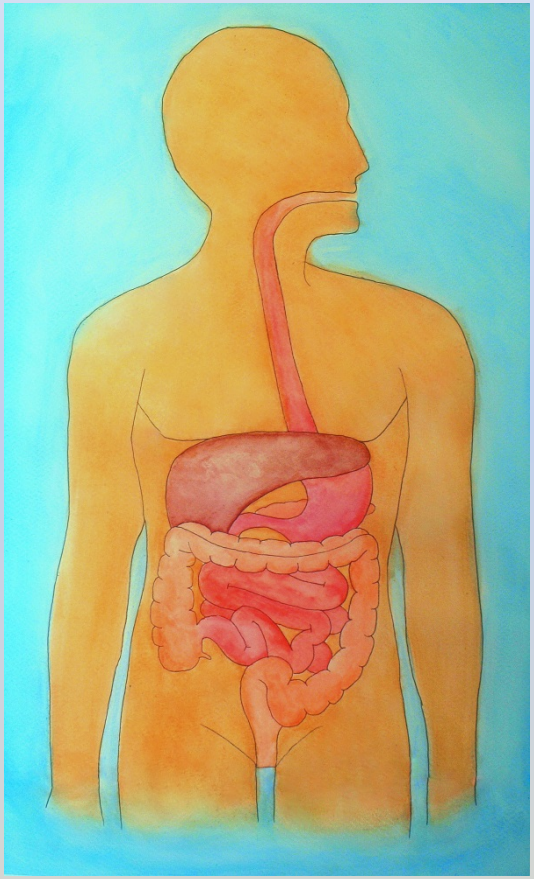
Suoliston mikrobitasapaino

Luento Helsingin yliopiston
Luomuinstituutissa, Mikkelissä
15.3.2016

Dos. Elias Hakalehto, FT, MMM



”Alimentary Microbiome”

- käsite v. 2000, Prof . Joshua Lederberg

Lederberg, J. Infectious history. *Science*, 2000; 288, 287-293.

”Human Microbiome” –projektista on nyt tulossa vastaavanlainen jättihanke kuin ”Human Genome” oli viime vuosisadan lopulla.

” A joint effort of the scientific community to set up a governmental-led strive for better understanding on the intestinal and other microbiome constitution”
Zimmer, C. Scientist urge national initiative on microbiomes. *The New York Times* 28.10.2015.

⇒ On alettu ymmärtää, miten olennaisesti mikrobit liittyvät ihmisten terveyteen, ja osatekijöinä kaikkiin sairauksiin.

Muita termejä esim.:

Digestive tract microflora

Gut microbiota

Intestinal microbes

Host-microbe interaction

Molecular communication

Ihmisen terveys on seurausta/riippuvainen tasapainosta hänen elimistönsä ja sen mikrobien välillä

Bacteriological Intestinal Balance (BIB)

-Käsite kuvaa sitä olotilaa, johon terveen ihmisen tasapainoinen, luonnollinen suoliston bakteeriyhteisö pyrkii.
-Tähän voidaan vaikuttaa siten, että hoitamalla mikrobiflooraa vaikutetaan epäsuorasti ihmisen terveyteen myönteisellä tavalla.

Hakalehto, E. (Ed.). 2012. *Alimentary microbiome - a PMEU approach*. New York, NY, USA: Nova Science Publishers, Inc.

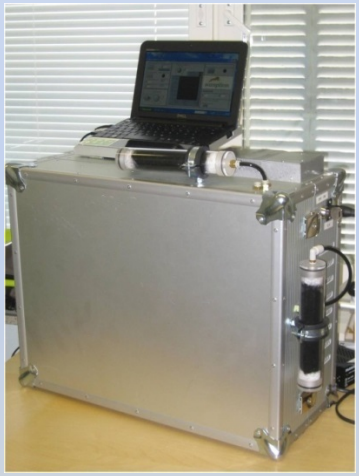
Ruuansulatuskanavan mikrobitutkimuksen pääkohdat:

1. Mikrobiflooran kartoittaminen
2. Mikrobien osallistuminen ravinnon hyödyntämiseen
3. Mikrobien väliset vuorovaikutukset
4. Mikrobien ja ihmiselimistön vuorovaikutukset
5. Mikrobiomin terveysvaikutukset

PMEU-laitteen hyödyntäminen tutkimuksessa on avannut näköaloja

PMEU Spectrion ®

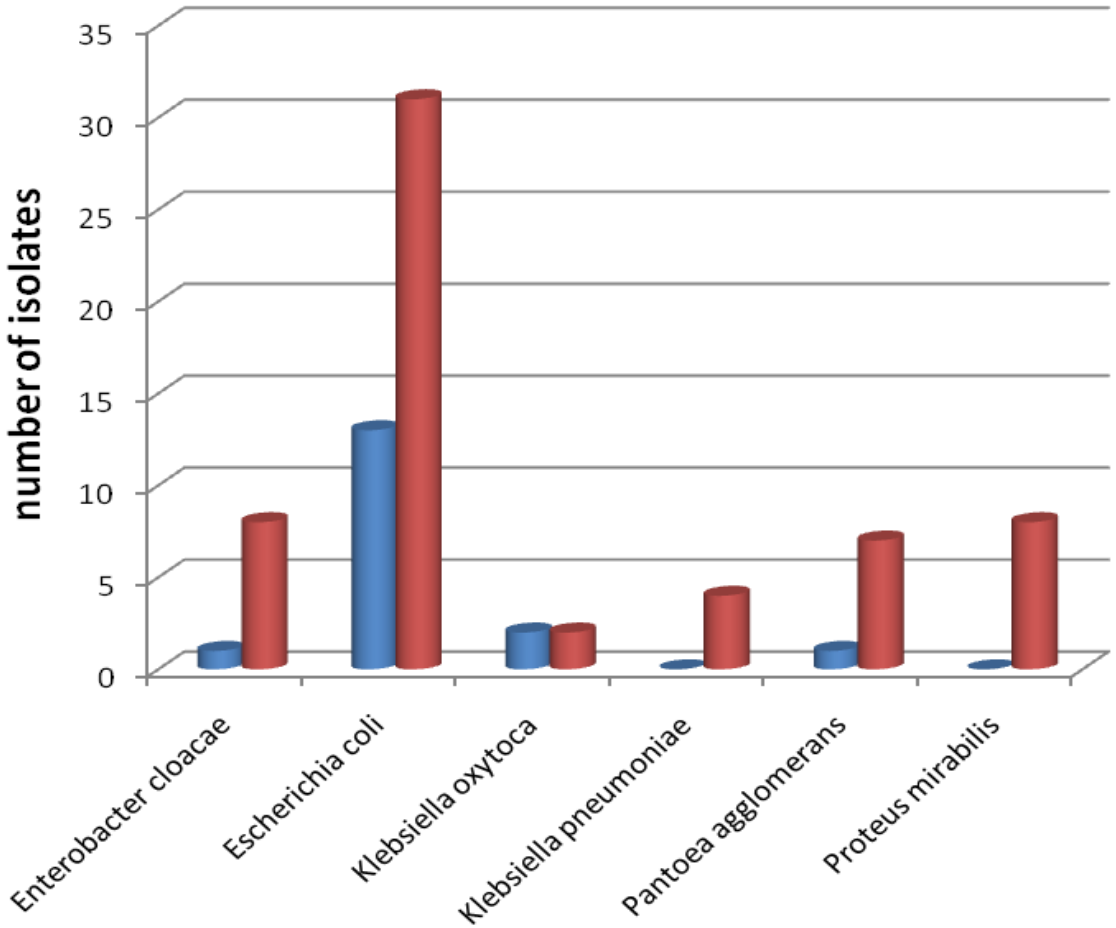
PMEU Scentrion ®



PMEU automaattisella näytteenotolla varustettuna

Laite on kehitetty Finnoflag Oy:n tekemien tutkimusten työvälineeksi vuosisadan vaihteesta alkaen.

The importance of PMEU enrichment



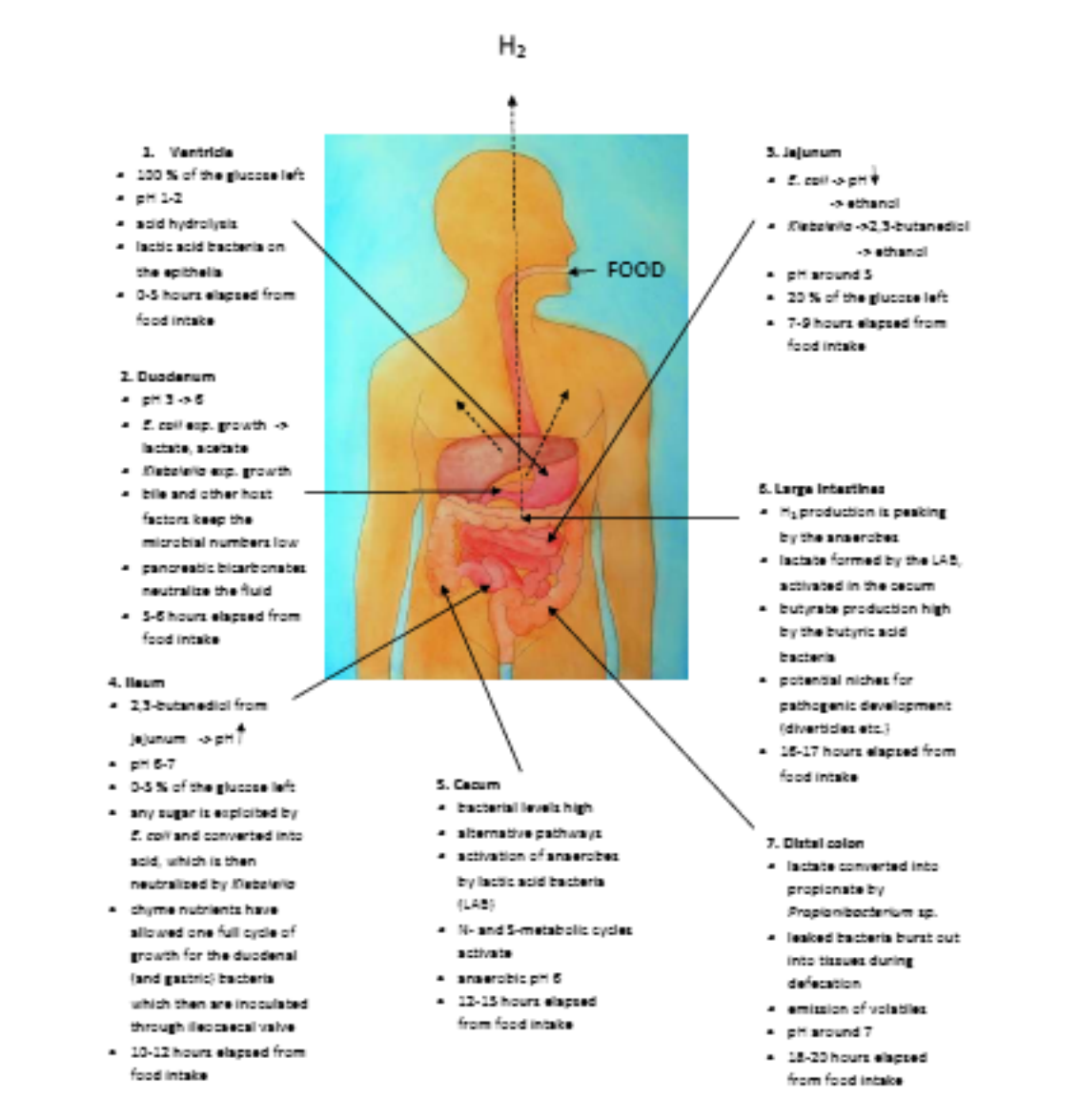
PMEU-rikastusviljelyn merkitys

by only direct plating

by combination of plating and enrichment

Pesola, J., & Hakalehto, E. 2011. Enterobacterial microflora in infancy - a case study with enhanced enrichment. *Indian J Pediatr, 78*, 562-568.

Kokonaiskuva mikrobitasapainosta



Hakalehto, E. (Ed.). 2015. *Microbiological Food Hygiene*. New York, NY, USA: Nova Science Publishers, Inc.

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Helsingin yliopisto, Luomuinstituutti, Mikkeli 15.3.2016
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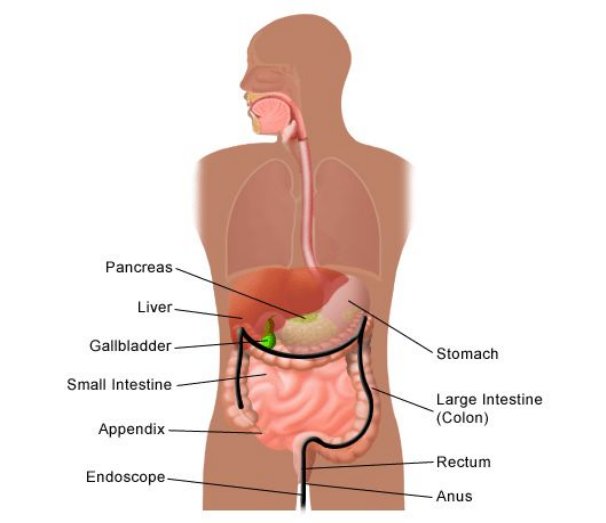
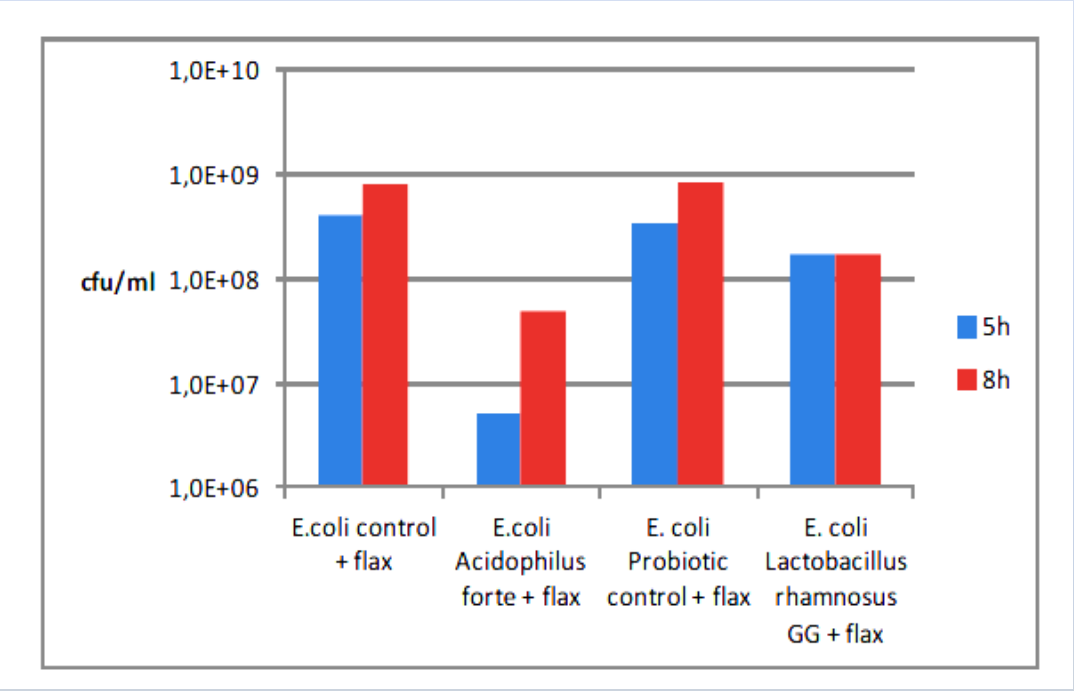
Patient no	Cardia	Antrum	Pylorus
1	*Enterococcus faecalis* (2)[a]	*L. salivarius ssp.*	*L.s salivarius ssp.*
	Str. thermophilus		
	L. salivarius ssp. salivarius	*salivarius (2)*	*salivarius*
2	*S.. salivarius* (2)		
4	*S..sanguinis* (2)	*S. salivarius*	
	S..salivarius		
	E. faecalis		
5	*S. salivarius*	*Str. salivarius*	*L. salivarius*
6		*L. reuteri*	*L.reuteri*
		L.casei 97%	
7		*L. reuteri* (3)	*L. reuteri*
		S.salivarius	
8		*Str.sanguinis*	*Lactobacillus reuteri* (2)
9	*Lactococcus lactis ssp. lactis (2)*		*S. thermophilus*
10	*S. sanguinis*	*S. sanguinis*	*S.. sanguinis*
11	*S.salivarius* (2)	*Str. salivarius* (2)	*L. reuteri* (3)
12	*S. salivarius*	*Str.salivarius* (3)	*S.salivarius*
		L. reuteri (2)	
13	*S. salivarius* (2)		*S. salivarius*

Identified LAB (lactic acid bacteria) species enriched from the biopsies obtained from different gastric sites and cultivated with PMEU



Hakalehto, E., Vilpponen-Salmela, T., Kinnunen, K., von Wright, A. 2011. Lactic Acid bacteria enriched from human gastric biopsies. *ISRN Gastroenterol.* 2011, 109183.
doi: 10.5402/2011/109183.

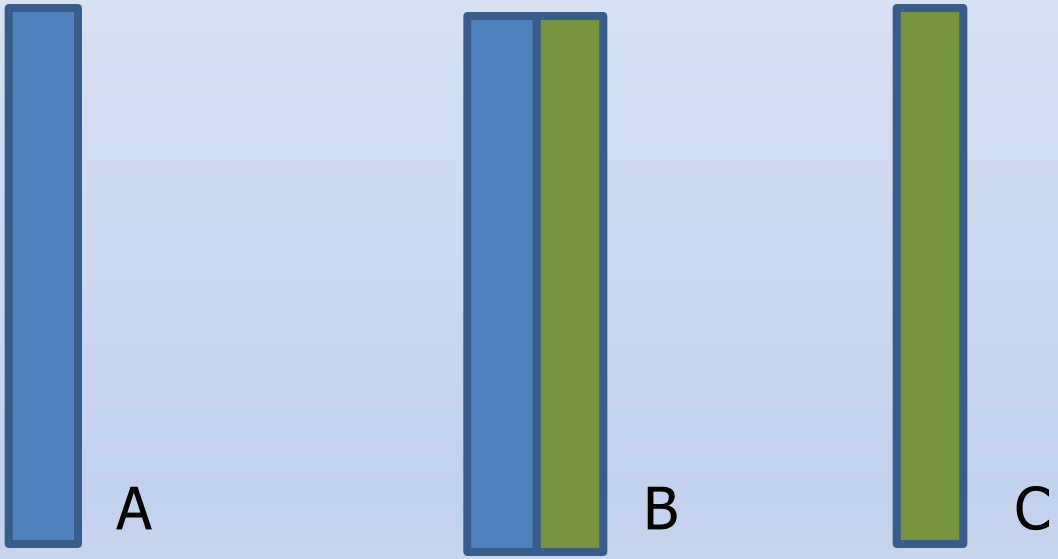
The joint effect of a probiotic mixture of *Lactobacillus acidophilus, Bifidobacterium bifidum,* and *B. lactis* with prebiotic flax on *Escherichia coli* growth in the PMEU. Note that the logarithmic scale indicates approximately 100-fold restriction of the colibacteria.



Hakalehto E. & Jaakkola K. 2013. Synergistic effect of probiotics and prebiotic flax product on intestinal bacterial balance. Poster in 35th ESPEN Congress on Clinical Nutrition and Metabolism, September 2013, Leipzig, Germany. *Clinical Nutrition* 2013 Vol. 32, Supplement 1, S200.

Hakalehto, E., Jaakkola, K., Pesola, J., Heitto, A., Hell, M., Hänninen, O. 2015. Tendencies in probiotic treatments. In: Hakalehto, E. (ed.) *Microbial clinical hygiene*. New York, NY, USA: Nova Science Publishers, Inc.

DUALISTIC BALANCE IN THE SMALL INTESTINES



Esherichia coli alone

E.coli and *Klebsiella* sp. together

Klebsiella alone

GROWTH LEVELS IN PURE CULTURES (A,C) AND IN A MIXED CULTURE (B) *Klebsiella* and *E. coli* GROW UP TO SAME CONCENTRATION AS EACH OF THEM SEPARATELY. TOGETHER THEY KEEP THE PH OF DUODENUM AT 6 WITH SIMULTANEOUS EMISSION OF VOLATILES, CO_2 AND H_2.

Hakalehto, E, Humppi, T., Paakkanen, H. 2008. Dualistic acidic and neutral glucose fermentation balance in small intestine: Simulation *in vitro. Pathophysiology* 15: 211-220.

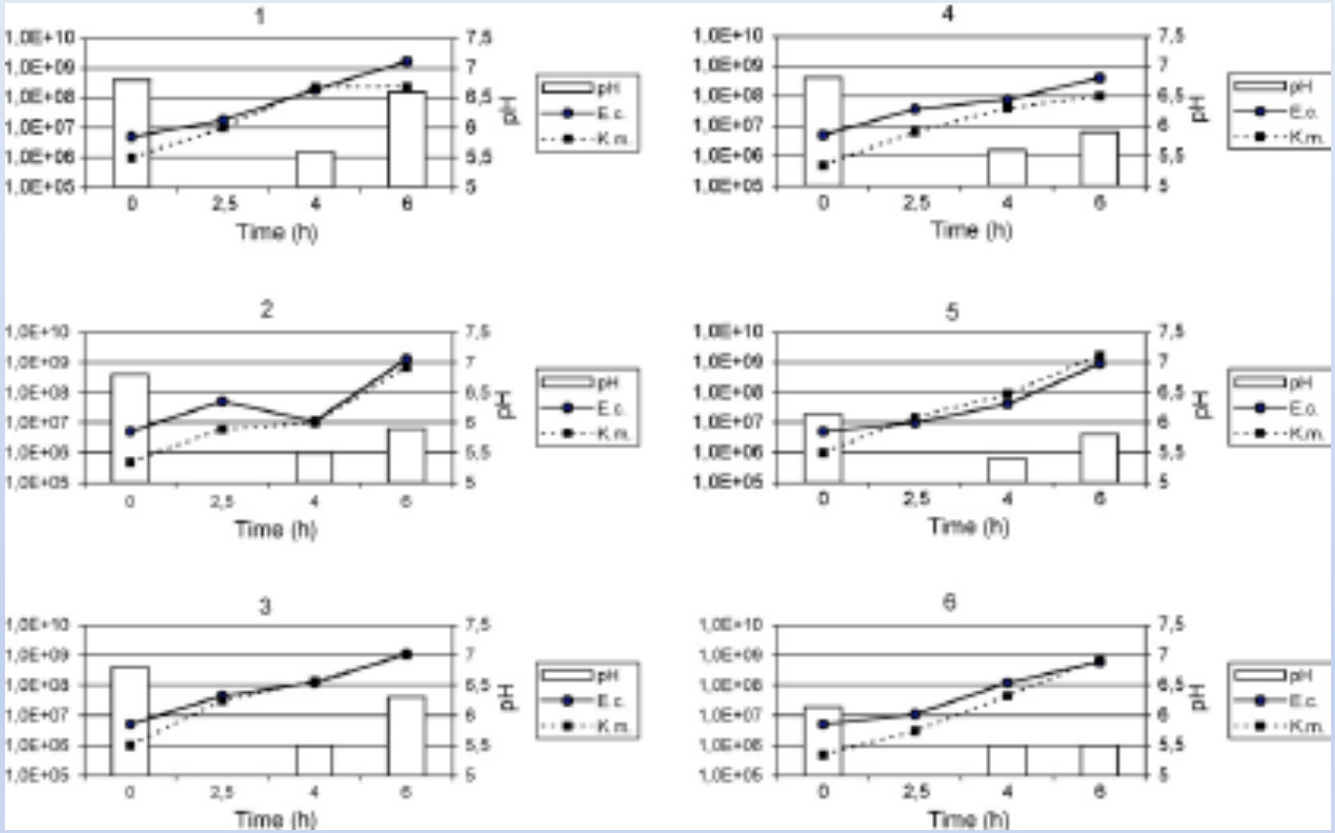


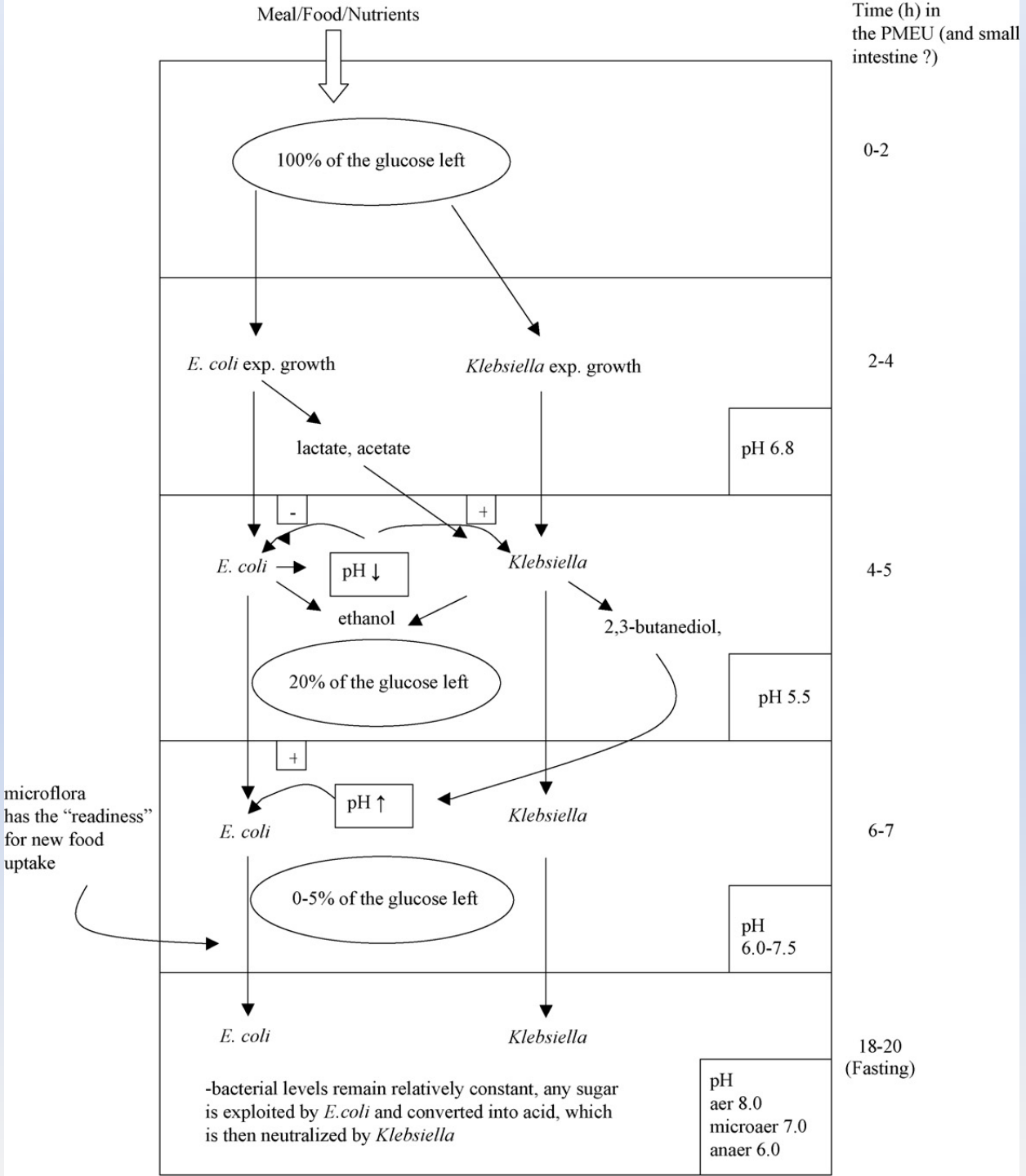
Fig. 1. Mixed aerobic cultivation of *Escherichia coli* (E. c.) and *K. mobilis* (K. m.) sp. strains at 37 °C in the PMEU. The inoculated cell numbers were about 5 × 10E6 for *E. coli* and for *K. mobilis* about 1 × 10E6 (in panel cultures 1, 3 and 5) and about 5 × 10E5 (panel cultures 2,4 and 6) per ml of the medium. The initial pH of the liquid medium was 6.8 in 1–4 and 6.14 in 5 and 6. Ethanol (1%) was added to cultures 3–6.

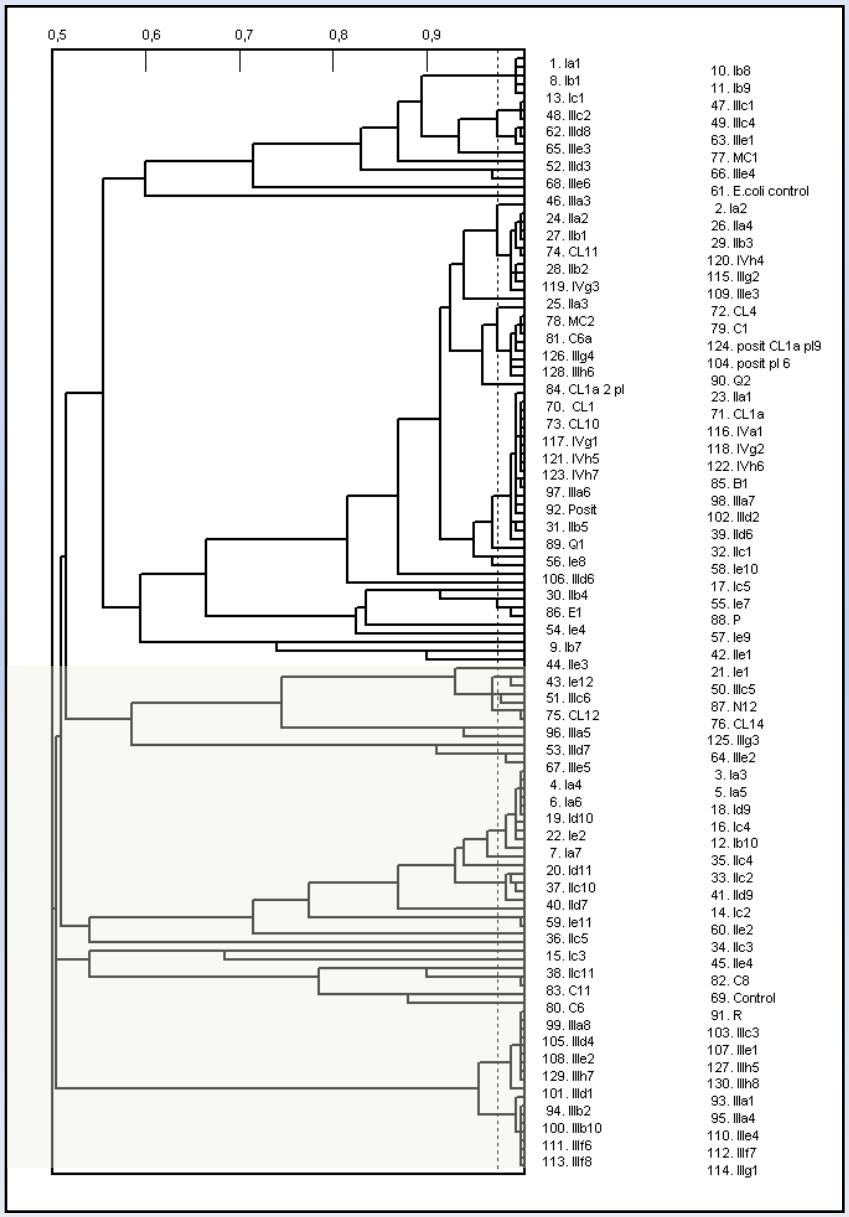
Hakalehto, E, Humppi, T., Paakkanen, H. 2008. Dualistic acidic and neutral glucose fermentation balance in small intestine: Simulation *in vitro. Pathophysiology* 15: 211-220.

Maintenance of dualistic fermentation balance in small intestine simulated in PMEU

The mixed acid fermentation pattern was accomplished by *E. coli,* and 2,3-butanediol and ethanol fermenting bacteria were represented by *K. mobilis*.

Hakalehto, E, Humppi, T., Paakkanen, H. 2008. Dualistic acidic and neutral glucose fermentation balance in small intestine: Simulation *in vitro.* Pathophysiology 15: 211-220.





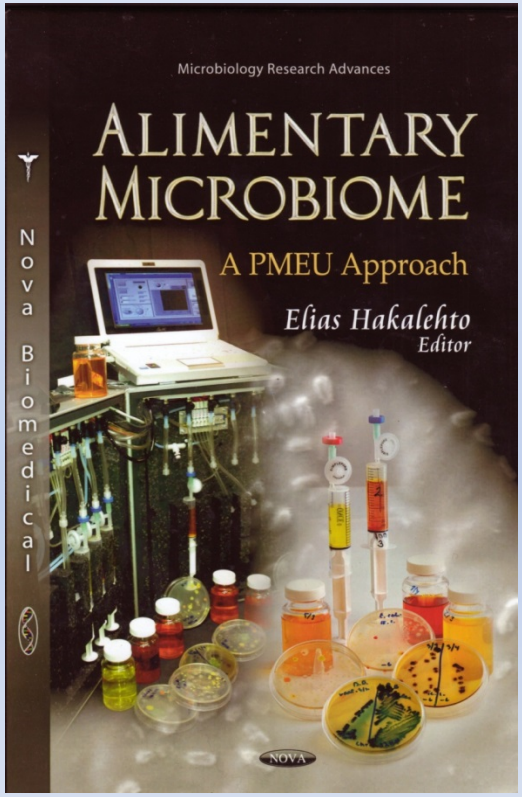
The dendrogram showing phenotypic homologies of the enterobacterial strains isolated in the Experiments I - VI studied by PhenePlate™ -RS plates. A division into two major groups is visualized by shading. This kind of dualistic characteristics of enterobacterial microbiota has been recently presented by Hakalehto *et al.* [2008] in the regulation of small bowel pH and interspecies commensalism. It is noteworthy that the upper group in this graph included *E. coli* and the other group (in the shaded area) *Enterobacter, Klebsiella* and *Pantoea* isolates, the former species being a mixed acid fermenting one and the latter ones carrying out 2,3-butanediol fermentation. The dotted line illustrates the level of similarity of 97.5% of the strains.

Pesola, J., & Hakalehto, E. 2011. Enterobacterial microflora in infancy - a case study with enhanced enrichment. *Indian J Pediatr, 78*, 562-568.

”Bacteria belonging to the genus *Klebsiella* have a dual role in human pathophysiology. Some of the strains are potent opportunistic pathogens capable of causing severe illnesses, whereas a majority of the klebsiellas belong to our normal flora, particularly in our alimentary tract.”

Hakalehto, E. 2013. Interactions of *Klebsiella* sp. with other intestinal flora. In Pereira, L.A. & Santos, A. (eds.) *Klebsiella infections: Epidemiology, pathogenesis and clinical outcomes*. Nova Science Publishers, Inc. New York, USA.

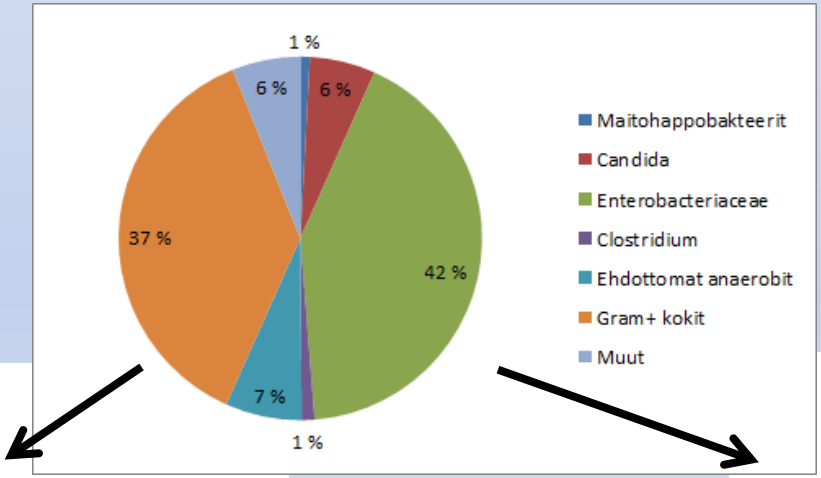
MIKROBIN TAUDINAIHEUTTAMISKYKY ON SEURAUSTA, PAITSI SEN INFEKTIIVISISTÄ OMINAISUUKSISTA, MYÖS SEN ASEMASTA MIKROBIYHTEISÖSSÄ SEKÄ IHMISELIMISTÖN VASTUSTUSKYVYSTÄ JA PUOLUSTUSMEKANISMEISTA.

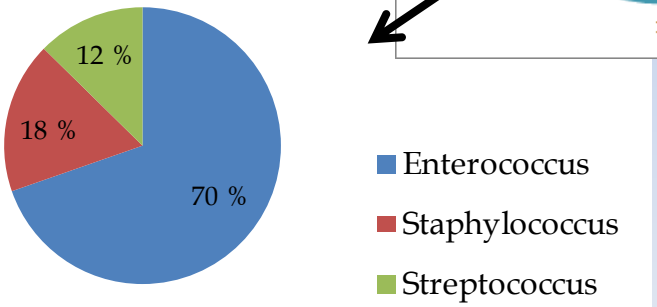


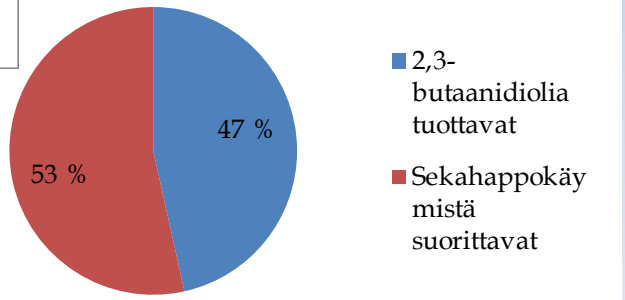
Hakalehto, E. (Ed.). 2012. *Alimentary microbiome - a PMEU approach*. New York, NY, USA: Nova Science Publishers, Inc.

Suoliston mikrobitasapaino
Helsingin yliopisto, Luomuinstituutti, Mikkeli 15.3.2016
Dos. Elias Hakalehto

Sappitulehdusten tms. yhteydessä eristetyt bakteerikannat kolmen vuoden ajalta SALK-sairaalassa (Universitätsklinikum Salzburg, Austria)

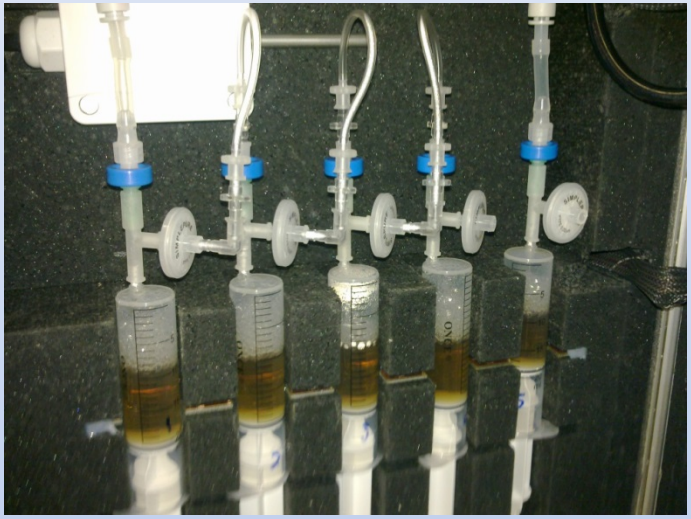






Hakalehto, E., Hell, M., Bernhofer, C., Heitto, A., Pesola, J., Humppi, T., & Paakkanen, H. 2010. Growth and gaseous emissions of pure and mixed small intestinal bacterial cultures: Effects of bile and vancomycin. *Pathophysiology, 17*, 45-53.

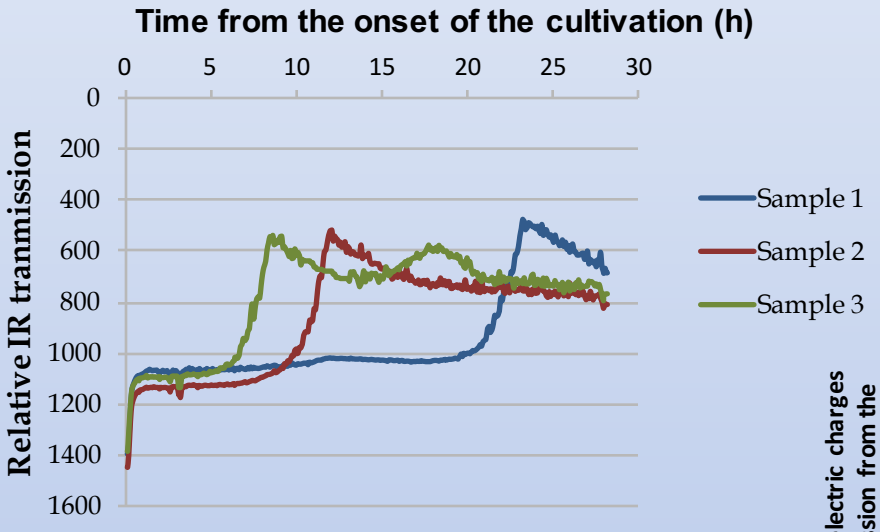
GUT-MICROBE INTERACTIONS, CARBON REUSE



The PMEU Spectrion® device used for cultivating, detecting and monitoring bacterial and other microbial growth by optional UV, visible light and IR sensors. In the figure on the right a closer look at the chained microbial cultivation syringes in the PMEU Spectrion®. The gas flow emitted from the preceding culture is directed as a bubbling flow into the next one.

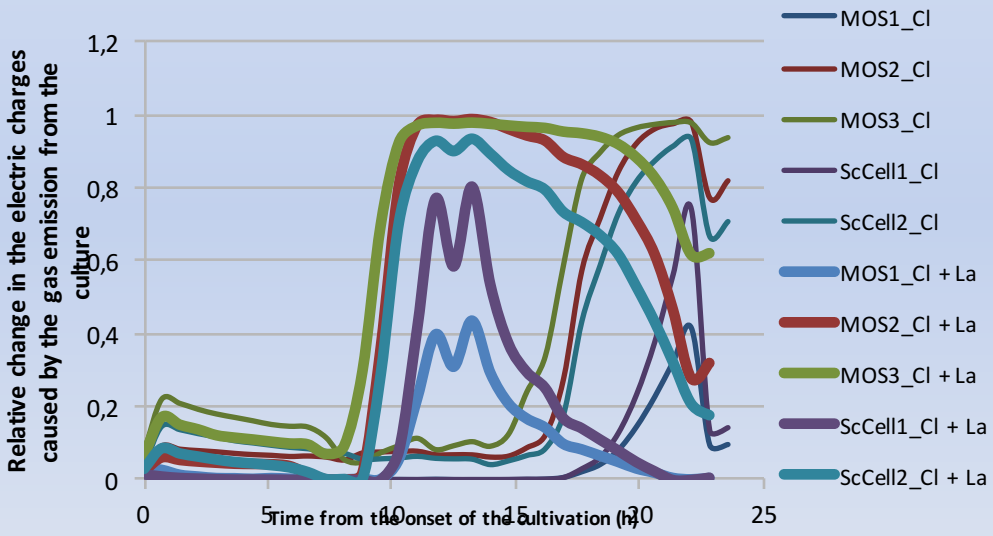
Hakalehto, E. 2013. Interactions of *Klebsiella* sp. with other intestinal flora. In Pereira, L.A. & Santos, A. (eds.) *Klebsiella infections: Epidemiology, pathogenesis and clinical outcomes*. Nova Science Publishers, Inc. New York, USA.

CARBON DIOXIDE SPEEDS UP THE GROWTH



Effect of CO_2 from preceding PMEU syringe culture on the onset of clostridial growth

Thin lines = *C. butyricum* alone
Thick lines = *C. butyricum* connected from *L. brevis* culture



Hakalehto, E. & Hänninen, O. 2012. *Can. J. Microbiol*. 58: 928-931.

Gaseous CO2 signal initiates growth of butyric-acid-producing *Clostridium butyricum* in both pure culture and mixed cultures with *Lactobacillus brevis*

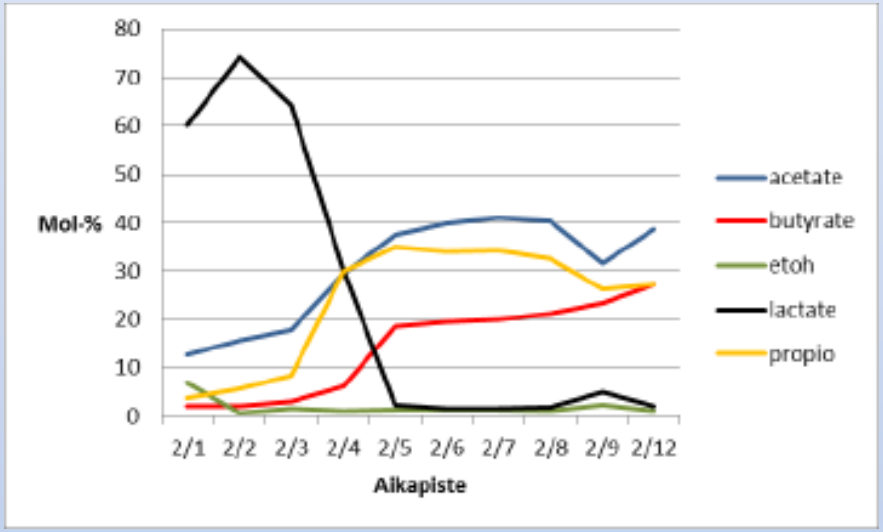
CO_2 IS BOOSTING THE BIOPROCESS

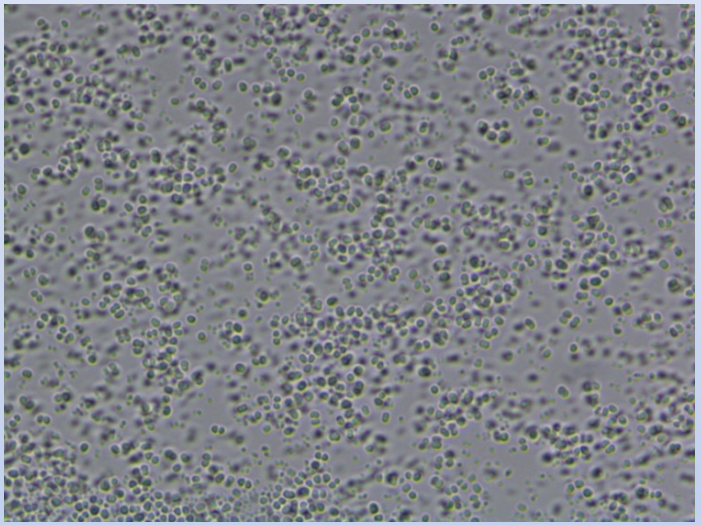
PMEU Spectrion® cultures of *Clostridium acetobutyricum* strain ATCC 185 were cultivated in a constant nitrogen (100 %) flow into the cultivation syringes (see also Hakalehto & Hänninen 2012). This flow was in some cases interrupted with pulses of 45 % CO_2 with 15-30 min duration in or near the beginning of the cultivation. The triggering CO_2 impulses produced bacterial growth in few hours as illustrated below.

No of cultures	Time of CO_2 impulse(s) (from the inoculation of PMEU enrichment syringes)	Onset of growth (in hours from the inoculation of PMEU enrichment syringes)
1	None	No growth in 49 hours
2	None	No growth in 49 hours
3	0-30 min	33
4	0-30 min	31,5
5	90-105 min	32
6	90-105 min	30
7	90-105 min, and 180-195 min	25
8	90-105 min, and 180-195 min	29,5
9	120-135 min	30,5
10	120-135 min	no data

Hakalehto, E. 2015. Enhanced microbial process in the sustainable fuel production. In Jinyue, Y. (Ed.). Handbook of Clean Energy Systems. Wiley JR & Sons. Inc, Chichester, West Sussex, UK.

Conversion of Lactate into Other Organic Acid in Slaughterhouse Waste Treatment

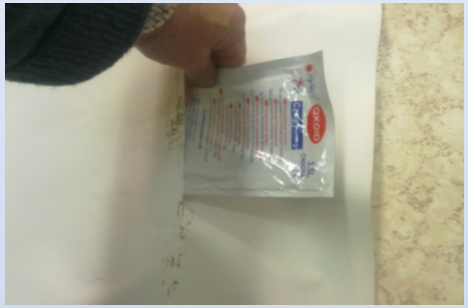




Propionibacterium acidipropionici is accepted as safe production organism by EFSAn, (European Food Safety Association). It is used for conserving wheat or chicken meat, for example.
http://www.journalofdairyscience.org/article/S0022-0302(98)75663-2/abstract

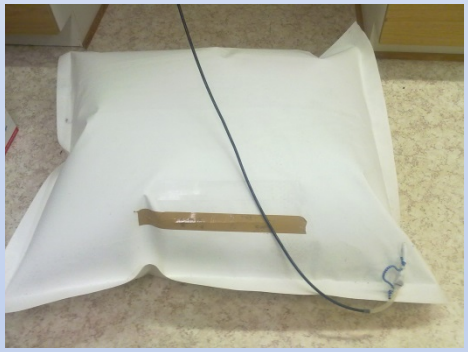
Hakalehto, E. 2015. Hygienic lessons from the dairy microbiology cases. In: Hakalehto, E. (ed.) *Microbial food hygiene*. New York, NY, USA: Nova Science Publishers, Inc. In Print.

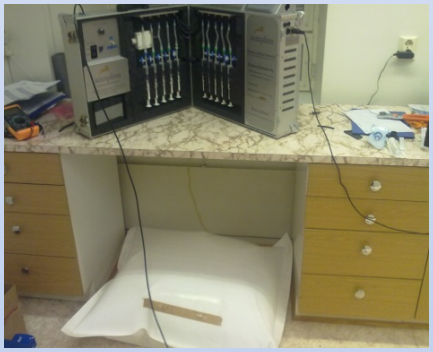
A



B

C





D

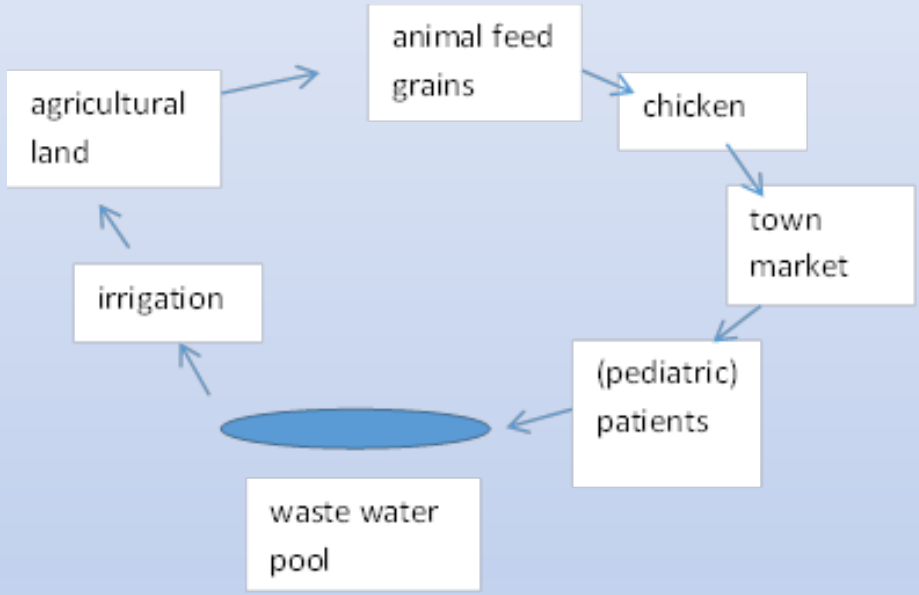
A. 30 pieces of 3,5 l CampyGen gas sachets (for 3.5 liters of gas mixture) were added into the sack (110 l). The sack was closed air-tightly with tape.

B. Air is pumped into the sack through the valve.

C. The sack is connected to the PMEU and gas flow adjusted.

D. In the sack there was enough gas-air mixture for 24 hours of microearobic cultivation in the ten enrichment syringes of the PMEU Spectrion®.

Hakalehto, E., Nyholm, O., Bonkoungou, I.J.O., Kagambega, A., Rissanen, K., Heitto, A., Barro, N. & Haukka, K. 2014. Development of microbiological field methodology for water and food-chain hygiene analysis of *Campylobacter* spp. and *Yersinia* spp. in Burkina Faso, West Africa. *Pathophysiology* 21: 219-229.



Hypothetical distribution of *Campylobacter* sp. during outbreaks in Burkina Faso, according to Hakalehto et al. 2014.

Hakalehto, E., Nyholm, O., Bonkoungou, I.J.O., Kagambega, A., Rissanen, K., Heitto, A., Barro, N. & Haukka, K. 2014. Development of microbiological field methodology for water and food-chain hygiene analysis of *Campylobacter* spp. and *Yersinia* spp. in Burkina Faso, West Africa. *Pathophysiology* 21: 219-229.

Journal of proteome research

Article

pubs.acs.org/jpr

Composition and Antimicrobial Activity of the Skin Peptidome of Russian Brown Frog *Rana temporaria*

T. Yu. Samgina,[1] E. A Vorontsov,[1] V. A. Gorshkov,[1] E. Hakalehto,[2] O. Hanninen,[3] R. A. Zubarev,[4] and A. T. Lebedev[1,*]

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[2]Department of Biosciences, University of Eastern Finland, P.O.B. 1627, FI-70211 Kuopio, Finland

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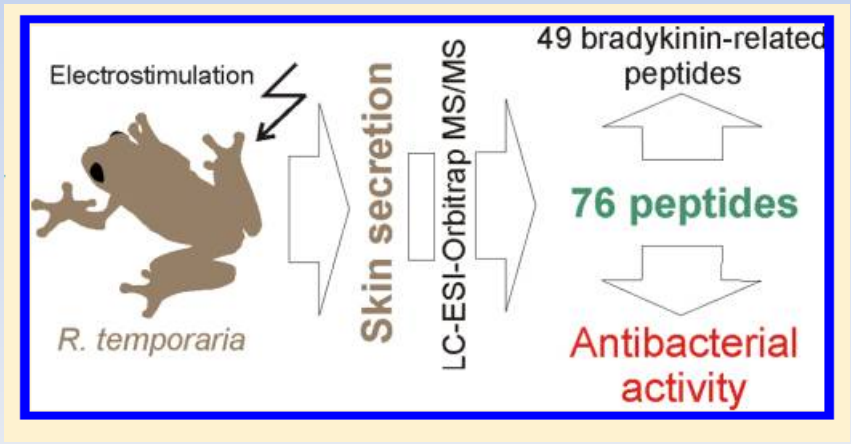
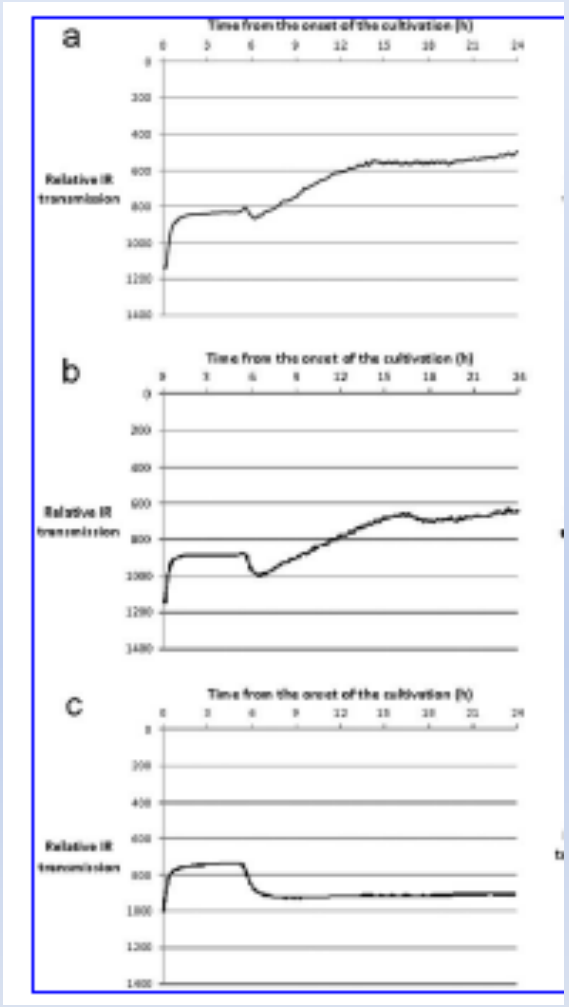
[4]Department of Medicinal Biochemistry and Biophysics, Division of Molecular Biometry, Karolinska Institutet, Stockholm, Sweden

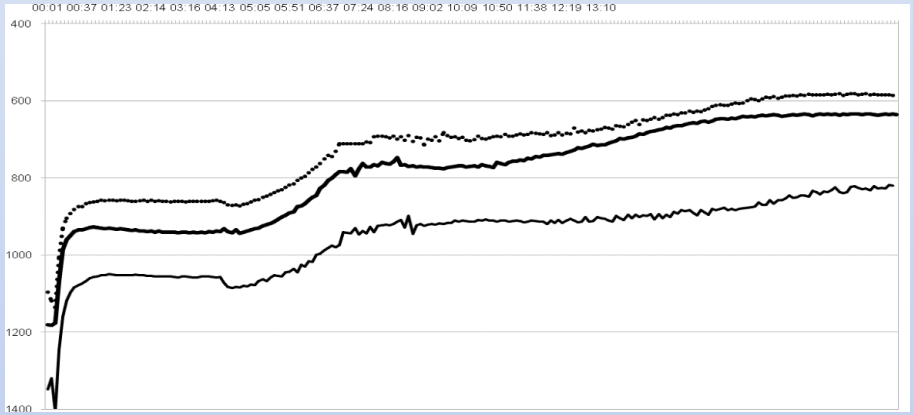




Samgina, T. Yu. et *al*. 2012. *Journal of Proteome Research,*
American Chemical Society

Figure 4. (a) Growth of *Staphylococcus aureus* strain in the TYG medium without peptide additions; (b) growth of *Staphylococcus aureus* strain in the presence of 0.01 mg/mL of M3 sample; (c) growth of *Staphylococcus aureus* strain in the presence of 0.03 mg/mL of M3 sample.

Kuten magaiini-peptidit sammakon iholla, samoin defensiini-peptidit suoliston limakalvoilla torjuvat infektioita.



The *Escherichia coli* patient strain isolated from the Neonatal Intensive Care Unit of the Kuopio University Hospital, Finland, was cultivated at 37 °C in TYG broth in the PMEU Spectrion® (Hakalehto, 2011). This cultivation was carried out in constant anaerobic N_2 flow without any addition (the uppermost curve), and with concentrations of 4ng/ml (middle curve) or 50ng/ml (lowermost curve) of human beta-defensin-2 (HBD-2). Interestingly, the initiation of growth was not delayed by the defensins, but the growth was somewhat attenuated by these small additions. The final cell content after 21 hours of the PMEU culture was about 1.7 x 10E9 per ml of medium in the control culture and with the lower defensin concentration, whereas with the higher addition the final cell concentration was 1.1 x 10E9 per ml of medium. The final pH´s were 6.90, 6.87 and 6.79, respectively, the original pH of the pure medium being 6.95.

Hakalehto, E. 2011. Simulation of enhanced growth and metabolism of intestinal *Escherichia coli* in the Portable Microbe Enrichment Unit (PMEU). In: Rogers MC, Peterson ND (eds.) *E. coli infections: causes, treatment and prevention.* New York, USA: Nova Science Publishers, pp. 159-175.

ENHANCED BACTERIAL ENRICHMENT IN THE MICROBIAL DIAGNOSTICS OF PEDIATRIC NEUTROPENIC SEPSIS

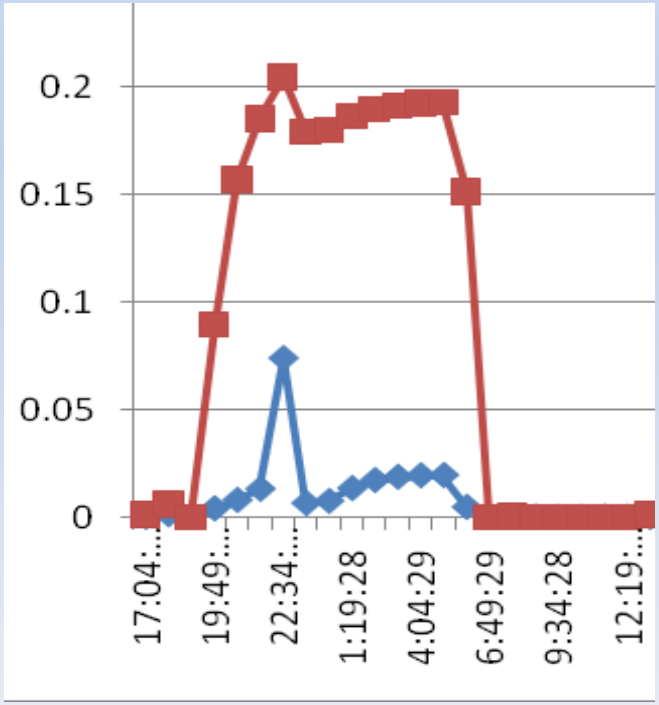
Pesola, J., Heitto, A., Myöhänen, P., Laitiomäki, E., Sankilampi, U., Paakkanen, H., Riikonen, P. & Hakalehto, E. 2011. Enhanced bacterial enrichment in the microbial diagnostics of pediatric neutropenic sepsis. *In*: NOPHO 29th Annual Meeting, May 21-24, 2011, Turku, Finland. Abstract book, abstract P4, pp. 59.

Introduction

- Neutropenic sepsis (NS) is an important reason for treatment related morbidity and mortality of pediatric hematologic and oncologic patients.
- Early diagnosis is crucial for the survival.
- Blood culture is the golden standard in the diagnostics of the NS.
- However, the bacterial agents are detected only in 10–20% of all NS cases.
- More efficient methods for the analysis of blood culture samples are warranted.

Case #26

- A. Standard protocol: aerobic alert 12 h; anaerobic alert 10 h; isolates: *Streptococcus mitis*, *Staphylococcus epidermis, Enterococcus faecium*
- B. Study method: anaerobic alert 4 h; isolates: *Streptococcus mitis*, *Staphylococcus epidermis*.



Growth curve of Case #26 by PMEU Scentrion®

Sample was taken at 5:30 p.m. (shortly before the raise of the red curve)

Suoliston epiteeli on yhden solukerroksen paksuinen. Sen vuoksi sen täytyy

A. olla suojattu elimistön omilla puolustusmekanismeilla, ja

B. olla tasapainoisen mikrobiflooran kanssa vuorovaikutuksessa.

Ihmisen normaalifloora vakiintuu 6. ikävuoteen mennessä.

Enhanced mycobacterial diagnostics in liquid medium by microaerobic bubble flow in Portable Microbe Enrichment Unit

Elias Hakalehto*

Pathophysiology 20 (2013) 177–180

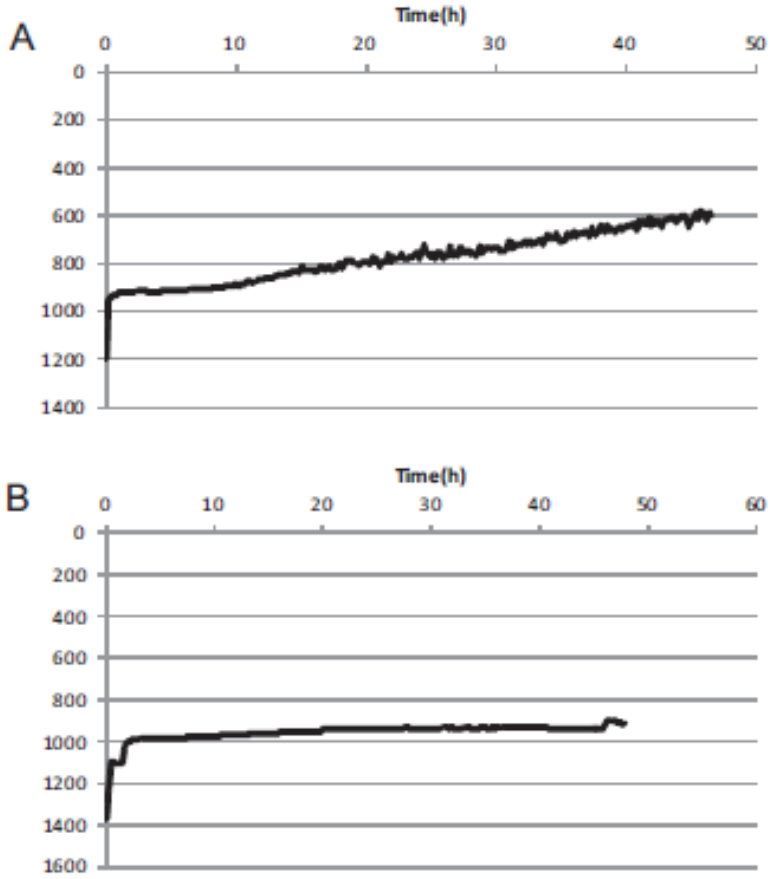


Fig. 1. Growth curves on *M. fortuitum* from PMEU Spectrion® analysis. The culture with gas bubble flow above (A), with static reference culture below (B). In the former one A case, the onset of growth was recorded in less than 12 h.

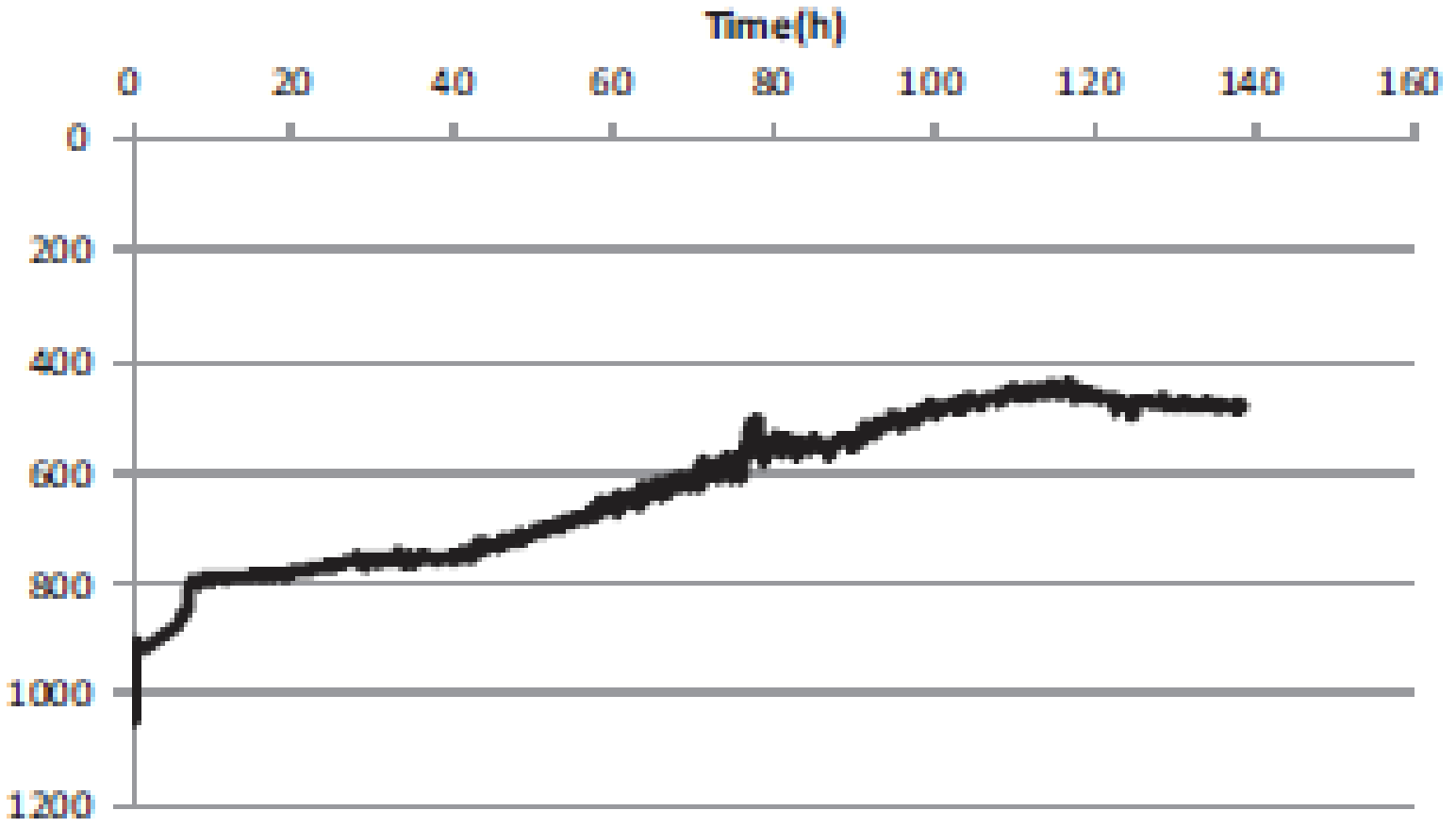
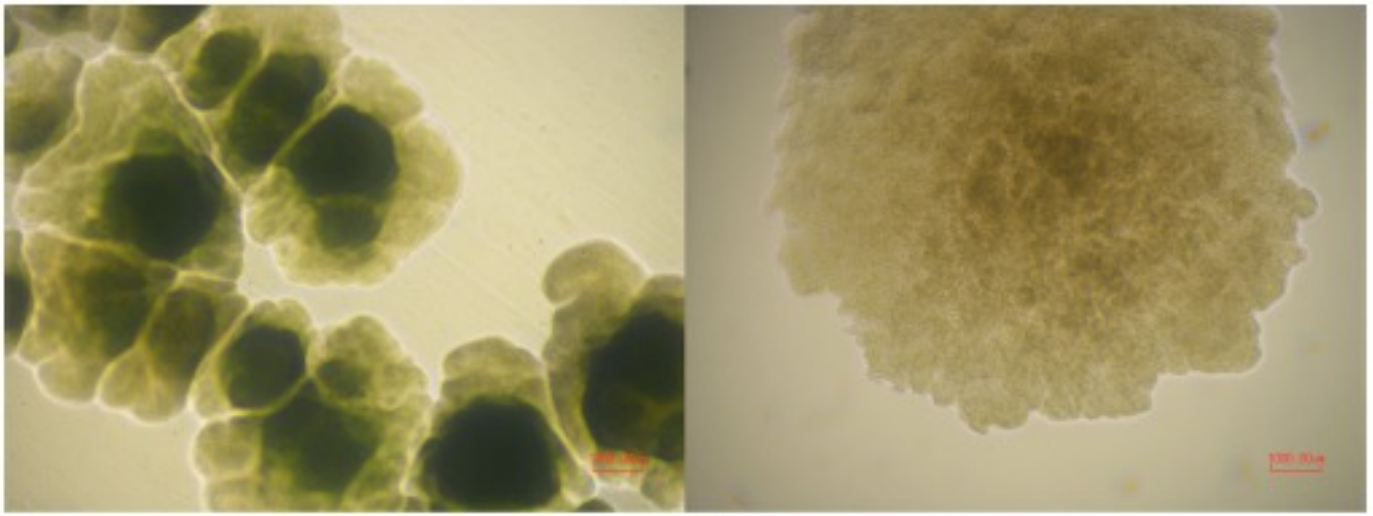


Fig. 2. Development of a *Mycobacterium* sp. culture during 6 days of PMEU Spectrion® cultivation. The early signs of increasing density were seen after 24 h, and clear onset of growth occurred in 48 h.



A B

Fig. 3. (A and B) Close images of the mycobacterial colonies. On the left growth of *Mycobacterium* sp. E40 isolated from river water on CromAgar™ medium. On the right a colony of *M. marinum* strain ATCC 927 grown on M7H9 agar (1.25%).

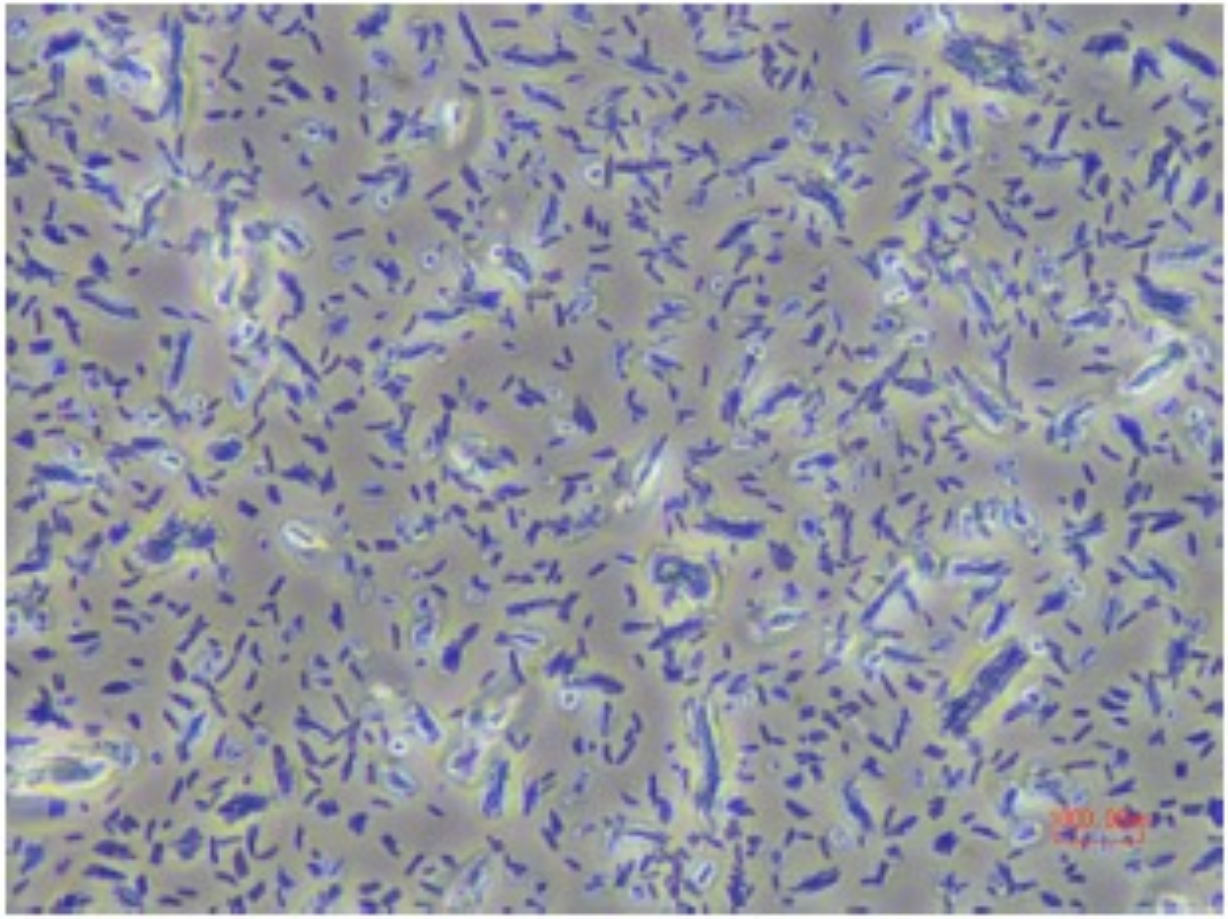


Fig. 4. Dark field image of *M. marinum* ATCC 927 cells photographed by Nikon Eclipse E3 microscope (1000×). Capsular structures were visible around some cells.

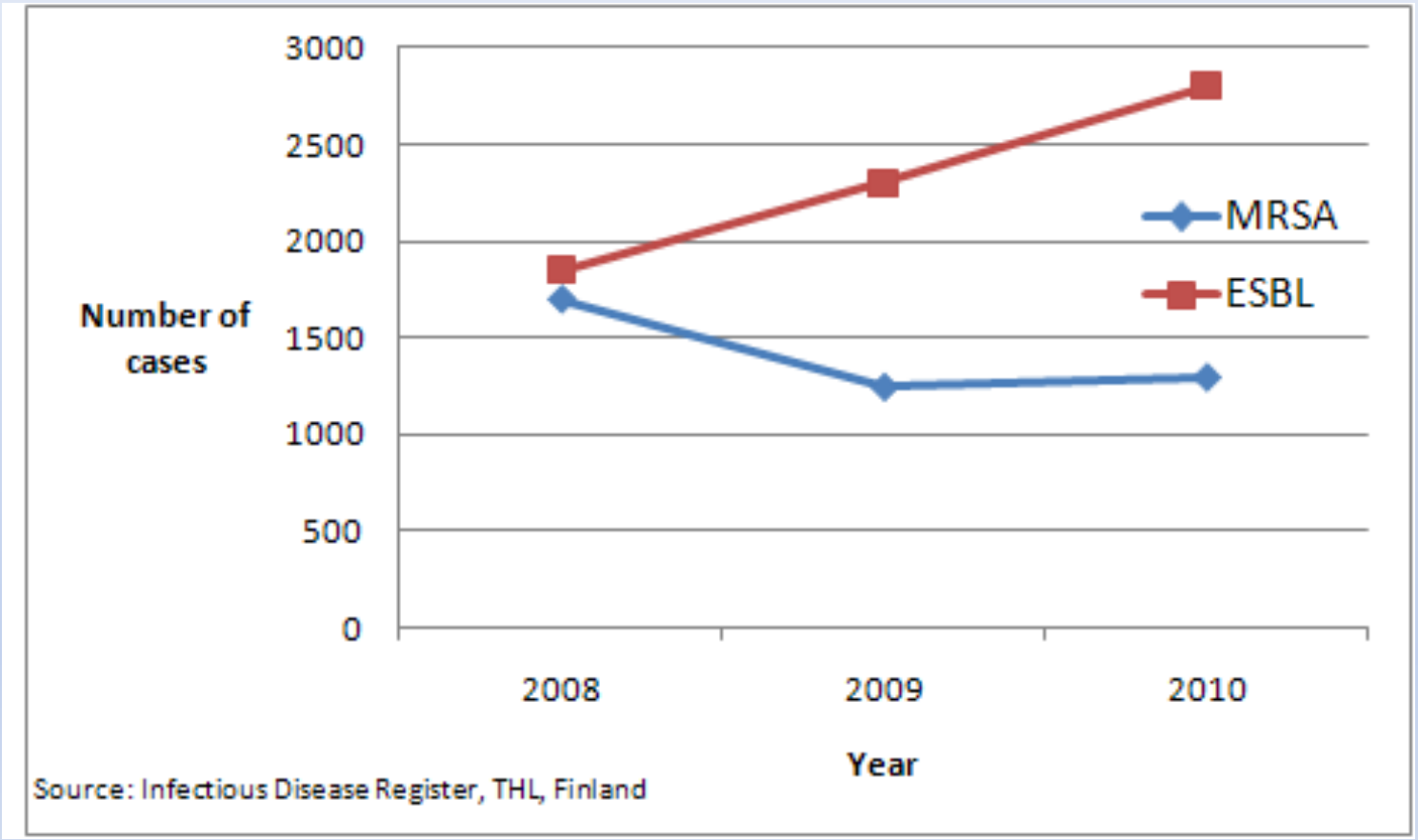
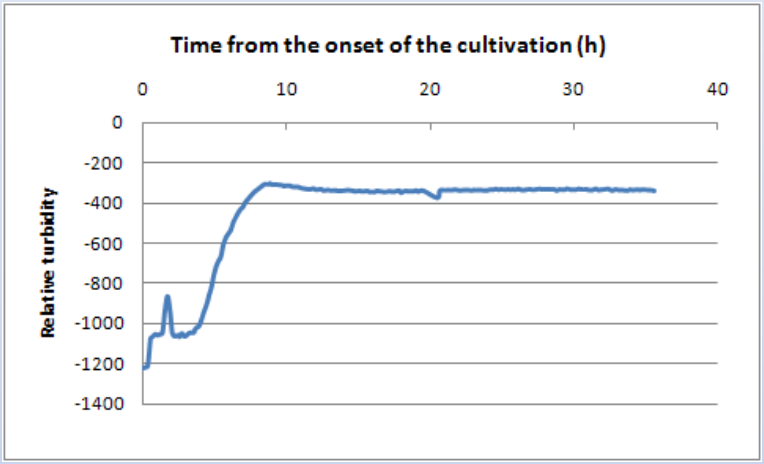


Fig. 1. Number of MRSA and ESBL cases in Finland 2008-2010

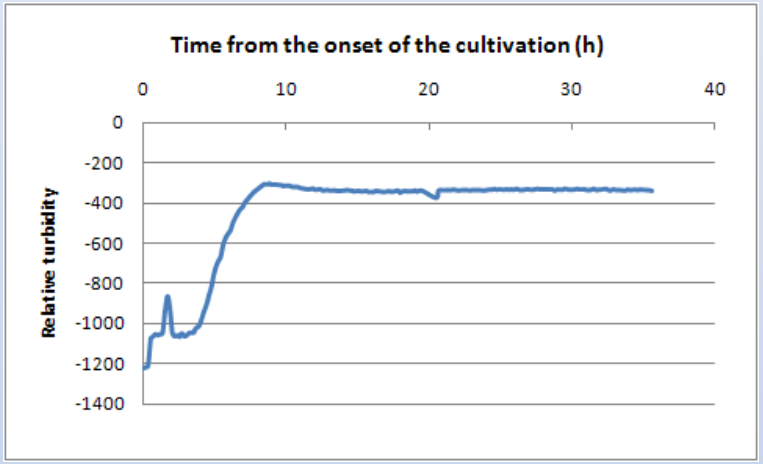
Hakalehto, E. 2011. Antibiotic resistance traits of facultative *Enterobacter cloacae* strain studied with the PMEU (Portable Microbe Enrichment Unit). In: Antonio Méndez-Vilas (ed.) *Science against microbial pathogens: communicating current research and technological advances*, Formatex Research Center, Badajoz. Spain: Microbiology Series N:o 3. Vol. 2. pp. 786-796.

Fig. 2. PMEU Spectrion® in operation with the sampling and cultivation syringes. On the right top the control unit with wireless and Ethernet connections.

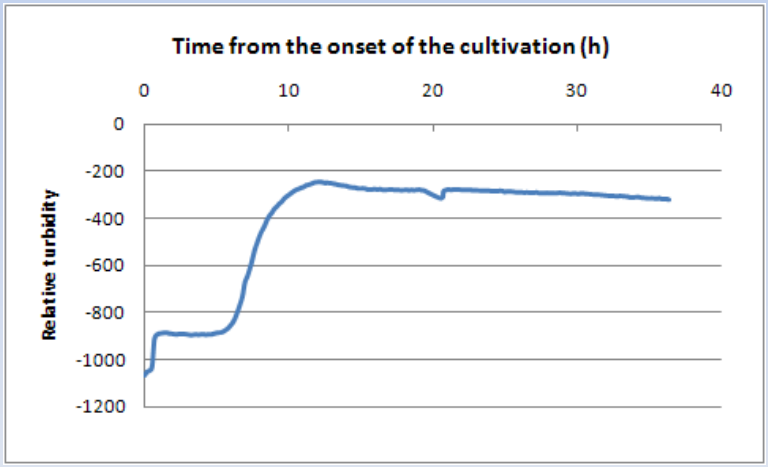
a)



b)



c)



d)

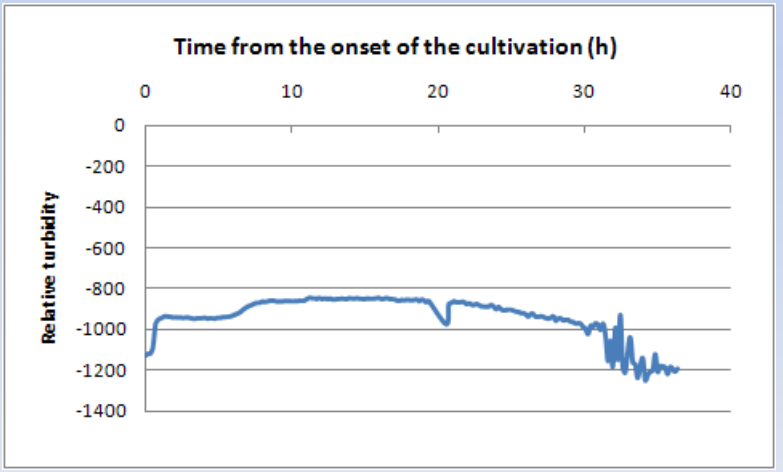


Fig 4.a-d. The influence on growth of *Enterobacter cloacae* in the PMEU Spectrion® in TYG medium in presence of different antibiotics. a) *E. cloacae* in TYG medium (control), b) with penicillin G, c) with cefuroxime, d) with netilmicin.

Suoliston mikrobitasapaino
Helsingin yliopisto, Luomuinstituutti, Mikkeli 15.3.2016
Dos. Elias Hakalehto

Hakalehto, E. 2011. Formatex Research Center, Badajoz. Spain: Microbiology Series N:o 3. Vol. 2. pp. 786-796.

Figure 1. Homeostasis, damage, barrier/function-loss and repair of the gut epithelium: role of microbiota and probiotics.
SCFA = short chain fatty acids; GALT = gut-associated lymphoid tissue.

Hell, M. et al. 2013. Probiotics in *Clostridium difficile* infection: reviewing the need for a multistrain probiotic. *Beneficial Microbes,* 4: 39-51.

Mikrobit eivät läheskään aina pyri tunkeutumaan suolistosta muualle elimistöön.

Tällöin ne kulkeutuvat ruokasulan (engl. chyme) mukana.

Ruuan mukana suolikanavaa pitkin liikkuvat mikrobit saavat täydennystä suolen seinämiltä.

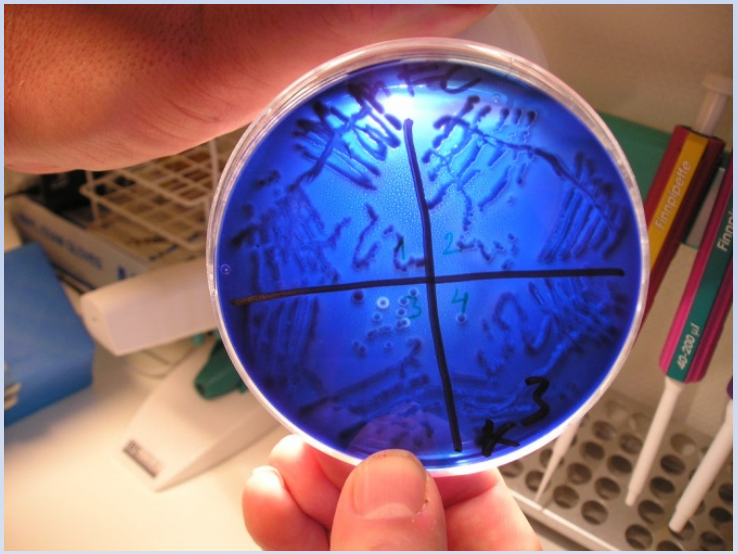
Tämän populaation tasapainotila on tärkeä, kun ehkäistään mm. ärtyneen suolen oireyhtymää.

IBS = Irritable Bowel Syndrome
SIBO = Small Intestinal Bacterial Overgrowth

Suoliston mikrobitasapaino yhdessä puhtaan ravinnon ja antioksidatiivisen ravitsemushoidon kanssa (uranuurtaja Suomessa tri Kaarlo Jaakkola) edesauttavat terveyden ylläpitämisessä ja palauttamisessa.

SUOLISTON MIKROBITASAPAINO ENNALTAEHKÄISEE SUOLISTON JA KOKO KEHON SAIRAUKSIA !

KIITOS !



Suoliston mikrobitasapaino
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