucleotidases.
In our study, we observed notable signs of retinopathy in $MITF^{mi\text{-}enu22(398)}$ mice. These mice can form retinal lesions with abnormal stratification of cells occurring in the outer nuclear layer, in addition to the innermost retinal layers undergoing extensive astrogliosis and microglial migration. To deepen our knowledge about neurovascular coupling in the onset and progression of ocular diseases, we further investigated purinergic signaling and therapeutically relevant proteins, such as CD39, CD73 and connexin 43, in ocular diseases.

Keywords: astrogliosis, ectonucleotidase, melanosome, microphthalmia-associated transcription factor, multiplexed imaging, neurovascular coupling, retina, retinopathy

96-well plate compatible assay format for screening ankyrin repeat proteins on filter paper

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Plastic waste is nowadays a rising problem worldwide. In the lab, disposable plastic items are a common sight which are petroleum-based and non-recyclable as GMO waste. Our mission is to develop paper-based assay which could replace plastic 96-well plates in recombinant binder protein screening applications. The main aims in the project were to assess cellulose binding domains (CBM) to bind different filter papers and to control capillary flow of the paper between separate wells.
CBMs' binding was studied as a fusion protein with anti-green fluorescent protein (GFP) ankyrin repeat domain called clamp. First in the protocol clamp-CBM was bound to different filter papers. Next GFP label was added to the complex and the unbound fraction was washed. Lastly the papers were detected with Bio-Rad imager and analyzed with its integrated tool and later with python code which detects well circles and counts areas' green pixels.
In the assay the clamp was used as a binding control to confirm that CBM is needed for the binding. We developed a proof-of-concept assay where a clamp-CBM binds to cellulose in range 25 µg/ml to 800 µg/ml without unspecific binding of clamp or GFP. We demonstrated that increasing GFP up to 800 nM does not increase signal and saturation starts after 400 nM of GFP. Another aspect in the work was to measure whether adding second CBM in the clamp-CBM fusion would increase the binding affinity or reduce dissociation, and as conclusion it did not improve assay kinetics. A plate washing experiment revealed that CBM binds tightly to cellulose without significant decrease in signal upon increased washing steps. Despite our many experiments we did not find a perfect solution to avoid capillary flow between wells. However, we found out that the optimal sample volume is 5 µl on filter paper with 3D printed wells.

Keywords: paper-based assay, Cellulose binding domain, assay development, ankyrin repeat protein, 3D printing

Mammalian display screening for increased antibody developability

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Mammalian display is a technique where antibody libraries are expressed on the surface of mammalian cells. This allows the selection of desired antibody charasteristics from a large number of library variants. Mammalian display is often used as a tool in therapeutic antibody discovery and development. In addition to target binding properties of a therapeutic antibody, it is important that its biophysical properties are optimal to increase the likelihood of success in drug development process. These developability aspects should be considered in early stages of antibody discovery to avoid wasted time, resources, and money. It could be beneficial to have a screening step using a deselection method for antibodies with poor biophysical properties.
In this project the developability of antibody variant libraries were analyzed. This was done using a group of deselection reagents that bind to antibodies due to certain problematic biophysical properties. First, error-prone PCR was used to create variants from three different commercial antibodies. Variable light and heavy domains were mutated separately so that there were two different libraries created from each antibody. After cloning the variant plasmids in Escherichia coli -cells, they were transfected into Chinese hamster ovary cells. The cells that expressed antibodies were analyzed with flow cytometry to see their display levels and which, if any, deselection reagents they bound. Interesting variants were further analyzed with next-generation sequencing to see what types of mutations they had.

Keywords: mammalian display, antibody developability, deselection method, error-prone PCR, flow cytometry, next-generation sequencing

RT-PCR-kemian optimointi uudelle infektiotestijärjestelmälle

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Infektiotaudit eli tartuntataudit ovat ihmisten yleisimpiä sairauksia ja merkittävä taakka yhteiskunnalle ja yksilölle. Taudinaiheuttajan tunnistaminen luotettavasti ja oikea-aikaisesti mahdollistaa tehokkaan hoidon ja tartuntaketjun katkaisun. Kvantitatiivinen käänteiskopiointipolymeraasiketjureaktio (RT-PCR) on vakiintunut pikadiagnostiikkaan soveltuva molekulaarinen menetelmä.
Toimeksiantona oli optimoida RT-PCR-kemiaa terveysteknologiayhtiö Uniogen Oy:ssä kehitteillä olevalle infektiotautien pikadiagnostiikkaan tarkoitetulle testijärjestelmälle. RT-PCR-kemian keskiössä ovat entsyymit, joiden toimivuus vaikuttaa menetelmän suorituskykyyn. Tuotteen valmistuksessa entsyymit ja muut PCR-reagenssit kuivataan reaktiokammiona toimivalle muovilastulle. Tavoitteena oli löytää kuivaukseen soveltuvia glyserolittomia käänteiskopioijia ja DNA-polymeraaseja, jotka toimivat tehokkaasti nopeassa RT-PCR-määrityksessä ja säilyvät lastulle kuivattuna huoneenlämmössä vähintään vuoden ajan.
Työssä testattiin 46:ta käänteiskopioijan ja DNA-polymeraasin muodostamaa paria analysoimalla useita mallianalyyttejä yksivaiheisella noin puolen tunnin pituisella RT-PCR-määrityksellä, joka perustui hydrolyysikoetinteknologiaan. Entsyymien vertailu pohjautui signaali-taustasuhteeseen ja kynnyssykliin. Optimointia jatkettiin lupaavimmilla entsyymeillä. Lisäksi valmistettiin 10 kuivalastuerää, joiden säilyvyyttä huoneenlämmössä (+28 °C) ja jääkaapissa (+4 °C) seurattiin 60 päivän ajan.
Signaali-taustasuhde ja kynnyssykliarvot olivat 60 päivän säilytyksen jälkeen tavoitellulla tasolla, eikä tulosten perusteella havaittu merkittävää eroa huoneenlämpösäilytyksen ja jääkaappisäilytyksen välillä. Lopputuloksena löydettiin hyvin yhteensopivia entsyymejä ja PCR-puskureita, jotka alustavien tulosten perusteella soveltuvat käytettäväksi uudessa infektiotestijärjestelmässä. Laajemmat tulkinnat eri entsyymien ja reaktiokompositioiden välisistä eroista voidaan tehdä vasta tulevien optimointien ja pidemmän säilyvyystestauksen jälkeen.

Asiasanat: DNA-polymeraasit, infektiotaudit, kuivakemia, pikadiagnostiikka, polymeraasiketjureaktio

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Anti-hAFP / anti-hCGβ kuoppalevyn tuotantoprosessin kehitys

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Alfa-fetoproteiinia (AFP) ja vapaata koriongonadotropiinin β-alayksikköä (hCGβ) käytetään raskauden aikana sikiön kromosomipoikkeamien seulontaan. Määrityksissä käytettävien kuoppalevyjen tulee olla tasalaatuisia ja täyttää tuotteelle asetetut laatuvaatimukset. Kuoppalevyjä voidaan käyttää sekä molempien merkkiaineiden havainnointiin samanaikaisesti että vain hCGβ:n havainnointiin.

Anti-hAFP / anti-hCGβ -kuoppalevyt valmistetaan tällä hetkellä Wallac oy:n vanhalla tuotantolinjalla. Prosessivaiheet ovat vasta-aineliuoksen annostelu, pesu- ja kyllästysvaihe ja imuvaihe. Prosessivaiheiden välillä levyjä inkuboidaan. Työn tavoite on siirtää kuoppalevyjen tuotanto uuteen tuotantotilaan ja varmistaa niiden toimivuus käyttötarkoituksessaan. Työssä optimoidaan prosessin parametrit ja testataan niiden äärirajat uudella tuotantolinjalla.

Tuotettuja kuoppalevyjä tutkitaan homogeenisuus-, tausta-, ja kapasiteettitesteillä. Homogeenisuustestissä tarkastellaan levyn sisäistä CV-prosenttia, joka kertoo levyn kuoppien tasalaatuisuudesta. Taustatesti tehdään 0-kalibraattorilla, jotta varmistetaan, että kuoppalevy ei tuota määritykseen taustasignaalia. Kapasiteettitestillä saadaan tietoa signaalin kehittymisestä, ja siinä tulos on merkkiaineita sisältävän kalibraattorin ja sen 1:5 laimennoksen signaalien suhde.

Työn tuloksena odotetaan saatavan uudella tuotantolinjalla valmistettuja kuoppalevyjä, jotka ovat toimivuudeltaan identtisiä vanhan tuotantolinjan kuoppalevyihin. Lisäksi odotetaan saatavan optimoidut tuotantoparametrit kuoppalevytuotannon pesu- ja imuvaiheiden imupään korkeudelle ja imuvaiheen nopeudelle, sekä tietoa pidennetyn kyllästysinkuboinnin vaikutuksesta tuotteen tasalaatuisuuteen ja laatuun.

Asiasanat: Raskaudenaikainen seulonta, AFP, hCGβ, kuoppalevy, tuotannon kehitys

Integroidun sylkinäytteenoton kehittäminen kotitestaukseen

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Sylkinäytteistä tehtävät hormonien lateraalivirtaustestit ovat nopeasti yleistyvä pikatestausmenetelmä. Sylkinäytteen ottaminen soveltuu kotioloissa tehtäväksi ja siinä havaittavat hormonipitoisuudet ovat tyypillisesti verrannollisia veritesteihin. Diplomityön tarkoituksena oli kehittää integroitua syljenkeräysjärjestelmää hormonipikatesteihin. Tutkimuksen ensisijaisena tavoitteena oli saada sylki siirtymään luotettavasti näytelastulta lateraalivirtausliuskaan ilman lisättyä puskuria.
Syljen kerääminen tapahtui pitämällä näyteliuskaa suussa, minkä jälkeen sylkeä imenyt liuska vedettiin kasetin läpi. Kasetin sisällä oleva rakenne puristi sylkeä näyteliuskalta kasetin näytekaivoon. Niistäjältä sylki valui näytekaivoon. Lateraalivirtausliuska oli näytekaivon yläpuolella siten, että sen näytetyyny voidaan kastaa näytteeseen haluttuna ajankohtana.
Diplomityössä havaittiin, että näyte liikkui paremmin pesemällä muovipintaa detergentillä. Näytelastun materiaaliksi valittiin polyvinyylialkoholi, joka litistämisen jälkeen palautui alkuperäiseen muotoonsa vasta kosketuksessa nesteeseen. Kasetin eri versioiden nopealla mallintamisella ja 3D-tulostamisella saatiin kasetin sisärakenteen muoto ja koko optimoitua tehokkaasti.

Asiasanat: Sylki, lateraalivirtaustesti, hormoni, 3D-tulostus, polyvinyylialkoholi

HIDEX

Troponiini T:n eri muotojen samanaikainen tunnistus sydäninfarktin diagnosoinnissa

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Sydänperäinen troponiini T on yleisesti käytetty biomerkkiaine sydäninfarktin diagnostiikassa. Troponiinikompleksi koostuu kolmesta osasta (I, T ja C) ja sillä on rooli lihaksen supistumisen säätelyssä. Sydänlihasvaurion seurauksena troponiinia vapautuu verenkiertoon, jossa se alkaa ajan kuluessa hajoamaan pienemmiksi fragmenteiksi. Nykyiset troponiini T testit tunnistavat myös nämä fragmentit. Kuitenkin veren troponiinipitoisuus voi olla koholla muistakin sairauksista tai terveydentiloista johtuen, mikä vaikeuttaa sydäninfarktin diagnosointia. Sydäninfarktin alkuvaiheissa vapautuva troponiini T on ensin kompleksina tai vähän fragmentoituneena muotona, minkä vuoksi eri troponiini T:n muotojen tunnistus voisi parantaa testin diagnostista käytettävyyttä, sillä esimerkiksi loppuvaiheen munuaistautipotilailla ja maratoonareilla on havaittu pääosin fragmentoituneita muotoja. Pitkän troponiini T:n osuuden troponiini T:n kokonaispitoisuudesta on havaittu olevan myös korkeampi sydäninfarktipotilailla verrattuna loppuvaiheen munuaistautipotilaisiin.
Työn tavoitteena oli parantaa sydäninfarktin diagnosointia kehittämällä heterogeeninen kaksipuoleinen immunomääritys, jolla voidaan tunnistaa eri troponiini T:n muotojen pitoisuudet potilasnäytteistä yhdellä määrityksellä. Troponiinin eri muotojen yhdenaikainen määrittäminen voisi vähentää sydäninfarktin diagnosointiin tarvittavaa työmäärää. Eri troponiini T:n muotojen samanaikainen tunnistus toteutettiin käyttämällä kahta eri väriä emittoivaa käänteisviritteistä nanopartikkelia, joista toiseen konjugoitu vasta-aine tunnisti ainoastaan troponiini T:n pitkän muodon ja toinen myös fragmentoituneet muodot. Käänteisviritteisten nanopartikkeleiden tuottama emissiovalo on viritysvaloa lyhyemmällä aallonpituudella, mikä mahdollisti matalan taustasignaalitason. Kaivoon kiinnitetty sitojavasta-aine pystyi sitomaan kaikkia troponiini T:n yleisimpiä muotoja. Työssä kokeiltiin eri vasta-ainekombinaatioita, optimoitiin määritysprotokollaa ja -olosuhteita. Määrityksellä saatiin mitattua yhtä aikaa troponiini T:n eri muotoja, mutta plasmamatriisissa oli vaihtelevaa epäspesifistä sitoutumista, mikä johtui todennäköisesti leima-aineiden epätäydellisestä pinnoituksesta.

Asiasanat: immunomääritys, käänteisviritteinen nanopartikkeli, multiplex-määritys, sydän infarkti, troponiini T

Hydrofobiseen vuorovaikutuskromatografiaan perustuvan karakterisointimenetelmän kehittäminen Eu-kelaateilla leimatuille vasta-aineille

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Aikaerotteista fluorometriaa, jossa käytetään luminoivia lantanoidikelaatteja, voidaan hyödyntää immunomäärityksissä. Fluoresoivat leima-aineet konjugoidaan vasta-aineisiin ja niiden avulla voidaan tunnistaa halutun analyytin läsnäolo näytteessä. Vasta-aineissa on useita funktionaalisia ryhmiä, joita voidaan käyttää lantanoidikelaattien kovalenttiseen konjugaatioon. Yleinen konjugaatiokohta on lysiinitähteiden aminoryhmät, joiden kanssa reagoidessaan leimat kiinnittyvät satunnaisesti osaan vasta-aineiden lysiiniryhmistä. Näin syntyvä tuote on heterogeeninen. Herkän havaitsemisen mahdollistamiseksi on toivottavaa leimata mahdollisimman monta lantanoidileimaa lopulliseen tuotteeseen eli tuotteelle halutaan mahdollisimman korkea leima-aste. Tämä on mahdollista, sillä suuren Stokes-siirtymän vuoksi lantanoidikelaatit ovat harvoin alttiita itsesammuttamiselle. Kuitenkin liian korkea leima-aste voi vaikuttaa leimatun vasta-aineen immunologiseen sitoutumiseen.
Tällä hetkellä leima-aste määritetään menetelmällä, joka antaa vain keskiarvon leimatun vasta-aineen leima-asteesta. Tutkimuksen tarkoituksena oli tutkia hydrofobiseen vuorovaikutuskromatografiaan perustuvan menetelmän soveltuvuutta leima-asteen tarkempaan karakterisointiin. Menetelmän haluttiin erottelevan leimattuja vasta-aineita niiden leima-asteen perusteella. Menetelmän toimivuutta vertailtiin tutkimalla erilaisia pylväsmatriiseja sekä optimoimalla suolan, pH:n ja liuottimen vaikutusta erotteluun. Lopulta menetelmä sovitettiin korkean erotuskyvyn nestekromatografiaan.
Tutkimuksen tulokset osoittivat, että menetelmä on tehokas erottamaan leimatut ja leimaamattomat vasta-aineet sekä tunnistamaan erilaisia leimauksia. Menetelmä on erityisen hyödyllinen alhaisen leima-asteen tunnistamisessa, kun taas korkean leima-asteen vasta-aineiden erotteleminen on haastavampaa liian voimakkaan sitoutumisen vuoksi. Lisäksi suhteelliset erot korkean leima-asteen vasta-aineiden hydrofobisuudessa ovat pienempiä kuin matalalla leima-asteella. Tutkimuksessa kehitetty menetelmä mahdollistaa uuden tavan parantaa leimattujen vasta-aineiden laadunvalvontaa.

Avainsanat: Aikaerotteinen fluorometria, hydrofobinen vuorovaikutus-kromatografia, immunomääritys, lantanoidikelaatti, leima-aste

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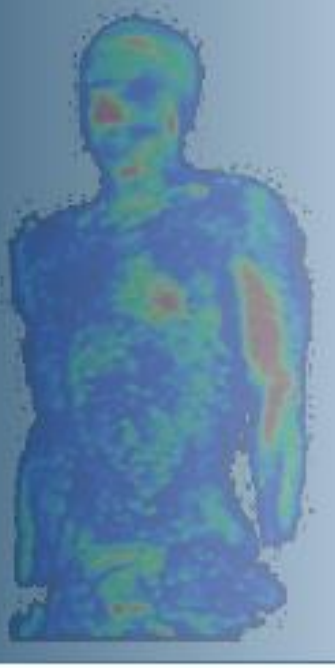

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TYKS

Lääkeaineiden kuljetukseen tarkoitettujen polymeerimikropartikkeleiden valmistusmenetelmät

Ella Suistala
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Biohajoavat polymeerimikropartikkelit mahdollistavat lääkeaineen pitkäaikaisen ja kontrolloidun vapautumisen elimistössä. Monet prosessiparametrit vaikuttavat mikropartikkeleiden ominaisuuksiin, kuten kapselointitehokkuuteen ja lääkeaineen vapautumiseen. Mikropartikkeleiden valmistusmenetelmistä ja niihin vaikuttavista prosessiparametreistä ei ole vielä tarpeeksi tutkimustietoa. Siksi tässä työssä tutkittiin kahta mikropartikkeleiden valmistusmenetelmää erilaisin prosessiparametrein.

Mikropartikkelit valmistettiin biohajoavasta polylaktidi-polyglykolidi (PLGA) -kopolymeeristä. Mikropartikkeleita valmistettiin sekä perinteisellä emulsio/liuottimen haihdutus -menetelmällä, että mikrofluidistisella menetelmällä. Molemmilla menetelmillä valmistettiin sekä yksittäis- että kaksoisemulsioita. Prosessiparametrit, joita tutkittiin, olivat muun muassa polymeerikonsentraatio, sekoitusnopeus, malliaineen konsentraatio, käytetty liuotin ja polymeerin moolimassa. Kapselointitehokkuutta tutkittiin kapseloimalla kahta malliainetta (Fluoreseiini-isotiosyanaatti (FITC) dekstraani ja Coumarin 6). Mikropartikkeleille suoritettiin vapautumistutkimukset, jossa FITC dekstraanin vapautumista tutkittiin yli 100 päivän ajan.

Tulokset osoittivat, että malliaineet oli mahdollista kapseloida korkein kapselointitehokkuuksin (>90 %). Malliaine kapseloitui tehokkaammin kaksoisemulsiopartikkeleihin kuin yksittäisemulsiopartikkeleihin. Burst eli ensimmäisen päivän aikana tapahtunut malliaineen vapautuminen oli suurempaa yksittäisemulsiopartikkeleilla kuin kaksoisemulsiopartikkeleilla. Mikrofluidistiikalla ei onnistuttu kapseloimaan FITC dekstraania. Coumarin 6 kapseloitui mikrofluidistisella prosessilla, mutta kapselointitehokkuus oli noin puolet siitä, mitä emulsiomenetelmällä saavutettiin. Mikrofluidistiikalla valmistetut plasebopartikkelit olivat tasalaatuisempia kuin emulsiomenetelmällä valmistetut.

Avainsanat: emulsio/liuottimen haihdutus -menetelmä, kapselointitehokkuus, lääkeaineiden kuljetus, mikrofluidistiikka, polylaktidi-polyglykolidi-kopolymeeri, polymeerimikropartikkelit

RNA-polymeraasin ω-alayksikön rooli *Synechocystis* syanobakteerin sopeutuessa typen puutostilaan

Joonas Silmu
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MOLEKULAARINEN KASVIBIOLOGIA

Syanobakteereihin kuuluva *Synechocystis* on laajasti tutkittu fotosynteettisten prokaryoottien malliorganismi. Sen RNA-polymeraasista on havaittu, että siihen kuuluva ω-alayksikkö ei ole välttämätön polymeraasin toiminnalle. ω-alayksikön toimintaa ei täysin tunneta, mutta sillä tiedetään olevan vaikutus σ-faktorien affiniteettiin kiinnittyä polymeraasiin. σ-faktoreita on *Synechocystiksellä* 9 erilaista ja niiden tehtävä on tunnistaa erilaisia promoottorialueita. ω-alayksikön geenin (*rpoZ*:n) poistaminen muuttaa RNA-polymeraasiin kiinnittyneiden σ-faktorien suhdetta ja näin ollen vaikuttaa solun geenien ekspressioon.
Aikaisempi tutkimus on osoittanut ω-alayksikön olevan välttämätön korkeaan CO_2:n sopeutumisessa. Tässä työssä vertasin ω-alayksikön vaikutusta *Synechocystiksen* sopeutuessa typenpuutosstressiin vaihtamalla sen kasvatusliuoksen typettömään kasvatusliuokseen. Happielektrodilla mitattu fotosynteettinen aktiivisuus oli ΔrpoZ-mutantilla hieman kontrollikantaa alhaisempi normaaliolosuhteissa. Happielektrodimittauksista havaittiin fotosynteesiaktiivisuuden eksponentiaalinen lasku molemmilla kannoilla. Hapentuotto putosi puoleen muutamassa tunnissa ja 2 päivän typenpuutteen kuluttua tuottoa ei havaittu. Kontrollikannalla oli myös taipumus suurempaan soluhengitysaktiivisuuteen pimeässä typenpuutteen aikana. Typenpuutostilalle olennainen vihreyden häviäminen kesti ΔrpoZ-soluilla kontrollikantaa kauemmin. Jatkan tutkimusta mittaamalla keskeisten fotosynteesikompleksien määrien muutoksia tutkimalla niiden proteiinien määriä western blot –tekniikalla. Proteiinimäärää mitataan valoreaktiokompleksien klorofylliä sitovista CP43 ja PsaB proteiineista, valoenergiaa keräävän fykobilisomikompleksin fykosyaniinista ja allofykosyaniinista, soluhengitysproteiini NDH-1:ä ja happea kuluttavista flavodiiron-proteiineista eri typenpuutteen vaiheessa.
Tutkimus on vielä kesken, mutta toistaiseksi jo aiemmin tiedossa ollut fykobilisomin hajoaminen typenpuutoksen aikana on havaittu myös ΔrpoZ-mutantissa. Western Blot -kokeiden lisäksi tuloksia on tarkoitus tukea mittaamalla edellämainittujen proteiinien mRNA-transkriptien määrä kvantitatiivisella PCR:llä.

Asiasanat: happielektrodi, RNA-polymeraasi, syanobakteerit, typenpuute, ω-alayksikkö

Kaurapohjaisten maidonkorvikkeiden tuotannon CIP-pesujen optimointi ennen lämpökäsittelyä

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Kasvipohjaisten elintarvikkeiden kysynnän kasvu on monipuolistanut kauran hyödyntämistä elintarviketeollisuudessa. Uusien elintarvikeprosessien ja tehtaiden nopea pyrkimys käynnistää tuotanto ei ole mahdollistanut prosessien huolellista optimointia. Diplomityön tavoitteena oli optimoida uuden kaurapohjaisia maidonkorvikkeita valmistavan tuotantolaitoksen CIP-pesujen veden käyttöä. Työ toteutettiin Raisio Oyj:n Algot tehtaalle. CIP-pesujen optimointiin vaikuttivat puhdistettava tuote, käytetty kemikaali ja vesi sekä lämpötila, paine ja virtaus. Työ toteutettiin osana normaalia tuotantoa ottamalla huuhteluvesinäytteitä pesukohteiden koepesuista. Pesukohteita oli yhteensä 15. Koepesun onnistuminen todennettiin fysikaalista, kemiallista ja mikrobiologista puhtautta mittaavien analyysien avulla. Optimoinnin tuloksissa merkittävimpiä olivat tuotejäämiä ilmaisevat kemialliset analyysit, kuten pitoisuuden titraus ja johtokyvyn mittaus.
Työn tuloksien mukaan pesukohteiden huuhteluihin kuluvan veden määrä ylitti selkeästi riittävään puhtauteen vaaditun veden määrän. Tulokset vastasivat ennakko-oletuksien veden runsaasta käytöstä. Parametrimuutoksien avulla veden kulutus laski yhteensä 24,8 %, mistä merkittävimmän parametrit olivat huuhteluvaiheiden (13,0 %), kierron täytön (8,5 %) ja sekoitusalueen (3,3 %) parametrimuutokset. Virtausnopeuden nostaminen riittävälle tasolle nosti veden kulutusta, mutta sen vaikutus suhteessa muihin parametrimuutoksiin ei ollut merkittävä. Myös pesujen huuhteluiden teoreettinen enimmäiskokonaiskesto lyheni parametrimuutoksien vaikutuksesta kohtalaisen merkittävästi (25,2 %). Satunnaisesti pesujärjestelemä ei kuitenkaan toteuttanut pesuja asetettujen parametrimuutoksien mukaan, mikä heikensi parametrimuutoksien vaikutusten luotettavuutta. Pesujen puhdistuksen laatu ei heikentynyt huuhteluiden toteutuessa alkupäisen ohjelman mukaisesti.
Parametrimuutoksien myötä vettä pystyttäisiin säästämään 2023 vuoden tuotantovolyymien mukaan jopa 2,5 miljoonaa litraa, mikä lisäisi teoreettisesti vuoteen jopa 200 tuntia tuotantoaikaa. Muuttuvan tuotannon ja tuotantojärjestelmän luotettavuuden takia tulokset ovat suuntaa antavia. Diplomityö mahdollisti kaikesta huolimatta veden kulutuksen vähentymisen ja siitä seuraavat taloudelliset säästöt, jotka tukivat myös yrityksen ympäristötavoitteita kohti kestävämpää tulevaisuutta.

Asiasanat: CIP, puhdistus, optimointi, kaura.

Lipidiluokat ja rasvahapot valituissa suomalaisissa järvimikrolevissä

Elsa Haloila
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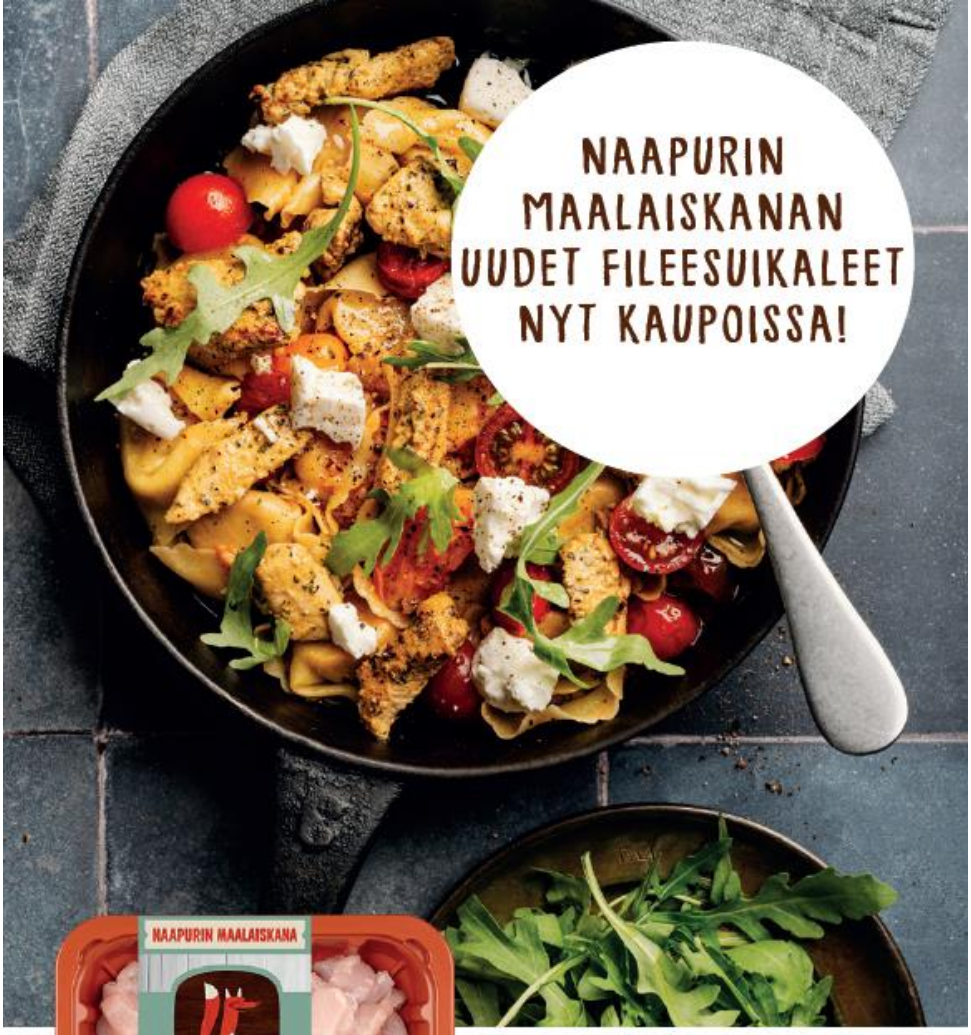
ELINTARVIKEKEHITYS (DI)

Mikrolevät ovat hyviä pitkäketjuisten omega-3 rasvahappojen lähteitä. Monet lajit sisältävät paljon esimerkiksi eikosapentaeenihappoa (EPA) ja dokosaheksaeenihappoa (DHA), jotka vaikuttavat positiivisesti hermoston, sydämen ja verisuonien normaaliin toimintaan. Tyypillisesti EPA:n ja DHA:n tärkein lähde ravinnossa on kala tai kalaöljytuotteet, mutta EPA:n ja DHA:n riittävän saannin takaamiseksi olisi hyvä tutkia muitakin lähteitä pitkäketjuisille omega-3 rasvahapoille. Tässä työssä tutkittiin suomalaisista järvistä eristettyjen mikrolevien *Gymnodinium impatiens*, *Cryptomonas sp*. ja *Euglena velata* lipidi- ja rasvahappokoostumusta, ja selvitettiin voisiko niitä käyttää omega-3 ravintolisien raaka-aineena. Levät valittiin yhteistyökumppani apulaisprofessori Sami Taipaleen (Jyväskylän yliopisto) aikaisempien tutkimusten pohjalta.
Levistä eristetyt öljyt fraktioitiin kiinteäfaasiuutolla polaarisiin ja neutraaleihin lipideihin, ja tutkittiin nestekromatografia-tandemmassaspektrometrilla (LC-MS/MS) lipidiluokkien tunnistamiseksi ja määrän määrittämiseksi. Kokonaisöljyn sekä polaaristen ja neutraalien lipidien rasvahapot tutkittiin kaasukromatografialla, jossa oli liekki-ionisaatiodetektori (GC-FID).
Eniten DHA:ta oli *G. impatiensissa* (18,6 %) ja vähiten *C. sp*:ssa (2,2 %). Eniten EPA:a oli *C. sp:ssa* (21,7 %) ja vähiten *E. velatassa* (4,8 %). EPA:n ja DHA:n yhteismäärä oli suurin *G. impatiensissa* (38,1 %). EPA ja DHA löytyi pääosin polaarisista lipideistä. Polaarisista lipideistä tunnistettiin fosfatidyylikoliineja (PC), monogalaktosyylidiasyyliglyseroleita (MGDG) ja digalaktosyylidiasyyliglyseroleita (DGDG). Neutraaleista lipideistä tunnistettiin triasyyliglyseroleja ja diasyyliglyseroleja. Kaikissa levissä suurimmat lipidiryhmät olivat neutraaleissa lipideissä triasyyliglyserolit ja polaarisissa lipideissä monogalaktosyylidiasyyliglyserolit.
Tämän tutkimuksen perusteella lajeista *G. impatiens* olisi potentiaalisin jatkotutkimuksille ravintolisien raaka-aineeksi. Käytettyjä tutkimusmenetelmiä voisi optimoida ottamaan paremmin huomioon, miten eri lipidit ionisoituvat, ja miten polaariset lipidiluokat saataisiin eroteltua toisistaan paremmin. Tutkimuksella saatiin silti arvokasta tietoa vähän tunnettujen levälajien lipideistä.

Asiasanat: DHA, EPA, mikrolevä, monogalaktosyylidiasyyliglyseroli, omega-3, rasvahappo, ravintolisä, triasyyliglyseroli



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A2- ja hydrolysoidun laktoosittoman A1A2-maidon vaikutukset tulehdukseen ja suolisto-oireisiin

Liisa Hokkanen
Ohjaajat: FM Enni Mannila, Prof. Kaisa Linderborg
ELINTARVIKEKEMIA

Maito on olennainen osa monien ihmisten ruokavaliota, mutta se voi aiheuttaa vatsavaivoja joillekin yksilöille. Laktoosin lisäksi maidon A1- ja A2-kaseiinien erilaisesta suolistokäyttäytymisessä on viitteitä. A1-fenotyypin maidosta peräisin olevan beetakaseiinin hajoaminen voi tuottaa beetakasomorfiini-7 (BCM-7) -nimistä yhdistettä, joka saattaa aiheuttaa suoliston tulehdusta ja motiliteetin muutoksia. A2-maidosta BCM-7-molekyyliä ei muodostu yhtä paljon. Lisäksi maidon proteiinihydrolysointi, jossa maitoproteiineja hajotetaan entsyymien avulla, muuttaa proteiinien pilkkoutumista ruoansulatuskanavassa.
Tutkimuksen tarkoituksena oli selvittää proteiinihydrolysoidun laktoosittoman sekä A1 että A2 -muotoja sisältävän maidon, A1A2-maidon, sekä vain A2-muotoa sisältävän maidon (A2 -maidon laktaasilisällä tai ilman) vaikutuksia koettuihin suolisto-oireisiin sekä tulehdusmerkkiaineisiin. Tutkimus oli satunnaistettu, kaksoissokkoutettu vaihtovuorotutkimus, jossa osallistujat nauttivat maitoja (A2-maito, A2-maito laktaasientsyymillä ja hydrolysoitu laktoositon A1A2-maito) kolmen päivän ajan. Tuloksia tarkasteltiin sekä koko 36 hengen ryhmänä, että jaettuna laktoosia sietäviin ja laktoosi-intolerantikoihin.
Tuloksia arvioitiin vatsaoirepäiväkirjojen perusteella sekä veri- ja ulostenäytteistä. Kun tarkasteltiin laktoosia sietäviä ja laktoosi-intolerantikoita yhtenä ryhmänä, koettiin maitoa sisältävinä jaksoina enemmän vatsaoireita verrattuna maidottomaan ruokavalioon. Laktoosi-intolerantikoiden ryhmässä vatsaoireissa havaittiin eroa A2-maitojen ja maidottoman ruokavalion välillä, kun taas hydrolysoidun laktoosittoman A1A2-maidon kohdalla eroa ei havaittu. Laktoosia sietävien ryhmässä vatsaoireissa ei havaittu eroa. Tulehdusmerkkiaineissa havaittiin ero laktoosi-intolerantikoiden ryhmässä joissakin merkkiaineissa eri maitojen välillä. Näitä olivat esim. interleukiini-8 ja interleukiini-33. Laktoosia sietävien ryhmässä eroja tulehdusmerkkiaineissa ei havaittu. Tämä tutkimus osoitti, että hydrolysoitu laktoositon A1A2-maito on yhtä hyvin siedetty kuin A2-maito.

Asiasanat: A2-maito, laktoosi-intoleranssi, matala-asteinen tulehdus, vaihtovuorotutkimus, vatsavaivat

Ravitsemusinterventio kaupallisilla kasviproteiinivalmisteilla: Terveysvaikutukset glukoosi- ja lipidiaineenvaihduntaan terveillä koehenkilöillä

Emmi-Lotta Virta
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ELINTARVIKEKEMIA

Kasvipohjaisten proteiinivalmisteiden kulutus on viime vuosina kasvanut, jonka myötä markkinoille tulee jatkuvasti uusia proteiinipitoisia täysin kasvipohjaisista ainesosista valmistettuja elintarvikkeita. Eri astein prosessoitujen kasviperäisten elintarvikkeiden vaikutuksista ihmisen terveyteen on kuitenkin saatavilla vain rajallinen määrä tieteellistä tietoa, jonka takia kaupallisten kasviproteiinituotteiden terveysvaikutuksien tutkiminen on tärkeää. Tämän tutkimuksen tavoitteena on tutkia kaupallisten kasviproteiinia sisältävien tuotteiden kulutuksen vaikutusta ihmisten terveyteen aineenvaihdunnan toiminnan kannalta. Tutkimus on tehty osana Newplant-tutkimuskokonaisuutta.

Kasvipohjaisten proteiinilähteiden vaikutuksia ihmisen terveyteen tutkittiin vaihtovuorotutkimusasetelmalla viiden viikon ravitsemusinterventiossa. Interventioon osallistui yhteensä 36 tutkimushenkilöä, joiden ruokavalioiden järjestys oli satunnaistettu. Interventioruokavaliot koostuivat kolmen eri prosessointiluokan elintarvikkeista. Ensimmäinen ruokavalio sisälsi prosessoimattomia kasviproteiinituotteita (palkokasvit), toinen ruokavalio sisälsi kevyesti prosessoituja elintarvikkeita (esim. nyhtökaura, falafelit ja soijarouhe) ja kolmas raskaasti prosessoituja elintarvikkeita (esim. nugetit, burgerpihvit ja nakit). Interventioruokavalioiden vaikutuksia tutkittavien glukoosi- ja lipidiaineenvaihduntaan analysoitiin paastoverinäytteiden avulla. Tutkimushypoteesina oli, että prosessoimattomat ja kevyesti prosessoidut elintarvikkeet saavat aikaan paremman vasteen tutkimushenkilöiden veressä raskaasti prosessoituihin elintarvikkeisiin verrattuna.

Jokainen tutkituista interventioruokavalioista vaikutti positiivisesti tutkittavien arvoihin kokonaiskolesterolin, LDL-kolesterolin ja HDL/kokonaiskolesterolisuhteen osalta. Lisäksi kolmas interventioruokavalio, joka sisälsi raskaasti prosessoituja kasviproteiinituotteita, auttoi laskemaan tutkittavien veren triglyseridiarvoja.

Asiasanat: glukoosi, glukoosiaineenvaihdunta, insuliini, kasviproteiinivalmiste, kolesteroli, lipidiaineenvaihdunta, triasyyliglyseroli

Determination of serum inflammatory markers in response to a plant-based protein dietary intervention

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FOOD CHEMISTRY

Increasing evidence supports the role of diet as a lifestyle and environmental factor that possibly could intensify or promote inflammation. Interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) are both pleiotropic inflammatory markers, which have a connection to the maintenance of homeostasis within the body. In addition, high sensitivity C-reactive protein (hs-CRP) is a marker of low-grade inflammation that is widely used in identifying inflammatory processes in clinical care. The aim of this randomized cross-over study was to examine whether short-term consumption of plant-based protein foods with three different processing levels (whole plant-based foods, mildly processed plant-based and refined plant-based foods) have notable effects on measured inflammatory markers. This study was done as a part of Newplant research.

Total of 37 healthy volunteers in the age range of 18-65 and in the BMI range of 18.5-27 participated in this study. Quantitative assessment of serum IL-6 and TNF-α was performed with enzyme linked immunosorbent assay (ELISA). The results were later compared with hs-CRP values provided by TYKSlab.

When comparing the three diets to their respective baseline values, the concentration of TNF-α increased on average by 0.20 pg/ml (25 %) and concentration of hs-CRP decreased on average by 0.70 mg/l (56 %) on diet containing mildly processed products. The results were statistically significant. Correlation analyses between diets and inflammatory markers showed that there is a moderately strong statistically significant positive correlation between both IL-6 and hs-CRP with the diet of mildly processed products, and between IL-6 and TNF-α with the diet of refined products. Comparing the three different diets did not have a statistically significant effect on inflammatory marker values of the participants.

Keywords: dietary intervention, ELISA, food processing, inflammatory markers, low-grade inflammation, plant-based products, plant proteins

Development of a novel food product by fermentation of faba bean flour

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FOOD DEVELOPMENT (TECH.)

Faba Bean (*Vicia faba* L.; FB) is a leguminous crop abundant in essential nutrients, offering potential health-promoting properties. Despite these health benefits, the consumption of FB is sparse due to the presence of certain antinutritional factors (ANFs; such as vicine and convicine and galacto-oligosaccharides). This study aims at developing a novel food product with potentially reduced ANFs by fermentation of FB flour with different lactic acid bacteria (LAB) cultures and investigating the impact of fermentation on its sugars and acids.

FB flour was inoculated with 1% pure *Lactiplantibacillus plantarum*, 25%/ 50% *Lactiplantibacillus acidophilus-Bifidobacterium sp.* fortified yogurt, and 15%/ 30% mixed curd cultures to create novel fermented products (n=6, 2 replicates). The fermentations were carried out for 24 hours at 30 °C. Finally, the products were prepared by microwaving the fermented batters at 500W for 8 minutes. The sugar and acid contents of the unfermented and fermented batters were analysed using a gas chromatogram coupled with a flame ionization detector (GC-FID).

An increase in the lactic acid content with a decrease in pH was observed in each sample, thereby confirming a successful fermentation. In general, there were significant differences in lactic acid, fructose, and sucrose before and after fermentation in every combination (t-test, $p<0.05$). Also, while the malic acid content increased in curd combinations, it decreased in the yogurt and pure *L. plantarum* combinations.

Lactic acid may help to mask the striking sour flavour of malic acid, improving the potential for consumer acceptance. Hence, FB flour fermented with pure *L. plantarum* and 25% yogurt possess a high potential for novel food product development due to a higher lactic acid content along with a lower malic acid content. In summation, this research elucidates the influence of LAB fermentation on the flavour characteristics of FB-based dairy analogues, thus laying the groundwork for further optimization of the novel food product to enhance its market potential.

Keywords: faba bean, fermentation, lactic acid bacteria, lactic acid, malic acid, *Lactiplantibacillus plantarum,* yogurt, curd, GC-FID

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Analysis of alkaloids in fermented and nonfermented blue lupin (*L. angustifolius*) products

Lakshmi Karath
Supervisors: Doc. Jukka-Pekka Suomela, Ph.D. Marika Kalpio, Doctoral Researcher Jasmin Raita
FOOD DEVELOPMENT

L. angustifolius, also known as blue lupin or sweet lupin belongs to the genus *Lupinus*, a member of the legume family Fabaceae. Lupins contain high amounts of protein and some of them have high alkaloid content. Sweet lupins contain antinutrients termed quinolizidine alkaloids which cause toxic effects on oral consumption. In a variety of food products, blue lupins are utilized either as a whole grain or as an ingredient. Therefore, the analysis of alkaloids in blue lupin products has become a necessity since quinolizidine alkaloid content in blue lupin products needs to be quantified before marketing as a final product.

The study aimed to analyze quinolizidine alkaloids such as angustifoline, alpha-isolupanine, lupanine, sparteine, and 13-hydroxylupaninein in commercial lupin flour, 4 nonfermented and 11 fermented, (*L. angustifolius*) lupin milk samples. Different lactic acid bacteria (LAB) starters were used either with or without a thickening agent to ferment lupin milk samples. The sample pretreatment method was optimized and after extraction, the samples were analyzed using gas chromatography with a flame ionization detector.

The data revealed that commercial blue lupin flour and some fermented lupin milk samples contained alkaloids more than the maximum limit of 200 mg/kg based on international regulations. The total alkaloid content in nonfermented lupin milk samples was observed to be lower than in fermented lupin milk samples. Nonfermented samples with a thickening agent showed lower total alkaloid content than those without. Notably, samples containing a thickening agent (barley starch, 4% w/v) fermented with Mix E; an in-house (Natural Resources Institute Finland) isolate, originating from the plant, exhibited a total alkaloid content lower than other fermented samples. While comparing the different fermentations without the thickening agent, total alkaloids were found to be the lowest (146.00 mg/kg) when fermented with the LAB starter SYAB1(containing LAB species commonly used in yogurt production) followed by 199.92 mg/kg, when fermented with *Lactococcus lactis ssp. lactis* LAB labeled B2_1B and highest when fermented with MixE; 223.88 mg/kg.

Based on this study it could be inferred that total alkaloid content was observed to be much lower in nonfermented lupin milk samples containing a thickening agent as well as those fermented with LAB in-house isolate (MixE). This study also emphasizes the importance and need to use optimal sample pretreatment and analysis methods to reliably study the alkaloid content in blue lupin ingredients and products during product development.

Keywords: blue lupins, fermented, GC-FID, nonfermented, quinolizidine alkaloids

Development, metabolite profile, and sensory qualities of water kefirs with sucrose and non-sucrose sweeteners

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FOOD DEVELOPMENT (TECH.)

Water kefir, a fermented beverage based on sugared water or juices, has become more popular due to its potential health benefits attributed to its microbiota. However, maintaining microbial viability may potentially cause alterations in product qualities as a result of extended fermentation during storage. One possible solution to this challenge is introduction of non-fermentable sweeteners to substitute part of sucrose during production. On the other hand, such a change in the fermentation medium may potentially influence the metabolism of microorganisms in the water kefir and alter the final metabolite profile, potentially affecting the sensory attributes of the beverage.

The aim of this study was to develop prototypes for birch sap-based water kefirs flavoured with raspberry utilising sucrose and natural non-sucrose sweeteners and to compare their sensory attributes and metabolite profiles. Fermentation as the basis of water kefir production was monitored by measuring physicochemical parameters at different product stages. To evaluate the sensory characteristics, descriptive sensory analysis with a trained panel was conducted for a selected number of water kefir prototypes. Gas chromatography with flame ionisation detector (GC-FID) and headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS) were used for the identification and quantification of organic acids, sugars, and volatile compounds in the product prototypes.

A perceived difference in sweetness and sourness was observed between different water kefirs, and an increase in fizziness and sourness with storage time was detected for water kefirs containing sucrose. However, no statistically significant difference was found between recipes for other attributes. This signifies that an alteration in the sweetener composition affects the sensory qualities pertaining to the concentration of sucrose and other sweeteners but not those attributes of odour and flavour that are mostly conditioned by microbial metabolites.

The study shows that production of water kefirs with non-sucrose sweeteners presents a viable possibility from the sensory perspective. Further product development can focus on adjusting sweetener concentrations and studying their preference among consumers.

Keywords: water kefir, fermentation, sucrose, sweeteners, sensory evaluation, volatile compounds, organic acids.

Volatile compounds in juices and ciders made from Finnish apple cultivars

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FOOD DEVELOPMENT (TECH.)

Apples (*Malus domestica Borkh.*) are globally significant, ranking second in consumption and third in production. While primarily consumed fresh, a substantial portion is processed into value-added products like apple juice and cider. Cider, defined as a fermented alcoholic beverage, has gained popularity for its refreshing attributes and gluten-free nature.

This study investigates how Finnish apple cultivars and the choice of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* yeast strains influence the volatile organic compounds of alcoholic fruit beverages. The juice and cider samples utilized in this investigation were derived from a prior study conducted last year and have been stored at -22 °C since then. The apple cultivars were harvested in autumn 2022 from the Marie Priest's Garden orchard (Turku, Finland), managed by the Natural Resources Institute Finland (Luke). Juice samples from six native apple cultivars, including Antonovka, Mustialan Iso Venäläinen, Aleksanteri, Rambo, along with two unidentified cultivars, were separately inoculated with *S. cerevisiae* and *T. delbrueckii*. The current study investigates 18 juice samples and 108 cider samples using headspace solid-phase microextraction gas chromatography-mass spectrometry.

Juice samples presented 36 volatile compounds, whereas cider samples revealed 49. While certain volatile compounds were common to both juice and cider samples, such as esters, higher alcohols, and volatile acids, cider samples boasted a more extensive range of these compounds compared to juice. Moreover, ciders featured distinctive volatile groups, like acetals and lactones, absent in juice samples. Through principal component analysis, correlations were identified between *S. cerevisiae* and esters as well as volatile acids, while *T. delbrueckii* exhibited correlations with a broader range of volatile groups, including acetals, ketones, terpenes, and aldehydes. In addition to yeast strains, the choice of apple cultivar played a vital role in the formation of volatile compounds. Certain cultivars, such as Mustialainen iso venäläinen, Rambo, and Antonovka, exhibited significant correlations with specific volatile compound profiles. This study unveils insights into crafting premium apple-based beverages from specific cultivars and catalyzing the commercialization of native Finnish apple varieties.

Keywords: Finnish apple cultivar, apple cider, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, volatile compounds

The variation of protein, sugars, and organic acids in oat hulls after bioprocessing with enzymes

Sambavi Amirthalingam
Supervisors: Dr. Ying Zhou, Docent. Dr. Oskar Laaksonen,
Prof. Baoru Yang

FOOD DEVELOPMENT (TECH.)

The production of oats (*Avena sativa*) is steadily increasing due to growing awareness of its health benefits and a shift towards plant-based diets. However, this rise in production generates a significant byproduct in the form of oat hulls (OH), often discarded as waste. This study focused on exploring a green method to improve the extraction of nutrients in oat hulls for their potential in the application of innovative food products. To accomplish this goal, enzymatic hydrolysis was employed to break down the recalcitrant structure of oat hulls, converting fibers into oligosaccharides and monomeric saccharides while increasing protein solubility. Eventually, the research aims to enhance the efficiency of utilization of food resources and promote sustainability of the food system.

In this study, samples were prepared using ultrasound-assisted enzymatic treatments in parallel with the corresponding controls. Different enzymes such as commercial food grade carbohydrase, feruloyl esterase, their combination, and proteases were used. The supernatants obtained from them were subjected to further analysis. Protein content was determined using the Kjeldahl method. The composition and quantification of free sugars and organic acids were performed using GC-FID. Oligosaccharides were investigated with reference standards using UHPLC-ELSD. Statistical analysis was conducted with analysis of variance by SPSS and principal component analysis by Unscrambler.

The results revealed a notable increase in protein content, free sugars, and organic acid levels in the supernatants of all enzyme treated samples. Furthermore, the combination of enzymes treatment exhibited greater effectiveness compared to individual enzyme treatments. The highest protein content (68.9 g/100 g OH DM) was obtained with the enzymes combination treatment. It contained free sugars (65.8 g/100 g OH DM) and organic acids (1.7 g/100 g OH DM). Moreover, the semi-quantitative analysis of oligosaccharides using protease enzymes showed certain compounds could be tentatively identified as oligosaccharides. Subsequent analysis to validate these findings could potentially enable the utilization of the fiber fraction of oat hulls in novel food applications. In summary, these results affirm the potential of oat hulls utilization in innovative food products.

Keywords: *Avena sativa,* food-grade enzymes, free sugars, oat hulls, oligosaccharides, organic acids, proteins, ultrasonication


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Phenolic compounds in selected oat and rice products

Md Zakir Hossain
Supervisors: MSc Enni Mannila, Doc. Annelie Damerau, Prof. Kaisa Linderborg
FOOD DEVELOPMENT (TECH.)

Oat and rice have significant nutritional value by containing high amounts of macro- and micronutrients, dietary fibers, and phenolic compounds. Finland is one of the biggest producers and third largest exporter of oat worldwide. The phenolic compounds of these sustainable cereals have been mostly studied in their natural form. Several researchers have shown that food processing has significant effects on phenolic compounds. Therefore, it is important to study the impact of food processing on phenolic compounds in commercial products.

This study aims to identify and quantify the phenolic acids and avenanthramides of selected oat and rice products. The products were chosen from the research project OAT-GUT-BRAIN. All the sample sets were prepared as they were provided to the participants. Oat and rice products were divided into six sample categories. Oat samples were oat flakes, oat grains, oat ready-meal, oat muesli, oat snack bars, and oat meal. Rice samples contained rice flakes, rice grains, rice ready-meal, rice crispies, rice cakes, and rice meal. The samples were extracted with 80% methanol and analyzed using High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). The statistical analysis included the analysis of variance (ANOVA) and principal component analysis (PCA).

In the analysis, eight phenolic acids and five avenanthramides were detected based on standards. All the avenanthramides were detected in oat samples. The most abundant phenolic acids in oat and rice samples were *p*-coumaric acid and ferulic acid. In oat samples, total phenolic-acids content (TPC) was 1.59 – 404.38 mg/100 g of dry weight (dw) samples, and total avenanthramides content (TAC) was 0.68 – 12.23 mg/100 g (dw). In comparison, rice samples provided 2.33 – 82.01 mg of TPC in 100 g (dw). Oat muesli contained the highest amount of TPC and TAC. There were strong relationships between the phenolic contents and nutritional composition of the samples, and the samples were significantly different ($p<0.05$). Phenolic contents of unknown compounds were measured by a semi-quantitative approach using maximum absorption range and literature review. Unknown compounds will be identified using Liquid Chromatography-Mass Spectrometry (LC-MS).

In conclusion, more phenolic compounds were quantified in oat samples than in rice samples, even after processing. A greater number of phenolic compounds were identified in oat than in rice. Avenanthramides were only present in oat samples.

Keywords: avenanthramides, HPLC-DAD, oat, rice, PCA, total phenolic content

Impact of Latitude and Environmental Conditions on the Tocopherol and Phenolic Content of Sea Buckthorn Leaves

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Supervisors: Assist. Prof. Maaria Kortesniemi, Adj. Prof. Jukka Pekka Suomela
FOOD DEVELOPMENT (TECH.)

Leaves of sea buckthorn (*Hippophaë rhamnoides*) are known to contain many health-promoting compounds such as phenolics and tocopherols. As secondary metabolites, they offer a defence mechanism against environmental stress and pathogenic diseases. Interestingly the concentration of these compounds is influenced by latitude, harvest time, genotype, and environmental conditions. This study beams a light on the impact of latitude and environmental conditions on the tocopherol and phenolic content of sea buckthorn leaves of two Finnish cultivars of *Terhi* and *Tytti* from Kittilä (North) and Kakskerta and Paattinen (South) analyzed using UHPLC-FLD and HPLC-DAD respectively.

α-Tocopherol (α-T) was the most dominant of all three tocopherols detected in the leaves, accounting for about 47% of total tocopherols while the proportion of γ-T was 35% and δ-T was 18%. Samples cultivated in the South showed a clear discrimination from the samples grown in the North. Samples cultivated in the south had a higher α-T ($p < 0.05$) and Vitamin E activity ($p < 0.05$) when compared to the northern counterparts. The northern samples had a higher δ-T content of 200.65 ± 13.8 mg/100 g of leaves on a dry weight basis (DW) when compared to the two growth locations in the south with total tocopherol content of 108.75 ± 12.4 mg/100 g DW. Cultivar did not have a statistically significant impact on the samples and the concentration of tocopherols ($p > 0.05$). The principal component analysis (PCA) scores plot showed a clear separation between samples from northern and southern locations based on the year of harvest. PCA also revealed that precipitation sum and storage time correlated positively with samples from the north with lower levels of tocopherols, while temperature sum correlated positively with samples from the southern location with higher levels of tocopherols indicating that warmer climate promotes tocopherol production. Preliminary qualitative result of phenolics shows phenolic content in the northern samples is more prominent than their southern counterparts, with quercetin 3-*O*-rutinoside (rutin) and kaempferol 3-*O*-rutinoside being the dominant phenolic compounds.

This study will provide further insight into how plants adjust to their growth environments, the impact of post-harvest handling, and the intricate relationship between environmental variables, geographical variance, and storage time and their impact on the tocopherol and phenolic content of sea buckthorn leaves.

Keywords: UHPLC-FLD, HPLC-DAD, sea buckthorn leaves, latitude, tocopherols, phenolic compounds, principal component analysis

Identification of carotenoids and phenolic compounds in bacteria-based protein using LC and MS techniques

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ELINTARVIKEKEHITYS (DI)

Global food demand is predicted to increase significantly in the future. Therefore, discovering new protein sources of sustainable origin is important. One of these is bacteria-based protein (PBM), which has minimal environmental impact compared to traditional animal agriculture. In this study we aim to identify the carotenoid and phenolic compound profile of five PBM samples from different cultivations to identify key color pigments and possible off-flavor compounds as well as observe the differences between different samples. The samples were received from Aistila, where they had undergone sensory analyses.

The qualitative analysis of carotenoids and phenolics was done using HPLC-DAD and UHPLC-ESI-MS/MS. Carotenoids and phenolic compounds where extracted using solid-liquid extraction, using acetone for carotenoid extraction, and ethyl acetate, methanol, and water for phenolic compound extraction. Identification and characterization of compounds was carried out by combination of available reference standards, mass spectrometry data, retention time, UV spectral information and equivalent data from previous literature.

A total of 7 carotenoids and 16 phenolic compounds were observed; 4 out of 7 and 6 out of 16 of which were tentatively identified. Zeaxanthin and its glucosides zeaxanthin dirhamnoside and zeaxanthin monorhamnoside were observed as major carotenoids in all five samples; approximately 90-100% of observed carotenoid concentrations were identified as zeaxanthin derivatives. Furthermore, beta-carotene was observed in 4 out of 5 samples, accounting for roughly 1-5% of observed concentrations. Phenolic acid derivatives were observed to be the most common compound type in all proteins. Hydroxybenxoic acid, hydroxybenzoaldehyde, coumaric acid derivatives, phenylalanine, and dihydroxyphenyllactic acid derivatives were tentatively identified from all samples. Hydroxybenzoic acid and it’s derivatives were found to account for 20-50% of observed phenolic concentrations.

Future studies are needed for a thorough quantification and identification of all carotenoids and phenolic compounds in this bacteria protein, as well as to discover their influence on the product’s appearance, flavor, and odor.

Keywords: bacteria-based protein, carotenoid, HPLC-DAD, HPLC-MS, phenolic acid, phenolic compound, zeaxanthin

Behaviour of n-3 polyunsaturated fatty acid ethyl esters in INFOGEST *in vitro* model

Iida Valta
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FOOD CHEMISTRY

Omega-3 polyunsaturated fatty acids (n-3 PUFAs), such as marine-derived docosahexaenoic acid and plant-derived α-linolenic acid (ALA) are crucial for human health. In nature, n-3 PUFAs are mainly in triglyceride (TAG) form, but their content is often enriched in supplements through their transesterification to ethyl esters (EE). Concentrated EEs can be re-transesterified back to TAGs or included in supplements as such. Short-term clinical trials have suggested reduced bioavailability of EEs compared to TAGs, possibly resulting from slower hydrolysis of EEs than TAGs by pancreatic lipase. Previous research has indicated that the ingestion of n-3 PUFA EEs with fatty meal improves their bioavailability, possibly because 2-monoglycerides (2-MAG) are provided for resynthesis of TAGs in intestinal enterocytes.

This study investigated the influence of dietary fat, e.g. TAG and 2-MAG of oleic acid, on the hydrolysis of ALA-EE with the INFOGEST *in vitro* digestion model. In addition, the effect of encapsulation of linseed oil EEs on digestion was studied. Encapsulation protects n-3 PUFAs from oxidation, but the effect of polysaccharide-type coating materials on bioavailability is not well understood. EE hydrolysis and re-esterification in the digesta were evaluated with proton nuclear magnetic resonance spectroscopy. Lipid classes of digestates were analyzed with liquid chromatography combined with mass spectrometry.

Hydrolysis of ALA-EE increased from 6.6 % to 22.1 % and to 28.8 % when triolein and 2-monoolein, respectively, were incubated with excess of ALA-EE (1:10 ratio) in simulated digestion. Prolonging intestinal phase from 2 to 4 hours resulted in 54.9% ALA-EE hydrolysis with 2-monoolein. Enhanced hydrolysis in the presence of 2-monoolein occurs likely because 2-monoolein is already in bioavailable form, whereas triolein is also a substrate of lipase, thus limiting hydrolysis of EEs. Re-esterified TAGs and diglycerides containing ALA were formed with 2-monoolein and in minor amounts with triolein. These findings suggest that transesterification in small intestinal lumen would not be the main route for EE absorption. Microencapsulation improved EE hydrolysis 27.1-40.9 % depending on coating material. Emulsified form of EEs in microcapsules likely facilitates the action of pancreatic lipase.

Keywords: n-3 polyunsaturated fatty acids, α-linolenic acid, ethyl ester, *in vitro* digestion, INFOGEST, microencapsulation, bioavailability

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Variability in fatty acid concentration in hemp (*Cannabis Sativa* L.*)* seeds depending on soil conditions

Michaela Kotsiou
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FOOD DEVELOPMENT (TECH.)

Hemp (*Cannabis Sativa* L.*)* belongs to the *Cannabaceae* family and the *Cannabis* genus. Depending on its use *Cannabis* is categorized into two groups: marijuana and hemp. Marijuana is mostly used for recreational purposes because of its intoxicating characteristics, although it may also be used for medicinal purposes. Hemp, on the other hand, is used for medicinal purposes, fiber, and seeds. Hemp seeds are the fruit of *Cannabis Sativa* L. The whole hemp seeds consist of 25–35% lipids, 20–25% protein, 20–30% carbohydrates, and 10-15% insoluble fibers and minerals. The high concentration of polyunsaturated fatty acids (PUFAs) in hemp seeds is mostly responsible for their benefits on human health. Hemp seed oil does not have psychotic effects but instead displays health-beneficial activity due to its ideal ratio of n-6 linoleic acid and n-3 linoleic acid (3:1) which is recommended in a healthy diet.

The aim of the work is to study the effects that different soil conditions, such as processing with *Arbuscular Mycorrhizal Fungi* (AMF) and glyphosate residues, have on the lipid content and the fatty acid composition of hemp seeds. For this reason, four different soil types were used for the cultivation of the plants: control soil, control soil treated with mycorrhizal fungi, soil with glyphosate residues, and soil with glyphosate residues treated with mycorrhizal fungi. The lipids were first extracted in the form of oil from the hemp seeds using chloroform and methanol. After the extraction, the oil was methylated and analyzed using gas chromatography paired with a flame ionization detector (GC-FID).

The results of the study indicated that the total lipid content increased in the samples that were treated with mycorrhizal fungi. The main fatty acids that were found in all the samples were palmitic acid (~5%), stearic acid (~2%), oleic acid (7-8%), linoleic acid (~50%), γ-linoleic acid (~5%), and α-linoleic acid (~20%). There was also a statistically significant difference ($p<0.05$) between the four different soil conditions in terms of the total lipid content and the sums of saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs), and n-3 fatty acids. In conclusion, the treatment of the plants with mycorrhizal fungi increased the total lipid content but at the same time decreased the sums of polyunsaturated fatty acids and n-3 fatty acids.

Keywords: Hemp, Total lipid content, Fatty acids, Lipid extraction, GC-FID, n-3 to n-6 ratio.

Analysis of enantiomers and regioisomers of triacylglycerols using supercritical fluid chromatography

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FOOD DEVELOPMENT (TECH.)

The diverse structural nature of lipids present in real biological samples contributes to the complexity of lipid analysis, and sophisticated analytical methods are needed. Recently, several chromatography techniques including recycle high performance liquid chromatography (HPLC) have been reported in analyzing triacylglycerol (TAG) regioisomers and enantiomers. However, most of the existing chromatographic methods require more analysis time and they consume a high amount of organic solvents. Nowadays, supercritical fluid chromatography (SFC) in lipid analysis has gained more attention since it is known to be a low-cost and eco-friendly technique and can be applied to identify isomeric lipid species.
The overall aim was to familiarize oneself with the operating principles of SFC instrumentation. More specifically, this study aimed to develop and optimize a method to analyze regioisomers and enantiomers of TAG isomers (*sn*-PPO, *sn*-OPP, POP, *sn*-OOP, *sn*-POO, OPO, and ABC-type TAGs isomers). For that, the subcritical fluid chromatographic technique with mass spectrometric (MS) detection was applied with 2 similar CHIRALPAK® IG-U columns in series. The developed method was applied to find the relative abundance of *sn*-PPO, *sn*-OPP, POP, *sn*-OOP, *sn*-POO and OPO in selected vegetable oils (rapeseed oil, olive oil, oat oil, sunflower oil, avocado oil and rice bran oil).
Based on the obtained results, the most optimal SFC conditions were selected (flow rate, pressure, solvent gradient, injection volume and column oven temperature). This novel chiral SFC method was able to separate TAG isomers including *sn*-OOP/*sn*-POO/*sn*-OPO, *sn*-OPP/*sn*-PPO/*sn*-POP, *sn*-MPO/*sn*-OPM, and *sn*-OPS/*sn*-SPO. However, TAG isomers *sn*-OPL/*sn*-LPO and *sn*-LaPO/*sn*-OPLa were not appropriately separated by applying the developed method. All the separations were completed within 35 minutes. Based on the results of relative abundance and ratios of TAG isomers in selected vegetable oils, the enantiomers present in vegetable oils are not racemates. This approach of simultaneous analysis of TAG regioisomers and enantiomers is crucial in obtaining the metabolic, nutritional, and technological information on TAGs in foods.

Keywords: enantiomers, mass spectrometry, method development, regioisomers, supercritical fluid chromatography, triacylglycerols, vegetable oils

Fermentation of bladderwrack (*Fucus vesiculosus*) and its impact on composition

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FOOD DEVELOPMENT (TECH.)

In response to consumers demand for non-dairy products due to lactose intolerance and the growing preference of a vegan diet, production of plant-based fermented products such as kimchi, sauerkraut have increased. The majority of plant-based fermented products utilize terrestrial vegetables as their substrate as seen in the use of lactic acid fermentation in the processing of cucumber, cabbage and olives. However, research on the fermentation of aquatic plants such as seaweeds remains limited. Bladderwrack (*Fucus vesiculosus),* known and utilized for its distinctive saltiness (high iodine concentration) and richness in bioactive compounds is a small-sized brown seaweed. Its non-volatile compounds have been studied for their potential value in different industrial applications. However, a detailed identification of its volatile compounds is essential in defining desirable aroma and flavors to be enhanced during processing to meet consumer preferences.

The aim of this study was to investigate changes in the chemical composition (pH, organic acids, and volatiles) of fermented bladderwrack. In this study, fresh bladderwrack was processed at different conditions (blended and chopped) and treatments (raw, heat treatment and enzymatic hydrolysis) to prepare seaweed broth for fermentation. The broth was fermented with cells of *L. plantarum* strains for 120 h at 37 °C. pH was measured every 24 h, organic acids were analyzed with gas chromatography coupled with flame ionization detector after silylation and the profile of volatile compounds in the fermented seaweeds was investigated using headspace solid-phase microextraction with gas chromatography–mass spectrometry.

A decrease in pH is shown in heat treated samples compared to the raw samples. In the analysis of organic acids, lactic acid and malic acid were present in fermented samples. The volatile analysis of the raw and fermented samples detected thirty-eight compounds. Among these were identified two aldehydes, two benzenes, three acids, four esters, five hydrocarbons, seven ketones, and ten alcohols. Bladderwrack's complex polysaccharides composition presents intrinsic challenges for a successful fermentation, yet it remains a viable means for the development of innovative seaweed products. Nonetheless, additional research is necessary to optimize the process. This involves exploring different starter cultures, alternative seaweed pretreatments and enhancers to promote the fermentation process of bladderwrack for potential food use.

Keywords: bladderwrack, fermentation, lactic acid bacteria, volatiles, GC-MS

Fermentation of *Fucus vesiculosus*: Sensory evaluation and product innovations

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FOOD DEVELOPMENT (TECH.)

Fucus vesiculosus is a common macroalgae abundant in coastal regions of the North Sea, the western Baltic Sea and in both Atlantic and Pacific oceans. While traditional consumption of seaweeds is prominent in Asian cultures, trends of consumption of seaweed-based products are growing in Western cultures. However, consumption remains relatively low despite the recognized health benefits.

Fermentation is a natural bioprocess that can improve the shelf-life, sensory and nutritional quality of fresh macroalgae. The study initially trialed two different conditions of the macroalgae: chopped intro approximately 1-inch pieces and blended with an immersion blender. Both conditions were trialed using lactic acid bacteria (LAB) *Lactiplantibacillus plantarum* DSM 20174. Following this, different glucose concentrations (10 and 20 %) were trialed to attempted to optimize the fermentation. Variations were also made to the algae concentrations used within the fermentation mass. Finally, the fermented and fresh macroalgae were used to develop two different product innovations, a pesto and a sauerkraut respectively. Fermentation progress was monitored by chemical analysis. The sugars and acids were then analyzed through gas chromatogram coupled with flame ionization detector on samples across different fermentation durations. The sensory evaluation was carried out by a trained panel (n=6) and was split into two parts: likeness of the two product innovations compared to their controls and descriptive analysis on 4 samples which were subjected to different treatments, such as raw, heat treated, 2-day fermentation, and 12-day fermentation.

The preliminary results suggests that lactic acid concentrations were higher in 10% glucose compared to use of 20% indicating a more optimum sugar concentration for LAB. Despite a significant reduction in pH, no lactic acid was detected from the 2-day fermentation. Spontaneous fermentations with naturally occurring LAB (from the cabbage), contained significantly higher concentrations of lactic acid compared to controlled fermentations inoculated with the LAB strain. The preliminary result from the sensory evaluation suggests that fermentation reduced the overall aroma intensity in the 12-day fermentation compared to the raw untreated sample. Additionally, both 2 and 12-day fermentations significantly reduced grassy and seaweed aromas compared to raw and heat treated.

Keywords: aroma, fermentation, lactic acid bacteria, *Lactiplantibacillus plantarum*, macroalgae, sensory evaluation

Hyvinvointia rakentamassa

Lactic acid fermentation of crowberry juice: Effects on phenolic compounds

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FOOD CHEMISTRY

Crowberry (*Empetrum nigrum* L.) is an evergreen shrub native to many areas of Northern Hemisphere, including Finland. However, despite abundant harvests of dark purple-to-black berries each year, these anthocyanin-rich berries are currently underutilized commercially. As such, alternate processing schemes for crowberries should be investigated. Lactic acid fermentation has previously been used to modify physicochemical properties of various berry juices, though studies on crowberry juice are lacking. The purpose of this thesis was thus to study lactic acid fermentation of crowberry juice and its effects on various phenolic compounds.
In this study both non-pH adjusted (pH 3.7) and pH-adjusted (pH 5.0) samples of pasteurized crowberry juice were fermented for five days (sample collection on days 0, 3 and 5) at 30 °C using two strains of *Lactiplantibacillus plantarum*. Additionally, uninoculated juice samples were used as controls. Successful lactic acid fermentation was confirmed through a GC-FID analysis, in which lactic acid was detected in all Day 5 samples. To evaluate changes in the amounts of lactic acid bacteria during fermentation, viable colony counts were also performed on Day 5 samples. Anthocyanins were analyzed using HPLC-DAD, and will be further analyzed with LC-MS. Other phenolic compounds will be analyzed using the same methods.
Based on viable colony counts, pH-adjusted juice was a much more suitable growing medium for the two strains of *L. plantarum*: number of viable cells in both cases increased by a factor of 10^4. In contrast, tenfold decrease was observed in non-pH adjusted juice for both strains. Decrease in the number of bacteria was also observed during earlier optimization of fermentation conditions, and this was the main reason behind the use of pH-adjusted juice.
Preliminary HPLC-DAD results indicate that anthocyanin content decreased in all samples during fermentation, which was to be expected due to their poor stability. However, in some fermented samples the anthocyanin content appears to have been slightly higher than in controls, with the effect being strain-dependent. As such, fermentation by *L. plantarum* may have a stabilizing effect on anthocyanins.

Keywords: anthocyanins, crowberry, lactic acid fermentation, *Lactiplantibacillus plantarum*, phenolic compounds

Enzymatic and microbial solubilization of brewers' spent grain for sugar and phenolic compound extraction.

Jaakko Hakakari
Supervisors: MSc Oskar Laaksonen and senior lecturer Eija Kulju
FOOD CHEMISTRY

Brewer's spent grain (BSG) is a nutritionally valuable byproduct of brewing beer. BSG is rich in phenolic compounds, fiber and protein. Current methods of utilizing BSG range from animal feed to biofuel production. Issues with utilizing BSG in food production stem from how BSG spoils rapidly as it is a favorable growth medium for bacteria and fungi. Methods of sterilization prove costly and labor intensive, so immediate methods of processing are needed. Currently there are no in-house methods of gaining value from BSG within microbreweries.
The aim was to create an immediate method to utilize BSG within a microbrewery setting using bacteria and enzymes. By employing a method to release fermentable sugars and potential flavor-active phenolic compounds from BSG, these compounds can be reintroduced back into the brewing process. Onsite processing would rule out the need for heat treatment and cold storage. Fresh BSG was collected from local breweries, blended, and stored in water at room temperature. Multiple samples were collected in 30 ml batches with a variety of different lignocellulose-hydrolyzing enzymes and a *lactiplantibacillus plantarum* strain. Samples were incubated in 25° C for 20 hours before collecting the liquid fraction for analysis. For analysis, UHPLC-DAD is used to quantify phenolic acids, HPLC-MS/MS is used for phenolic acid identification and HPLC-ELSD is used to analyze the sugar content within the samples.
An analysis of the liquid fraction with UHPLC-DAD revealed an increase in ferulic acid and p-coumaric acid with samples treated with enzymes. An upcoming analysis of sugars within the samples is expected to show an increase in sugars produced by enzymes and a reduction in glucose within samples containing *lactiplantibacillus plantarum.* The goal is to produce a method of extracting components like lactic acid, complex carbohydrates, and phenolic acids from BSG and reintroducing said components into the beermaking process.

Keywords: Brewing, Beer, Brewer's spent grain, phenolic acids, lactic acid, recycling, circular economy,

Utilization of brewer's spent grain and other selected by-products to create edible cutlery

Marta Stachnik
Supervisors: M.Sc. Eija Kulju, P.hD. Doc. Oskar Laaksonen
FOOD DEVELOPMENT

Single-use plastic cutlery gained a bad reputation for its negative effect on the environment, being banned in many countries. This research utilizes 3D printing technology to develop prototypes of edible spoons from sidestreams of food and beverage production, being thus a solution to plastic pollution.
A set of six dough recipes (food inks) were developed and evaluated. The printability of the food ink was assessed through rheological measurements, while the spoons were tested for their water and oil absorption and water solubility. Additionally, a sensory evaluation of the spoons was performed.
Rheological evaluations in oscillatory mode, simulating the printing process, revealed that inks exhibit elastic behaviour at rest, becoming viscous under shear (phase shift angle from 14° to 80°). Both storage (G') and loss (G") moduli showed significant recipe-dependent variations. The recovery rate was notably low, despite the ink's ability to resist deformation, which was further reflected in the sagging of the dough after the print.
After 24 hrs. when submerged spoons absorbed large amounts of water, doubling their weight, but not oil – on average 20% increase in weight. There were no significant differences in water or oil absorption between samples. However, spoons made with brewers' spent gran flour had significantly higher water solubility than other recipes.
In the sensory evaluation, spoons made with fruit pulp received the highest ratings for colour, appearance, and smell. When used with plain yoghurt, the spoons did not noticeably alter the food's flavour. The texture of all spoons was considered to be appealing, with the 3D printing layers being either imperceptible or considered pleasant. However, some panellists observed that certain spoons were significantly more bitter, burnt, or sour than others.
Dough printing properties require improvements regarding its recovery and ability to hold shape. Also, water absorption needs to be lowered. Sensory tests confirm the need to improve printability and, thus shape of the spoons. Additional flavour enhancers could be used to offset bitterness and sourness.

Keywords: 3D printing, circular economy, edible spoons, side streams, plastic pollution

Kilpailevien mikrobien vaikutus Streptomykeettien geenien ilmenemiseen

Aleksi Kauro
Ohjaajat: Ph.D. Keith Yamada, Prof. Mikko Metsä-Ketelä
MOLECULAR SYSTEMS BIOLOGY

Luonnonyhdisteet ovat monimuotoisia kemiallisia yhdisteitä, joita eri organismit kuten kasvit, levät, sienet ja bakteerit tuottavat sekundaarimetaboliassaan. Näitä luonnonyhdisteitä voidaan käyttää sellaisenaan tai muokattuina esimerkiksi lääkeaineina. Mikrobeja on hyödynnetty monien lääkeaineiden tuotannossa jo vuosikymmeniä mutta, uusien hyödyllisten luonnonyhdisteiden etsiminen bakteereista perinteisin menetelmin tuottaa yhä useammin vain jo löydettyjä yhdisteitä.

Laajamittainen Streptomykeetti -suvun bakteerien genomien sekvensointi on paljastanut, että näillä bakteereilla on erittäin suuri geneettinen potentiaali erilaisten luonnonyhdisteiden tuottamiseen. Tästä syystä Streptomykeetit ovat kiinnostava kohde uusien lääkeaineiden etsimiseen. Ongelmaksi on muodostunut se, että Streptomykeetit tuottavat vain pienen osan näistä yhdisteistä puhdasviljelmissä laboratorio-olosuhteissa. Yhteisviljelmiä, joissa kaksi tai useampi organismi kasvaa samassa mediumissa, voidaan käyttää vaihtoehtoisena keinona muuttaa Streptomykeettien geenien ilmenemistä ja saada ne tuottamaan uusia luonnonyhdisteitä.

Tutkimuksen tavoitteena on selvittää transkriptomiikan keinoin minkälaisia muutoksia Streptomykeettien sekundaarimetaboliaan tulee yhteisviljelmän seurauksena. Tässä tutkimuksessa käytetään yhteensä kuutta eri Streptomykeetti lajia, joita kasvatetaan yhteisviljelmässä hiivojen (*Saccharomyces cerevisiae* ja *Candida krusei*) kanssa. Viljelmät analysoidaan ensin valomikroskoopilla sekä korkean erotuskyvyn nestekromatografilla (engl. *High Performance Liquid Chromatography,* HPLC) ja lopuksi soluista eristetty kokonais-RNA eli transkriptomi sekvensoidaan. Sekä mikroskopia että nestekromatografia näyttävät, että Streptomykeetit reagoivat hiivasolujen läsnäoloon eri tavoin. Yhteiskasvatusten vaikutus geenien ilmenemiseen ja kasvatusten eroavaisuuksiin perustuva transkriptomin analyysi on vielä meneillään.

Asiasanat: differentiaalinen ekspressio, luonnonyhdisteet, streptomykeetit, transkriptomiikka, yhteisviljelmä

LÖYDÄT SHOTIT MEHUHYLLYSTÄ

Marli

VAIKUTTAVAN HYVÄ SHOT

HELPPO ANNOS PÄIVITTÄISTÄ HYVINVOINTIA

VATSAN HYVIN-VOINTIIN*

Runsaasti hedelmä- ja kasviperäisiä **kuituja.**

MUISTIN TUEKSI*

B6- ja B12-vitamiinit edistävät normaaleja psykologisia toimintoja.

SYDÄMELLE*

Tyydyttymättömät **omega-3-rasvahapot** edistävät veren kolesterolitason pysymistä normaalina.

VASTUSTUS-KYVYLLE*

C-vitamiini vahvistaa vastustuskykyä ja auttaa vähentämään väsymystä.

*Huolehdi tasapainoisesta ja monipuolisesta ruokavaliosta sekä terveistä elämäntavoista.

Transcriptome analysis unveils regulatory landscape of *Spirochaeta africana*

Juho Kotikoski
Supervisors: M.Sc. Vilma Trapp, Assoc. Prof. Georgi Belogurov
SYSTEMS BIOLOGY

Transcription is the first and most regulated step in gene expression. Transcription is catalyzed by RNA polymerase (RNAP), a complex multisubunit enzyme. RNAP can read regulatory signals encoded in the DNA and respond to changes in the concentration of ribonucleoside triphosphates (NTPs). Most of the regulatory inputs come to RNAP via accessory protein factors and regulatory RNAs. Transcription systems of model organisms such as *Escherichia coli* and *Bacillus subtilis* have been studied in detail but little is known about transcription regulation in spirochetes. *Spirochaeta africana* is a feasible model for spirochetes since it is non-pathogenic and is relatively easy to culture.

To study the transcription regulation in *S. africana* we used two complementary approaches: (i) we expressed, purified, and reconstituted the core transcription system of *S. africana in vitro* and measured the activities of selected promoters; and (ii) we isolated total RNA from *S. africana* and sequenced the transcriptome. We sequenced six RNA samples, estimated the abundances of cellular transcripts, and compared the levels of RNAs encoding known transcription factors and RNAP subunits. The sum of transcripts encoding all 7 sigma factors constituted 4 times the amount of RNAP transcript in the exponential growth phase and 15 times in early saturation. During the log phase, the transcription initiation factor CarD and the elongation factor NusA were transcribed at 0.5 times and 0.6 times the amount of RNAP, respectively. In early saturation, CarD and NusA levels changed to 0.8 times and 0.2 times the amount of RNAP, respectively. The CarD/NusA ratios suggest that approximately half of the RNAP is engaged in transcription initiation and half in transcription elongation during the logarithmic phase. In early saturation, the fraction of elongating RNAP likely decreases at least twofold.

Our analysis also suggests that alternative sigma factors play a dominant role in *S. africana* because RNA encoding the primary sigma factor represented only 9% of all sigma-encoding RNAs during the exponential growth phase and only 1% in early saturation. This hypothesis is reinforced by the comparison of the *in vivo* and *in vitro* activities of several promoters featuring good -10 and -35 elements. Three out of seven promoters displayed abnormally low activity when assayed *in vitro* using the primary sigma holoenzyme, suggesting that they may be predominantly recognized by an alternative sigma factor *in vivo*.

Keywords: transcription regulation, RNA polymerase, spirochetes, transcriptomics, RNA-Seq, transcription factors, alternative sigma factors

Transcriptome of the SigB overexpression strain of cyanobacterium *Synechocystis* sp. PCC6803

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MOLECULAR SYSTEMS BIOLOGY

Synechocystis sp. PCC6803 (*Synechocystis*) is a photoautotrophic cyanobacterial species with great biotechnological potential for bioproduction of commercial metabolites. For practical applications robust host strains would be useful, and we have tested if modification of the main transcriptional regulators, σ factors, could improve the performance of *Synechocystis* as a host organism. A particular σ factor, SigB, activates transcription of many stress responsive genes when *Synechocystis* acclimates to different environmental conditions like high temperature or oxidative stress. We have inserted an extra copy of the *sigB* gene under the strong *psbA2* promoter into *Synechocystis* genome and studied the properties of the SigB-oe strain.

Real time qPCR analysis revealed that *sigB* transcripts were 6–fold more abundant in SigB-oe than in the control strain. We first compared the transcriptomes of the SigB-oe mutant and the control strain in an optimal growth environment. To do this, liquid cell cultures of the two strains were grown in standard conditions (photosynthetic photon flux density 40 µmol $m^{-2}s^{-1}$, 32 °C, ambient air, shaken at 90 RPM), the total RNA was isolated and sent for commercial sequencing. Then the reads were mapped to *Synechocystis* genome with Bowtie2, counted with HTSeq, and differential expression was analyzed with DESeq2 to compare the transcriptomes. No major differences were observed between the transcriptomes of SigB-oe and the control strain.

Next, we studied the σ factor content of the RNAP holoenzyme of the SigB-oe strain. For this, a histidine tag was added to the γ subunit of the RNAP core of both strains. Then, cells were grown in standard conditions, and RNAP complexes were pulled down using cobalt-coated magnetic beads. Western blotting was performed to compare the σ factor content in the RNAP holoenzymes of the two strains. The SigB content was low in both strains in standard conditions, and similar SigA, SigC and SigD contents were measured in both strains. Similar RNAP holoenzyme contents of the SigB-oe and control strains explain why the transcriptomes of these strains are similar.

Keywords: cyanobacteria, RNA polymerase holoenzyme, *Synechocystis*, SigB sigma factor, transcription

Physiological roles of the chloroplast acetyltransferase GNAT1 and GNAT2 in *Arabidopsis thaliana*

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The acetylation machinery in the chloroplast consists of eight acetyltransferase enzymes that belong to the General control non-repressible 5-related N-acetyltransferase (GNAT) superfamily. Loss of the GNAT2 enzyme has been shown to affect the regulation of photosynthetic light harvesting, thylakoid dynamics, plant phenotype, and acetylation level of chloroplast proteins, but the detailed effects on seed germination, root development, thylakoid protein acetylation, and *de novo* synthesis of Photosystem II (PSII), are yet to be studied. Therefore, the aims of my thesis are (i) to examine the effect of GNAT2 on thylakoid protein accumulation and acetylation of light-harvesting complex II (LHCII) proteins; (ii) to investigate germination and root morphology of wild type and *gnat2* knock-out mutant under standard conditions and osmotic stress; and (iii) to investigate the role of GNAT2 and GNAT1 in the early stages of PSII biosynthesis in *Arabidopsis thaliana*. So far, the results indicate that the loss of GNAT2 led to decreased seed germination (27% decrease both under standard and osmotic stress conditions) and an overall decrease in root growth both under standard (35%) and under osmotic stress induced by 200 mM mannitol (47%). Separation of thylakoid proteins by 2-D SDS-PAGE followed by Coomassie R250 staining showed changes in the pI of several protein spots. LC-MS/MS analysis of the spots revealed that the acetylation levels of LHCB 1, LHCB 2.1, LHCB 2.2, and LHCB 2.4 were decreased in the *gnat2* mutant. The MS data analysis is currently ongoing and the phenomenon is being further investigated. The analysis of PSII assembly utilizing clear native polyacrylamide gel electrophoresis is currently in progress. Overall, the results have the potential to bring in new insights into the physiological role(s) of chloroplast protein acetylation in higher plants.

Keywords: acetylation, acetyltransferase, Arabidopsis, chloroplast, light harvesting protein, Photosystem II, root morphology

New nanoparticle aided glycovariant biomarker tools to detect extracellular vesicles as a liquid biopsy for early diagnosis of bladder cancer

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Bladder cancer (BlCa) remains a significant health concern worldwide, with a high incidence rate and substantial morbidity and mortality. Early detection of bladder cancer is critical for timely intervention and improved patient outcomes. While current diagnostic methods such as cystoscopy and urine cytology play essential roles, unfortunately they have limitations in terms of invasiveness, sensitivity, and specificity. Therefore, there is an urgent need for non-invasive and reliable biomarkers that can enhance the accuracy and efficiency of bladder cancer detection.

The aim of this project is to develop and validate highly sensitive nanoparticle-based time-resolved fluorometry immunoassay (TRFIA) to detect bladder cancer patients compared to clinically challenging benign samples. This project focused on the development of a combination of biotinylated antibody as a capture and europium chelate-doped nanoparticles (NPs)-coated lectin as a tracer used in immunoassay for the detection of BlCa patients. Captures such as cancer-associated integrin (ITGA-3) and cell adhesion molecules (CAM1 & EpCAM) markers in combination with *Ulex Europeaus* Agglutinin (UEA) lectin were tested to find out the best functional biomarkers and their corresponding potential assays. Then the functional biomarker combinations were characterized and validated using cell culture medium (CCM) & isolated extracellular vesicle (EV) derived from cancer cell lines as well as pooled urine of PCa and BlCa patients. We have observed fold changes with various salts and buffer optimization. Specifically, when employing UEA lectin, we obtained p-values of .03, .0466 and .05 for EpCAM, CAM1 & ITGA-3 respectively.

The outcomes of this project highlight the significance of systemically screening of UEA lectin with different captures to improve the development of a glycovariant assay for the detection of bladder cancer patients.

Keywords: bladder cancer, extracellular vesicle, TRFIA, integrin, cell-adhesion molecule, nano-particles, lectin

Tunneling nanotubes enabling enterovirus cell-to-cell transmission – a novel way of spreading

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MOLECULAR SYSTEMS BIOLOGY

Enteroviruses are small, non-enveloped, positive-sense single-stranded RNA viruses. They are human pathogens, and some patients may develop neurological complications like aseptic meningitis, encephalitis, or cardiorespiratory failure. Non-enveloped viruses lytically burst from cells, releasing virions to neighboring cells. However, cell-free transmission encounters barriers like intrinsic immunities and neutralizing antibody evasion, impacting viral spread efficiency. There is evidence that some viruses for example HIV and SARS-CoV-2 use the cellular open membranous channels (Tunneling Nanotubes - TNTs), connecting neighboring cells, for direct cell-to-cell transmission and evade immune system recognition.
This project aimed to investigate the cell-to-cell movement of Coxsackievirus B3 and Coxsackievirus A9 through TNTs for spreading from one cell to another. This will shed light on how viruses spread in the central nervous system to infect cells lacking viral receptors, leading to severe neurological symptoms.
After virus infection in Green Monkey Kidney (GMK) cells, we used immunofluorescences and fluorescent small molecules to show infection, cellular morphology, formations of TNTs in infected and non-infected cells. To confirm intercellular connectivity via TNTs, we utilized split GFP and split sfCherry technology in a way that when different cell populations, harboring only parts of GFP or sfCherry, were connecting to each other we would see a fluorescent signal. Our results showed the evidence of increased TNT formation after virus infection. Actin staining with Phalloidin confirmed the presence of actin filaments within the cellular protrusions, indicating their identity as TNTs. However, we could not confirm that these structures were open ended, devoid of tubulin, or capable of transmitting the virus to neighboring cells. This work will continue to address these issues.

Keywords: enterovirus, fluorescent microscopy, green monkey kidney cells (GMK), split GFP, tunneling nanotubes

Using Shape-Focused Pharmacophore Modeling to Improve Docking Screening with Acetylcholinesterase

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Acetylcholinesterase is key in reducing acetylcholine signaling in cholinergic synapses by degrading the neurotransmitter into choline and acetic acid. Acetylcholine deficiency is related to neurodegenerative disorders such as Alzheimer's and Parkinson's disease. The first acetylcholinesterase inhibitors approved for clinical use were withdrawn due to harmful side effects; however, the emergence of the protein's 3D structure opened the door for structure-based drug discovery using affordable *in silico* methods. Computer-aided drug discovery is typically divided into ligand-based and structure-based methodologies such as pharmacophore modeling and flexible molecular docking, respectively. In this study, an in-house hybrid ligand- and structure-based method called O-LAP modeling was applied to improve docking-based virtual screening yield using human acetylcholinesterase as a test case. O-LAP is a graph clustering algorithm that builds shape-focused pharmacophore models by merging together overlapping atomic content. The non-clustered input can originate from docked active small-molecule ligands and/or cavity-based negative images. The shape complementarity of the O-LAP models with the flexibly docked ligand poses is compared using a similarity comparison algorithm. The O-LAP modeling improves docking yield for acetylcholinesterase massively based on the benchmarking done using a demanding and random training/test set division. The best docking boost is acquired by systematically exploring alternative clustering settings, carefully curating the cavity-filling atomic input, and optimizing the O-LAP model composition using a greedy search approach. In conclusion, the proof-of-concept testing with acetylcholinesterase indicates that the O-LAP modeling has the potential for wider usage in computer-aided drug discovery.

Keywords: acetylcholinesterase, cavity-based techniques, computer-aided drug discovery, graph clustering, greedy search optimization, molecular docking, O-LAP, pharmacophore, shape similarity, virtual screening.

Development of an LC-IM-MS method for profiling novel N-acyl amides in fecal samples

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MOLECULAR SYSTEMS BIOLOGY

Lipids are vital in multiple physiological functions, such as cell signaling and structure. Lipid profiles are sensitive to changes in the environment, immune system or diet. Fecal lipid analysis offers information on gut physiology, and lipid levels are known to be associated with microbial production. Lipidomic methods usually apply high-resolution mass spectrometry coupled with liquid-chromatogram (LC) separation. Ion mobility spectrometry (IM) provides a unique physiochemical property and additional separation dimension to the data. An improved separation technique is helpful for lipid analysis since lipids often have similar masses and multiple isomers. IM can also improve the signal to noise of the method due to the removal of overlapping background signals.
N-acyl amides (NAAs) are a large and diverse class of lipids comprising an acyl tail and an amine group. It is hypothesized that these molecules could facilitate various physiological functions, and revealing these mechanisms may offer better mechanistic understanding. Some NAAs, such as endocannabinoids, are already known to play an essential role in human physiology, for example, participating in immune responses. Strong evidence suggests these compounds may interact through several different receptors, such as metabolite sensing G-protein coupled receptors which are widespread in the body and are known to regulate gastrointestinal tract physiology, among other mechanisms. The NAAs may also carry other molecules across organs since the acyl tail helps enter cells through the lipid membrane. However, it is uncertain how many NAAs exist in biological systems. New validated methods of detecting them in biological samples would lead to better mechanistic understanding and probably reveal novel immunomodulators. This study used synthetic standard mixtures of 1,426 NAAs provided by our collaborators, who discovered this novel conjugation, to develop an LC-IM-MS method for profiling them in fecal samples. Fecal samples from in-vitro colon simulation vessels were analyzed to understand these compounds' dynamics. However, data analysis is still in progress, and the effect of fecal microbes on the NAA profile has yet to be investigated.

Keywords: fecal lipids, lipidomics, mass spectrometry, metabolomics, N-acyl amides

A comparative analysis of qualification process for pharmaceutical process equipment

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SUSTAINABLE BIOTECHNOLOGICAL PROCESSES (TECH.)

Pharmaceutical industry has a great responsibility in patient safety. Good Manufacturing Practice (GMP) is a set of codes, guidelines, and regulations for manufacturers, which aim to ensure good quality of pharmaceuticals. An important step while following GMP is qualification. Qualification is a process establishing the suitability of the equipment for the intended purpose. In this project, I performed qualification for two pharmaceutical process equipment types, mobile microbial air samplers and tube sealers, and compared their qualification workflows for pros and cons.

The qualification process can be separated into two sections: documentation and testing. Applicable documentation for this project included user requirement specification, validation plan, installation and operation qualification (IQOQ) protocol, and validation summary report. All documents were assessed against several regulatory requirements, such as Pharmacopoeias. For air samplers, all installation and operational qualification tests were performed in-house. For tube sealers, installation and operational qualification was bought from the supplier and only supplementary tests to cover all user requirements were performed internally. Installation qualification tests covered checking calibration reports, correct installation, and suitability of the equipment on paper. Operational qualification tests checked the functionality and suitability of the equipment in practice, including performance, accuracy, alarms etc.

The qualification workflow for air samplers was simpler and cheaper compared to tube sealer's qualification. All qualification steps were performed in-house and there were fewer steps in IQOQ documentation and testing approval. This also made the process fast. Tube sealers' IQOQ consisted of the supplier IQOQ and supplementary IQOQ. Preparing, reviewing, and approving of these documents was time-consuming. However, the supplier IQOQ and supplementary IQOQ combined were more thorough compared to air samplers' qualification, due to supplier's expertise with the equipment. In cases of malfunction, the tube sealers were fixed on-site during supplier's qualification, whereas air samplers had to be shipped to the supplier for repair. In conclusion, if there is expertise with the equipment to be qualified, performing all qualification in-house would be cheap and time efficient. If no such resources are available, buying qualification from a supplier could be useful.

Keywords: qualification, GMP, process equipment, pharmaceutical industry

Methane potential of oat husk waste streams in anaerobic digestion

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SUSTAINABLE BIOTECHNOLOGICAL PROCESSES (TECH.)

The production of oat grains produces side streams of oat husks that are mainly unsuitable for human consumption. In the world it reaches up to 28 million metric tons of agricultural waste, of which 6,5 million metric tons is oat husks. CH-Bioforce's process utilizes oat husks to extract biopolymers, however, oat husks also contain pulverized grain material (oat husks powder) which is removed before processing. The process also produces a hemicellulose permeate as waste stream.
Oat husks powder and hemicellulose permeate are analysed of their chemical composition. Oat husks powder consists of 1.22 % water, 6.53 % ash, 92.06 % volatile solids and 0.19 % lignin. Hemicellulose permeate consists of 97.38 % water, 1.07 % ash and 1.55 % of volatile solids.
Gas chromatography results of raw materials' methanolysis and hydrolysis showed more specific information about carbohydrate composition. Oat husks powder consists of 309.4 (mg/g) glucose, 154.6 (mg/g) hemicelluloses, explaining 46.4 % of the dry content. All of the hemicellulose permeate and some of the oat husks powder results are unreliable, so further conclusions cannot be made of the carbohydrate composition. The analytics must be reassessed and possibly modified for reliable results.
Methane production is conducted using anaerobic digestion with industrial inoculum. Methane production digestion setup included inoculum control, microcrystalline cellulose control, and sample, with three replicate samples of each. Digestion is located in a shaker incubator (35 °C, 80 rpm, 30 days). Biogas produced in the digestion is lead to sodium hydroxide (5 M) to capture carbon dioxide, after which methane is collected. Oat husks powder is expected to produce methane approximately 350 ml/g volatile solids.

Key words: agricultural waste stream, anaerobic digestion, biomethane, methane potential, methane production, oat husk

Fotoautotrofinen etyleenin tuotanto korkean solutiheyden syanobakteerikasvatuksessa

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KESTÄVÄT BIOTEKNISET PROSESSIT (DI)

Etyleeni (C_2H_4) on kaasumainen hiilivety, jota käytetään erityisesti muoviteollisuuden raaka-aineena. Sitä tuotetaan pääosin öljystä höyrykrakkaamalla, mikä kuluttaa paljon energiaa ja vapauttaa ilmakehään hiilidioksidia. Fotosynteettisiä syanobakteereja hyödynnetään kehitettäessä uuden sukupolven bioteknologian sovelluksia, joiden avulla orgaanisia kohdekemikaaleja, kuten etyleeniä pyritään valmistamaan suoraan hiilidioksidista valoenergian avulla. Tässä työssä käytettiin geneettisesti muokattua *Synechocystis* sp. PCC 6803 -syanobakteerikantaa, joka tuottaa etyleeniä soluun siirretyn heterologisen EFE-geenin (EC 1.13.12.19) avulla. Työn tavoitteena oli tutkia etyleenin tuottopotentiaalia erityisellä korkean solutiheyden kasvatuslaitteistolla ja hyödyntää uutta sensoriteknologiaa etyleenin tuoton mittauksessa. Näitä laitteistoja ei ollut aiemmin käytetty haihtuvien kohdekemikaalien fotobiologisessa tuotannossa.

Etyleeniä tuotettiin fotosynteettisten mikrobien kasvatukseen suunnitellulla kaupallisella HD100-laitteistolla (CellDEG GmbH), jonka avulla kasvatuksessa voidaan saavuttaa hyvin korkea solutiheys (OD_{750} jopa 40–50). Kasvatuksessa käytettiin korkeaa hiilidioksidipitoisuutta (5–6 %) ja hyvin korkeita valointensiteettejä (max 780 µmol/m^2/s), mikä oli mahdollista erikoisvalmisteisen kasvatuskaapin avulla. Solujen kasvua ja etyleenin tuottoa tutkittiin eri valo-olosuhteissa ja panoskasvatuksen eri vaiheissa. Etyleenin tuottoa seurattiin suoraan kaasutiiviin kasvatuskammion kaasufaasista kromatografisesti (GC-FID) sekä haihtuvien orgaanisten yhdisteiden kvantitointiin kehitetyn sensorin avulla.

Tulokset osoittavat, että kasvatuslaitteisto soveltuu etyleenin fotoautotrofiseen tuottoon korkeissa valointensiteeteissä. Alustavissa kokeissa korkein mitattu etyleenipitoisuus oli noin 4,5 ml/l solukasvatusta ja korkein tuotto vuorokaudessa noin 3,3 ml/l solukasvatusta, jotka ovat kasvatustilavuuteen suhteutettuna samaa suuruusluokkaa kuin parhaat kirjallisuudessa julkaistut arvot. Sensorin osoitettiin olevan tarpeeksi herkkä havaitsemaan etyleeni suoraan kaasufaasista, ja mahdollistavan tuotteen reaaliaikaisen mittauksen. Työn tuloksia voidaan hyödyntää esimerkiksi tekoälyyn perustuvan olosuhdeoptimoinnin kehityksessä, mikä edistää fotoautotrofisten bioteknologioiden kehitystä kohti suuremman mittakaavan sovelluksia.

Asiasanat: etyleeni, syanobakteeri, fotosynteesi, korkea solutiheys, sensori

Fotoautotrofinen 3-hydroksibutyraatin jatkuvatoiminen tuotanto ja optimointi muokatuissa *Synechocystis sp.* PCC 6803 -kannoissa

Frans Uusipaavalniemi
Ohjaajat: maist. Lauri Kakko, Samuli Pyytövaara, apul. prof. Pauli Kallio
KESTÄVÄT BIOTEKNISET PROSESSIT (DI)

Fotosynteettiset mikro-organismit valmistavat orgaanisia yhdisteitä käyttäen hiilidioksidia ja valoenergiaa, ja tätä mekanismia voi muokata ja hyödyntää bioteknologisissa sovelluksissa. Tässä työssä vertailtavat geneettisesti muokatut S*ynechocystis sp. PCC 6803* –kannat kykenevät tuottamaan ja erittämään biomuovien valmistamiseen sopivaa orgaanista happoa, 3-hydroksibutyraattia (3HB). Työn tavoitteena oli tutkia 3-hydroksibutyraatin tuotantoa jatkuvatoimisessa fotobioreaktorissa, mahdollistaen tasaisten tuotto-olosuhteiden hallinnan, optimoinnin sekä prosessin kvantitatiivisen arvioinnin.
Kahta *Synechocystis sp*. PCC 6803 –kantaa kasvatettiin fotoautotrofisesti solutiheydessä (OD=1) kahdeksassa rinnakkaisessa 100 ml kasvatuskanavassa, kaupallisesta fotobioreaktorista (MC1000; PSI) muokatussa jatkuvatoimisessa laitteistossa. 3HB:n tuottoa mitattiin kolmessa eri valointensiteetissä (100, 150, 220 µmol $m^{-2}s^{-1}$) ja korkeassa hiilidioksidissa (1–5 %). 20 päivää kestävistä kasvatusjaksoista määritettiin 3HB seitsemästä aikapisteestä. 3HB määritettiin derivatisoiduista näytteistä GC-MS-analyysilla. Bioprosessia seurattiin biomassan akkumulaation ja tuottokantojen pigmenttiprofiilien vertailulla spektrofotometrisesti absorptiospektrin avulla. Mediumina käytettiin BG11 ilman antibiootteja. Tuloksia vertailtiin perinteisellä pullokasvatuksella sekä toisella MC1000-laitteistolla suoritettuihin panoskasvatuksiin.
3-hydroksibutyraatin jatkuvatoimisesta tuottamisesta ei ole aiempia tieteellisiä julkaisuja. Tämä tutkimus tarjoaa merkittävää kvantitatiivista tietoa geneettisesti muokattujen syanobakteerien hyödyntämisestä jatkuvatoimisessa tuotantoprosessissa, 3HB:n tuottotehokkuudesta, valon konversiotehokkuudesta, jatkuvatoimisen bioprosessin optimoinnista sekä tuottokantojen geneettisestä stabiilisuudesta. Näiden ominaisuuksien tutkiminen on tärkeää siirryttäessä kohti teollisen mittakaavan bioprosesseja.

Avainsanat: *Synechocystis sp*. PCC 6803, 3-hydroksibutyraatti, jatkuvatoiminen kasvatus, fotoautotrofia, panoskasvatus, kestävä kehitys

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