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Chapter 1

INTERACTIONS OF *KLEBSIELLA* SP. WITH OTHER INTESTINAL FLORA

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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. They are usually rather bile-resistant and facultative, which characteristics give them opportunities to flourish both in small and large intestines. We have studied the interactions of some *Klebsiella* strains with other intestinal bacteria using the Portable Microbe Enrichment Unit (PMEU) technology. They are likely to have a relatively important role in the duodenum and other upper small intestines where their cocultures with other facultative coliformic bacterium, mixed acid producing *Escherichia coli*, seem to be mutually beneficial to both members of this symbiotic relationship. They contributed to the pH which was anaerobically settled to around 6 in cocultures, corresponding the pH of the duodenum. In the mixed flora of the small intestines, *Klebsiella* sp. seem to balance osmotical pressures by an overflow mechanism, thus aiding the flora to adapt to high sugar concentrations. In a joint culture the two

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enterobacterial strains, *K. mobilis* and *E.coli*, were maintaining their mutual balance even in the presence of other bacteria, such as staphylococci and *Bacillus cereus*. Together they were capable of exploiting the nutritional potential of the medium better than in separate PMEU cultures, and also of withstanding environmental stresses and antibiotic influences in an improved fashion. Interactions of the klebsiellas with the intestinal normal flora could partially determine the role of various strains in a pathological situation.

1. INTRODUCTION

Members of the *Enterobacteriaceae* belonging to the genus *Klebsiella* form an important and interesting group of human normal flora. Being facultatively anaerobic coliforms, they are found in the various parts of the intestinal system (Hakalehto 2012). In studies regarding the gastric isolates of lactic acid bacteria, some suspected klebsiellas were detected in the endoscopic samples but they were not reported (Hakalehto *et al.* 2011). As a matter of fact, *Klebsiella* sp. strains in the alimentary tract are seldom pathogenic, but almost exclusively of commensal nature. Even such species as *K. pneumoniae* causing severe pulmonary and urinary tract infections can belong to the gut microbiota as a harmless member. In this chapter we wish to introduce some mechanisms and interactions of the klebsiellas in the intestinal molecular communication (Hakalehto 2012). Some of them are clearly beneficial to the host.

In the duodenal tract, klebsiellas have been suggested to maintain the pH balance for their part in symbiosis with such mixed-acid fermenting microbes as *E.coli* (Hakalehto *et al.* 2008). Their mutual neutralizing balance and cooperation is not disturbed by other bacteria (Hakalehto *et al.* 2010). In fact, in the fecal isolations from a neonate during the first 2-3 years of his childhood, nearly equal numbers of *Klebsiella* sp. strains and *E.coli* strains were found in a PMEU follow up study (Pesola & Hakalehto 2011). In a survey of eight children, the *Klebsiella* isolates belonged to species *K. oxytoga* or *K.pneumoniae* (Pesola *et al.* 2009). In this study with a neonates and young babies, where various milk replacements were tested in childhood nutrition, six klebsiellas and four *E.coli* strains were observed at the age of 3 months, whereas at the age of 12 months the corresponding figures were 9 and 25 indicating some shift toward the activation of more *E.coli* strains in the intestines. Although most *Klebsiella* species are non-motile, we have studied also the flagellated *K. mobilis* strain (Hakalehto *et al.* 2008). One purpose of

this article is to give an idea of the role of the *Klebsiella* sp. facultative anaerobes in establishing the BIB (Bacteriological Intestinal Balance) (Hakalehto 2012).

As the duodenal tract is the starting point of the intestines, it is containing the microflora, which is forming the baseline of the entire intestinal microbiome (Kendall 1927). The bacterial and other microbial strains are transported in the chyme along the gut actively participating the degradation and processing of food materials (Hakalehto *et al.* 2013a). In fact, some probiotic lactic acid bacteria have been shown to balance the mixed cultures of klebsiellas and *E.coli*, especially with probiotic flax (Hakalehto & Jaakkola 2013). Correspondingly, a cycle of joint *K. mobilis* and *E. coli* culture in the PMEU cultivation is carried out in 6-7 hours, which period of time corresponds to the duration of food passing the small intestines (Hakalehto *et al.* 2008). During that period, the facultative bacteria regulate the pH if the structure of the microflora is in balance. If some species, such as *E. coli*, grow in an unbalanced fashion, this could cause disturbances in the BIB, such as the Small Intestinal Bacterial Overgrowth (SIBO) (Hakalehto 2011a, Hakalehto 2012).

Besides controlling each other´s growth and the intestinal pH, bacterial strains have many other balancing functions in the gut. For example, in case of elevated osmotic pressure due to high sugar concentrations, the klebsiellas degrade glucose by their overflow metabolism (Hakalehto *et al.* 2013a,c).

Since the klebsiellas are widely present in the alimentary tract, they can also transmit various metabolic and other traits to each other and other bacteria. With respect to antibiotic resistance, *K. pneumoniae* was among the first isolates of ESBL (Extended Spectrum Beta-Lactamase) producing strains (Jacoby *et al.* 1988). From this species, the genetic material responsible for the resistance has subsequently been transferred to other members of *Enterobacteriaceae* family (Livorsi *et al.* 2013). In Finland, the number of ESBL isolates have been increasing in recent years, although there is a clear drop in the numbers of MRSA (Methicillin-resistant *Staphylococcus aureus*) isolates during the same period (Hakalehto 2011b).

2. DIFFERENT INTESTINAL ECOSYSTEMS

In a normal environmental setting, the nutrients are often scarce and dispersed. Therefore, the microbes often act in isolated communities, as "scattered ecosystems" (Hakalehto 2012). In richer conditions, they establish

"enhanced ecosystems", where the metabolic rates and bacterial and other microbial growth is speeded up. This kind of environment is potentially generated in the PMEU system. In Figure 1 a-b, a PMEU Spectrion® device is set up for an investigation of the emitted volatiles from one culture into another one.

a)

b)

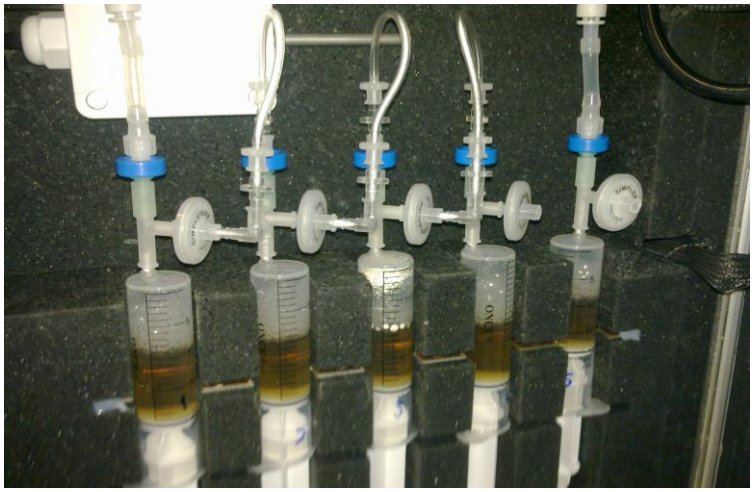


Figure 1. a. The PMEU Spectrion® device used for cultivating, detecting and monitoring bacterial and other microbial growth by optional UV, visible light and IR sensors. b. Closer look at the chained microbial cultivation syringes in the PMEU Spectrion®. The gas flow emitted from the preceding culture is directed as a bubbling flow into the next one. Photo Kevin King.

3. MICROFLORA SIMULATIONS WITH THE PMEU IN VARIOUS ENVIRONMENTS

Portable Microbe Enrichment unit (PMEU) has been in use for contamination control in water and food hygiene, and in air monitoring, as well as in agriculture, industries, and in the hospitals (Hakalehto 2010).

The PMEU method has been used for detecting water contamination in water departments, and also in source-tracking experiments, where the origins of specific strains and their routes for spreading up the contamination need to be evaluated (Hakalehto & Heitto 2012). The stand-alone PMEU Coliline™ system with ASCS (Automated Sample Collection System) has been used for monitoring the raw water, purification system, and the water distribution network at municipal plants (Hakalehto *et al.* 2011, Heitto *et al.* 2012). The PMEU´s have been used for hospital hygiene monitoring (Hakalehto 2006; Hakalehto 2010), as well as for environmental control of indicator species and pathogens (Heitto *et al.* 2009; Pitkänen *et al.* 2009). The PMEU Spectrion® has been validated for the analysis of hygiene indicating coliforms by the Finnish State Research Center (VTT) (Wirtanen & Salo 2010). Then it was stated that one cell of contaminating *Escherichia coli* was detected in about 9 hours by the PMEU when the gas bubbling of the PMEU Spectrion® was in use. The detection of single *Klebsiella* cells on Colilert® medium took place in 18h in automated PMEU Coliline™ device (Hakalehto *et al.* 2013b). PMEU monitoring has been applied for industrial contamination control at paper and pulp mills (Mentu *et al.* 2009, Backa *et al.* 2013). The klebsiellas are an important group of hygienic indicator bacteria both for fecal and industrial pollution.

The use of more sophisticated means for monitoring water sources will add safety of millions connected with the distribution systems of household water. Big cities can decide which pumping station has cleanest water for uptake at any time point (Hakalehto *et al.* 2011, Hakalehto *et al.* 2013b). The automated PMEU Coliline™ can transmit the information in real-time. It can also give an early warning for contaminated water inside the municipal purification system or in case of industry raw water. In the Finnish Polaris project the PMEU Coliline™ was successfully used for detecting artificially contaminated water in a pilot water department at Savonia University of Technology, Kuopio, Finland. Simultaneously the signal was sent by the PMEU to another equipment (by PAC Solution Oy, Helsinki, Finland)

designed for supplying the system with a disinfectant chemical. As an outcome, no contaminated water was pumped into the network.

Besides the coliformic bacteria, PMEU equipment have been used also for monitoring the distribution of fecal and industrial enterococci in lake water (Heitto *et al*. 2009; Heitto *et al.* 2012). Effective use of PMEU system could be preventing the dissemination of infections. *Campylobacter* sp. was detected with it in natural and in artificially contaminated waters (Pitkänen *et al.* 2009). In these studies, the PMEU method proved out to be faster than the ISO method (ISO17995:2005) in the detection of the campylobacteria.

In Burkina Faso, Africa, PMEU Spectrion® equipment was applied for the verification of *Yersinia* sp., *Campylobacter* sp. and other pathogens in a project by the Finnish Institute of Health and Welfare (results not shown here). By this kind of approach it is possible to monitor the quality of the irrigation water in the vegetable cultivation areas, and to prevent spreading of hazardous epidemics via the edible plants contaminated by water. The contamination control was extended from the water sources to the vegetable shelves in the local market in Ougadadougou.

Microbiological analyses of calcium carbonate slurries were carried out by IR (infra-red) sensing version of the PMEU Spectrion®. The slurries were diluted 1:10 000 which theoretically allowed the detection of total aerobic count > 10^3 cfu (colony forming units)/ml from the original samples (Hakalehto 2013). This was also the maximal contamination level accepted. The bacterial growth started after about 7 hours in the samples stored at room temperature (Fig) and after approximately 10 hours in refrigerated samples (Fig). The cultivation temperature in the PMEU Spectrion® was 30 ^{o}C. Later on the contaminants were identified as spore-forming *Bacillus* sp., which also is a common contaminant in the circulation water of the paper and pulp industries (Mentu *et al.* 2009). Also the klebsiellas occur as common inhabitants of these industrial systems, as well as their nitrogen-poor effluent pools. Their metabolic capabilities of the klebsiellas are rather remarkable. They can in general exploit both hexose and pentose sugars, as well as do fix atmospheric nitrogen (Gauthier *et al.* 2000). Due to these characteristics, such demanding surroundings as the effluents from wood industries, are occupied by klebsiellas.

In the studies in hospital environment, the PMEU technology has been used for monitoring *Clostridium difficile* infections, and the spread of this hazardous pathogen in the hospital rooms and equipments (Hell *et al.* 2010). As a conclusion, it was found out, that any strain in these studies was detected within 19 hours using the PMEU method. Also the improved recovery of the

searched bacteria has been reported. In case of enterobcterial isolates, 2.6 times more strains were documented after pre-enrichment with the PMEU, than by direct plating only (Pesola & Hakalehto 2011). The pistons of the PMEU syringes have been effectively used for the surface sampling in hospital laboratories (Hakalehto 2010), and their use in the improvement of the swipe sampling results has been recommended for various clinical uses (Hakalehto 2006).

4. *KLEBSIELLA* AS A MEMBER OF THE NORMAL FLORA

Although the genus *Klebsiella* contains several known opportunistic pathogens, it is of high importance to keep in mind that the species most often belong to our normal flora. A small baby may possess *K. pneumoniae* in his intestines, where the strain causes no harm. It constitutes one component of the flora. On the other hand, if this bacterium in his old age spreads into the lungs it may become fatal, especially if the person is subjected to some weakening factors such as consequences of alcoholism, immune deficiency, or weakness by ravaging disease.

Klebsiellas are gram-negative organisms. The outer surface of their outer membrane consists mainly of lipopolysaccharides (LPS). The LPS structure is an important antigen. The variation in this cell surface component often explains the different host-bacterium interactions and host reactions toward different strains. In case of *Klebsiella* sp., the LPS has been characterized in several studies structurally as it mediates interactions between bacteria and eukaryotic cells (Ferguson *et al.* 2000). Since LPS is also an activator of innate immunity system and potentially toxic ("endotoxin"). It can cause inflammations, resulting either from infectious diseases, or from continuous oxidative stress (Jaakkola & Hakalehto 2012). Interestingly, the 2,3-butanediol given as gastric intubation to rats significantly ameliorates acute lung injury (Hsieh *et al.* 2007). The structural variations in the LPS may influence in the sensitivity of the bacterium toward specific antibiotics, such as in case of *K. pneumoniae* with polymyxin (Helander *et al.* 1996). Microbes often provoke irritation and inflammation in our airways, caused by inhaled LPS (Helander *et al.* 1980). For example, both the lipid A part and the core part of the LPS of such gram-negatives as *Pseudomonas putida*, *Enterobacter agglomerans*, and *Klebsiella oxytoca* induced leukocytes invasion into the airways. The endotoxin can initiate these effects as a chemical component, detached from the bacterial cells. Different bacterial LPS has different toxigenic and

immunogenic properties on the host (Helander *et al.* 1984). With this respect, closely related bacterial strains belonging even to the same species can have entirely distinct impacts on the host. In case of such bacteria as *Pectinatus* sp. lipopolysaccharides bear unique features in their chemical composition (Helander *et al.* 1983). They are also at least equally active biologically as the *E. coli* and many of its relatives, even though *Pectinatus* sp. has been so far found only in the beverages, This illustrates the universally toxigenic nature of the LPS. In a culture broth, or inside industrial fluids, a strain not having the O-specific side chain, forms aggregates in the stationary growth phase (Hakalehto *et al.* 1984).

The cell surface structures of the intestinal members of the *Enterobacteriaceae* often contain fimbriae and adhesins. These appendiges are important in the non-specific and specific attachment of the bacterial cells onto the epithelial surfaces. Flagellae are important especially for the bacterial cell movement. The N-termal sequence of the repeating flagellin unit protein in the flagellum (flagellin filament protein) can be used for taxonomic considerations. In case of motile *Klebsiella*, this sequence is supposingly closely resembling that of *Salmonella* sp. or *E.coli* (Hakalehto *et al.* 1997).

Most importantly, it is crucial to understand that klebsiellas and other duodenal bacteria harbor the epithelial walls in relatively low numbers, but after the arrival of a new food or nutrient burst from the gastric port, they inoculate the chyme thus formed. The metabolic activities and interactions of the intestinal bacteria then occur largely during the movement of the chyme inside the alimentary canal. In these circumstances the members of the normal flora are protected from the powerful host defenses, such as leaching with bile acids, mucosa, and the effects of antibodies and defenses.

5. DUALISTIC BALANCE IN THE DUODENUM

After being neutralized in the duodenum, and subjected to the influence of several host, and also microbial, enzymes, the degrading food is moving on in the small intestines. It is forming, together with bile substances slimy fluid, containing microbes and their metabolites, as well as the said enzymes. This viscous substance is called intestinal chyme. The reactions and interactions inside this fluidic, particles containing slimy material are extremely difficult to be documented. However, we have tried to some extent simulate the possible sequence of events, as well as microbial interactions inside it (Hakalehto *et al.* 2008, Hakalehto 2011a).

Interestingly, the movement of intestinal chime from duodenum to the caecum takes about 6-7 hours which period of time corresponds to the time needed for one full cycle of nutrient utilization of a joint *E. coli* and *Klebsiella* culture in the PMEU (Hakalehto *et al.* 2008). During this period of about seven hours occurs also the intestinal part of the cycle of the bile substances (Donald *et al.* 1972). They are contributing to many essential digestive functions, such as fat uptake (Dawson 1967). *Klebsiella* and *E. coli* strains are usually devoid of capability of conjugating bile, which makes them suitable symbiotic organisms from the host point of view. The relatively large amounts of 2,3-butanediol produced by the *Klebsiella* sp. and related bacteria influence the qualities of the chyme. This diol substance is also non-toxic (Priscott 1985). It has been shown to activate the innate immunity system (Ebringer *et al.* 2006, 2007). *In vivo* it is degraded to *e.g.* ethanol and acetate, and other organic acids, which could then be exploited by the host for nutrition.

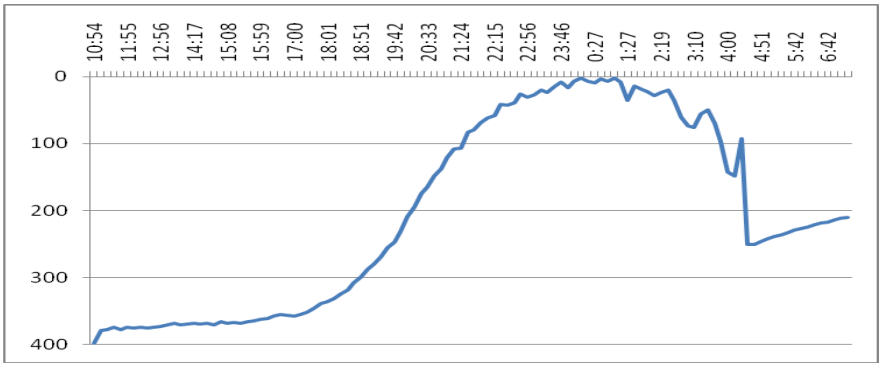
In a simulation with the PMEU it was proven that the balance and joint action of the mixed-acid fermenting *E. coli* ad neutral substances producing klebsiellas was not disturbed by other bacteria (Hakalehto *et al.* 2010). Together they also maintain the pH at about 6, which is beneficial for the host. *Klebsiella* sp. was also shown to maintain the osmotic balance in high sugar concentrations, which is essential for the microbiological balance in the gut milieu (Hakalehto *et al*. 2013a,c). On the basis of the above-mentioned evidence, we have suggested the concept of "Bacteriological Intestinal Balance" (BIB) to describe the various functions that the normal flora is performing in human digestive tract (Hakalehto 2011a, Hakalehto 2012). Members of the genus *Klebsiella* play an important role in this intestinal network of interactions.

When K. mobilis was cultivated on Colilert® medium in PMEU syringes chained by gas flow (Hakalehto 2011a; Hakalehto & Hänninen 2012; Hakalehto *et al*. 2013b) the increase in CO_2 concentration in subsequent five syringes provoked culture growth (Table 1). This enhancement was not as clearly as in the *E. coli* cultures promoting the earlier onset of growth (Hakalehto *et al*. 2013a,c), but it shortened the culture time required for peaking the growth. The differences in the growth curves of the first and fifth PMEU syringe cultures are presented in Figure 2 a-b.

Table 1. Cultivation of chained *K. mobilis* cultures connected by the bubbling gas flows from the preceding syringe cultures directed to the next ones (for reference, see also Figure 1 b, and Figure 2).

syringe culture	onset of growth	duration of exponential phase	growth peak
1	6h	4h	13h
2	4h	2h	10,5h
3	4h	2h	11h
4	4h	2h	10,5h
5	4h	2h	9h

a.



b.

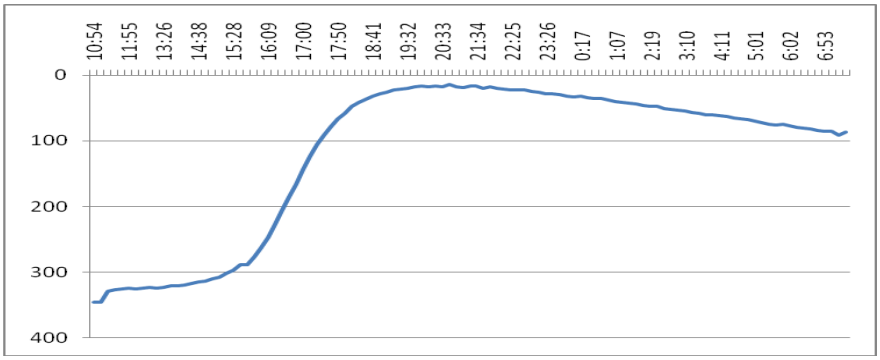


Figure 2. Growth in the carrier gas flow in the PMEU Spectrion® as the CO_2 concentrations increase by the emission from the cultures chained by tubes from preceding syringe cultures to the following ones (see also Fig. 1.b) (a) the first culture in sequence of five, and (b) the fifth culture where growth initiates about three hours earlier as a consequence of the CO_2 stimulation.

6. OSMOTIC AND ENERGY REGULATION

In overflow metabolism, *E. coli* excretes acetate (Vemuri *et al.* 2006), but in mixed cultures by *Klebsiella* sp. excess glucose is converted into ethanol and 2,3-butanediol. This is the case also in the presence of other bacterial strains (Hakalehto *et al.* 2010). Klebsiellas are thus likely to convert or avert the acidic fermentation products of *E. coli* and other mixed-acid producers into the neutral 2,3-butanediol and acetylmethylcarbinol (Hakalehto *et al.* 2013a,c). This cooperation of facultative coliforms continues also in the presence of other bacteria, such as *Bacillus cereus* or *Staphylococcus aureus*, or bile substance oxgall. This helps these symbiotic cultures to maintain the dualistic pH balance and prevents excess acidity as the bacteria rapidly increase in the chyme after the arrival of food substances into the intestinal tract (Hakalehto *et al.* 2008). Gram-negative facultative bacteria cope with increased sugar concentrations and other changes in the osmotic pressure by increasing the volume of their periplasmic space at the expense of cytoplasmic space (Nester *et al.* 1982). At the same time the excess sugar has to be metabolized to relieve the pressure, which has been shown to occur without major population growth or CO_2 production. The latter occurs in practically all fermentative pathways (Stanier *et al.* 1970). The formation of CO_2 causes acidification.

The CO_2 production actually occurs in some intracellular forms of bypass or overflow metabolism, such as methylglyoxal bypass (Teixeira de Mattos & Tempest 1983). The main end-product of this bypass route is lactate instead of 2,3-butanediol. The 2,3-butanediol, in turn, is believed to be produced by a branch of an anabolic pathway in normal growth conditions (Johansen *et al.* 1975). However, in the presence of high sugar concentrations these mechanisms seem to be replaced by another reaction sequence which cause the decrease in CO_2 production and the cessation of population growth during the pH increase. Earlier it had also been shown that in anaerobiosis *Klebsiella aerogenes* is producing high amounts of 1,3-propanediol from glycerol (Streekstra *et at.* 1987). In our recent studies, we have come into a conclusion that there has to exist a previously unknown overflow metabolic pathway, which lowers the osmotic pressure without pH decrease (Hakalehto *et al.* 2013a).

The above-mentioned examples give an idea about the specific events in the intestines that participate in the host-microbe interactions. In connection with the 2,3-butanediol production, considerable amounts of ethanol are formed (Hakalehto *et al.* 2013a). This substance is readily taken up by the host body, leading into energy production of chemical energy by the host.

Interestingly, the physical and chemical reactions contribute to the electrochemical flow and balances in the body. This links also the substance flow to the physiological energetics of an individual. It has been postulated that in all cells of a human individual put together, up to 60 kg of ATP is formed daily (McArdle *et al.* 2010). On the contrary, the body stores only 80-100g of ATP at any time. During physical exercises an adult could use about 200 kg of ATP daily (Brown *et al.* 2006). – Similarly, the bacterial cells also consume nucleotide phosphates for transmitting their intracellular energies from various reaction sites to another. The foundations of all this energy metabolism is based on properly functioning small intestines.

Thus it is possible to postulate that we live, together with our bacteria, in a remarkable energy field created by the functions of the living cells. These very same cells are themselves the objects in this field created by their own actions. In a way, the microbes in our alimentary tract are in a key role in absorbing the molecules and chemical energies in the food into fuels of this joint energy metabolism. Consequently, influencing food uptake via influencing the microbiota could thus contribute in a large scale to our nutrition, health and well-being (Hakalehto *et al.* 2013a,c). Therefore, it is essential to pay attention to the joint energy metabolism of human digestive system and its microbial ecosystems in order to improve general health and nutrition, and for avoiding malnutrition.

7. INTESTINAL CHYME AND HOST-BACTERIAL INTERACTIONS

Even though the bacterial content of the upper intestines is considerable lower than in the colon, the strains prevailing in the duodenal tract have a specific important task in the human digestion as a whole. Already on 1927, Kendall and his coworkers stated: “The duodenal tract of normal individual is an important part of the alimentary tract, not only it receives secretions from the neighboring glandular organs, but also because it is a place of the initial development of that vast column of bacteria, ..., which is excreted daily in the fecal mass ”. It is noteworthy that although the bacterial concentrations on the small intestinal epithelia are relatively low, their contribution to the degradation and processing of food substances within the chyme are forming an essential element in human nutrition. Interestingly the passage of food in the small bowel takes approximately 6-7 hours, which is also the duration of a

growth cycle of a joint *K. mobilis* and *E. coli* culture in the PMEU simulation (Hakalehto *et al.* 2008). These bacteria are actively metabolizing the food compounds, together with many other strains, in the intestinal chyme. They also produce volatile compounds which play a role in the intestinal regulation (Hakalehto & Hänninen 2012).

In the investigation of the gaseous emissions of pure *E.coli* and *Klebsiella mobilis* cultures, as well as in case of their mixed co-culture, the profiles of measurements from five different sensors of the PMEU Scentrion® were determined (Hakalehto 2012). For example, then the maximal relative "attenuation" of the charge on the metal Oxide Sensor 2 (MOS2) as a result of activities of *K. mobilis* culture was measured as 0.15 on the scale of the device roughly at time point of 5 hours. Corresponding value for *E. coli* at the same time point was about 0.65. One hour earlier the reading was close to 0.5 in the latter (about 75%), whereas in the culture of *K.mobilis* it was less than 0.1 (about 60%). This relative difference indicated the slightly slower onset of growth of the *K.mobilis* than *E.coli*, typical for their joint cultures (Hakalehto *et al*. 2008). In fact, the same values for a mixed culture in this case were 0.45 (5h) and 0.35 (4h), the latter value being close to 80% of the former one, indicating some summative change or accumulation in the emissions (Hakalehto 2012). However, in a pure culture of *K. mobilis*, although peaking on a lower level, the emission was much more long-lasting than the emission of the mixed culture which ceased approximately at time point 9h almost completely. In a pure culture, the measurable effect on MOS2 sensor remained at 0.04 or more (25% of the maximum) until the time point of 16 hours. The level of 0.08 exceeded until time point of 11h. The same level was achieved in the pure culture of *E. coli* at the same time point, but then the emission remained around 0.08-0.12 until the time point of 18h. In general, the levels of gaseous emissions from *E.coli* culture were considerably higher than from a pure *K. mobilis* culture. In a mixed culture, as indicated above, all metabolic functions seem to nearly vanish after 9 hours.

This clear shortening of a metabolically active period in a mixed culture of *K. mobilis* and *E. coli* could result from the more efficient use of the nutritional resources by these two bacteria together, which also maintains the culture pH at about 6,0 in an anaerobic conditions (Hakalehto *et al.* 2008). In our measurements with some probiotic substances in the PMEU together with either *E. coli* and *K. mobilis*, the probiotic products containing lactic acid bacteria seemed to rather provoke the facultative growth than to suppress it (Table 2). Then the cultivation took place in the PMEU Spectrion® at + 35 ^{o}C using gas mixture (N_2, 85 %, CO_2 10 %, O_2 5 %). In this experiment, it

seemed that *E. coli* and *Klebsiella*s moderated each other more effectively. However, if the probiotic action was combined with prebiotic flax, a clear restrictive joint effect was observed (Hakalehto & Jaakkola 2013).

Table 2. Effects of various probiotic bacterial preparated in cocultures with *E. coli* and *K. mobilis*. The inocula were 3 % of the initial concentrations (in parenthesis) of *E.coli* (3,8 x 10^8 cfu/ml), *K. mobilis* (3,7 x 10^8 cfu/ml). The cultivation in the PMEU Spectrion® took only 8 hours during which time the bacterial populations reached the maximal concentrations. It seemed evident that the probiotic lactic acid bacteria as such did not limit the growth of the facultatives. However, in specific conditions in the intestinal chyme, they could have the moderating effect (for details, see the text).

Table 2.

		5h time point/ cfu x 10^8	8h time point/ cfu x 10^8
1.	*E.coli* + probiotic mixture 1	7.0	3.4
2.	*E.coli* + probiotic mixture 2	6.0	nd
3.	*E.coli* + GG strain, *Lactobacillus rhamnosus*	8.9	6.6
4.	*Klebsiella* + probiotic mixture 1	9.0	10.3
5.	*Klebsiella* + probiotic mixture 2	6.5	10.1
6.	*Klebsiella* + *Lactobacillus rhamnosus* G6	6.7	8.0
7.	*E.coli* + *Klebsiella* + probiotic mixture 1	14.5 *E.coli* 8.2 *Klebsiella*	3.9 *E. coli* 2.6 *Klebsiella*
8.	*E.coli* + *Klebsiella* + probiotic mixture 2	2.9 *E.coli* 0.8 *Klebsiella*	4 *E. coli* 1.5 *Klebsiella*
9.	*E.coli* + *Klebsiella* + *Lactobacillus rhamnosus* G6	1.6 *E.coli* 1.7 *Klebsiella*	3.2 *E. coli* 1.9 *Klebsiella*
10.	*E.coli* + *Klebsiella* CONTROL	5.6 *E.coli* 1.6 *Klebsiella*	1.8 *E. coli* 1.3 *Klebsiella*

nd = no data

8. BACTERIAL INTESTINAL BALANCE (BIB)

During the movement of the chyme toward the caecum, the remaining food material in it is being digested by the joint action of the host enzymes and the microbes in the chyme. Simultaneously, the klebsiellas and other bacterial strains originating from the intestinal epithelial walls, multiply in the chyme

and participate in the process. Excessive growth of any particular microbe could now have adverse effects on the overall balance. In a recent experimentation with various lactic acid bacteria (LAB), such as *Lactobacillus* and *Bifidobacterium* strains, it was shown that together with prebiotic crushed flax seed and antioxidant extract from berries, some of these strains could clearly control the growth of facultative *E. coli* and *K. mobilis* (Hakalehto & Jaakkola 2013). This moderating effect was also demonstrated with *Staphylococcus aureus*, but not with *Salmonella typhimurium* Serovar *typhimurium* (results not shown in here). Reasons for the latter tolerating better the combined prebiotic and probiotic action in this test in the PMEU system is not known. However, it could be assumed that this illustrates the pathogenic salmonellas being more resistant to the control measures by other gut microflora. For them the intestinal chyme with its movements could offer a vehicle for transportation and means for infecting new areas of the gastrointestinal tract (Hakalehto *et al.* 2000). Even though the klebsiellas are taxonomically somewhat related to the salmonellas, they do not possess the mechanisms providing the latter with their high intrusion and infectivity capabilities.

In case of *Salmonella* infections, they are known to invade the tissues also outside the intestinal canal (Cohen *et al.* 1987). For example, *Salmonella* endocarditis has a fatality rate of 70%. The pathogenic salmonellas could take advantage of the host weakness, such as immunodeficiency, or leaky gut, or a weak normal flora including non-active klebsiellas in the chyme (Hakalehto and Jaakkola 2013). It is also possible that the salmonellas actively aim for the oxygen-rich tissues and bloodstream (Hakalehto *et al.* 2007). The opportunistic and relatively rare pathogenesis of the klebsiellas in the intestines is more resembling bacterial overgrowth type of action. The most common pathogenic species of the genus, *Klebsiella pneumoniae* is causing severe inflammations and infections of the human lungs, which could combine with other concurrent infections, such as with occurrence of MRSA staphylococcal antibiotic resistant strains (Okada *et al.* 2010). Some hypothesis based on the immunological findings have been presented regarding the role of klebsiellas in the Crohn´s disease and the ankylosing spondolytis (Ebringer *et al.* 2006). Then even the eradication of these microbes from the gut in patients with Crohn's disease with the use of low-starch diet and antibacterial agents has been suggested. However, as generally non-pathogenic and essential members of the intestines, the klebsiellas should be considered as important components of the intestinal flora at least in the healthy individuals. In case of intestinal infections caused by *Salmonella* sp.,

Shigella sp., or *Campylobacter* sp., the consequencies for the host are relatively tedious in long term including postinfectious diarrhea, bile acid malabsorption and irritable bowel syndrome (IBS) (Niaz *et al.* 1997, DuPont 2008). After five years of follow up, still 80% of the patients with postinfectious IBS following *Salmonella* infection still suffered from the symptoms (McKendrich 1996). This illustrates the "stubborn" nature of salmonellosis in the worst cases, and also poses a demand for the development of more effective probiotic supplementations with strains being able to eradicate the tedious infective agents from the alimentary tract. In a recent experimentation, it has been observed that the 2,3-butanediol produced by the *Klebsiella* sp. and other bacterial species is a potent activator of the cells of innate immunity system (Lai *et al.* 2012).

As like *E. coli* (Hakalehto 2011a, Hakalehto *et al.* 2013b), klebsiellas seem to be rather tolerant toward elevated carbon dioxide concentrations, as indicated by chained PMEU cultures (Figure 1). This kind of arrangement, where the gas emission from the preceding culture syringe is sterile filtered and directed into the next syringe broth culture, has been shown to provoke the onset of bacterial growth (Hakalehto & Hänninen 2012). Consequently, it could be postulated that CO_2 acts as an important signal molecule, and that the gaseous emissions from bacterial cell influence the others.

When the osmotic pressure is high due to *e.g.* sugary substances n the food supply, it has been demonstrated that the *Klebsiella* culture can convert the excess glucose into 2,3-butanediol and ethanol without simultaneous increase in the CO_2 production (Hakalehto *et al.* 2013b). The ethanol is then quickly absorbed by the host. As the gas emission is diminished, also the lowering of the pH by dissolving CO_2 is consequently blocked by this self-regulatory mechanism of gut microbiota. By this balancing action in the chyme, the klebsiellas are favouring the host, since the conditions remain relatively stable inside the small intestines. This is beneficial also for the other normal flora of the gut.

When the chyme arrives into the caecum, and further to the other large intestines, obligately anaerobic strains increase in number. In fact the higher presence of Firmicutes (mainly butyric acid bacteria) in the caecum of obese individuals has been recorded (Ley *et al.* 2006). This could be linked to the higher amounts of 2,3-butanediol in case of elevated ethanol product by the klebsiellas and their relatives (especially in the small intestines). In any case, it has been supposed that increased CO_2 in the large intestines by the so called "beneficial" bacterial species, such as the lactic acid bacteria (LAB) could prevent the anaerobes from rooting into the colon (Hakalehto & Hänninen

2012). Thus by keeping the anaerobic strains active and provoked to growth, they are prevented from starting secondary metabolism and toxin production potentially detrimental to the host. As in whole, this chain of events starting from the duodenum, is illustrating the importance of the BIB (Bacteriological Intestinal Balance) to the health and well-being of the host.

The surfaces of the microbial cells are often in close contact with each other, and with the host epithelia. The klebsiellas have proteinaceous structures, including various fimbriae, for mediating adhesion onto the surfaces. Close contact between the bacterial cells and the host body provoke various interactions and host reactions, often referred to as "molecular communication" (Hakalehto 2012).

In case of gram-negative gastrointestinal strains of *Bacteroides fragilis* residing mainly in the terminal ileum and the colon, zwitterionic polysaccharides (ZPS) such as polysaccharide A (PSA) serve as immunomodulators (Kasper 2009). They protect the host against inflammatory diseases by influencing with a specific mechanism on the T cell fraction. Correspondingly, there are good reasons to believe that many other members of the intestinal flora could have positive impacts on the host health and defenses, mediated by the molecular communication. Also the opposite is true, as some pathogens can provoke or cause events, which have adverse effects on the host defenses. For example, some bacteria have been shown to block vitamin D receptors, thus both restricting the innate immunity system and fat uptake in the intestines (Haussler *et al.* 2011; Castillo *et al.* 2012).

By keeping up the pH balance, and the BIB, in the small intestines, the klebsiellas balance the molecular communication and favour thus the well-being of the host (Hakalehto *et al.* 2008; Hakalehto *et al.* 2010, Hakalehto 2011a). On the other hand, these strains of *Klebsiella* sp. and other bile-tolerant facultative anaerobic members of *Enterobacteriaceae* originating from the upper small intestines, could become subjected to the control by lactic acid bacteria in the chyme (Hakalehto & Jaakkola 2013). These lactic acid bacteria can also enhance the mucosal IgA responses, both in humans and experimental animals (Brandtzaeg 2007). On the other hand, their lactic acid production could be neutralized by the generation of neutral end products as a result of *Klebsiella* metabolism (Hakalehto *et al.* 2008). This regulation is in operation with an intensified pace using an overflow mechanism of the klebsiellas with decreased gas production and ceased bacterial population growth (Hakalehto *et al.* 2013a).

9. WHY SOME *KLEBSIELLAS* ARE PATHOGENIC AS THE MAJORITY REMAIN HARMLESS OR BENEFICIAL MEMBERS OF THE NORMAL MICROBIOTA

As one of the few members of the otherwise commensal genus, *Klebsiella pneumoniae* is considered as nonopportunistic pathogen, its pathogenic nature properly being related to the thick antiphagocytic polysaccharide capsule (Levinson 2010). The pathogenic characteristics of *K. pneumoniae* are functioning almost exclusively outside the gastrointestinal tract, where according to our hypothesis, the klebsiellas have an important balancing role largely contributing to the BIB (Bacteriological Intestinal Balance) (Hakalehto 2011a, Hakalehto *et al.* 2008, Hakalehto 2012). *K. pneumoniae* causes infections in the respiratory and urinary tracts, but not in the intestines (Levinson 2010). Even these diseases are frequently associated with predisposing conditions such as advanced age, chronic respiratory disease, diabetes, or alcoholism. In healthy population 1 individual out of 10 is a carrier of *K. pneumoniae*. These strains can potentially turn into pathogens if host defenses are lowered. In fact, in the PMEU the klebsiellas have shown to interact with other bacteria in such a fashion that they could keep up the microbiological balance in the duodenum and in the other upper and lower intestines in normal circumstances (Hakalehto *et al*. 2010).

Some other members of the genus *Klebsiella* (*K. ozaenae* and *K.rhinoscleromatis*) are causing rare cases of rhinitis (Levinson 2010). On the basis of the above mentioned findings, it seems likely that the majority of the impacts of *Klebsiella* sp. could be considered as commensal or beneficial to the host, although there are some examples of a minor part of the *Klebsiella* strains being involved in diseases and other pathological developments. However, in these cases the causative agents and mechanisms are relatively well-known, and they do not generally occur in the intestinal areas.

Vibrio cholerae is an example of a pathogenic species, which has evolved through a significant alteration in its phenotype. However, this rapid change in the disease inducing strains of this bacterium is a sign of genetic variation within the species. Before the seventh cholera pandemia starting in 1961, the agents of cholera were mixed-acid fermenters. Then emerged the El Tor biotype which later on has displaced the other pathogenic forms (Yoon & Mekalanos 2006). Since then the pathogenic and virulent strains of *V. cholerae* have been predominantly carrying out 2,3-butanediol (or butylene glycol) fermentation, thus resembling the *Klebsiella* sp. The reason for this profound

and complete change in the fundamental metabolism is believed to be caused by the better survival options of the diol producing strains in the environment by e.g. better biofilm forming capabilities on some surfaces, such as chitin. According to this hypothesis, these strains would also be more capable of surviving in water and in other potential sources of contamination, as well as on the host epithelia. In this context, it is tempting to draw a conclusion that the 2,3-butanediol formation would also enable the klebsiellas to root into the intestines. Nevertheless, it is self-evident that this diol substance is an ingredient in the intestinal chyme.

On the level of actual molecular coordination of strain virulence, a mechanism exercised by *V. cholerae* to depress its MSHA (type IV mannose-sensitive hemagglutinin) pilus structure has been shown to be an essential trait for the pathogen to evade host defences and successfully colonize intestines (Hsiao *et al.* 2006). This also illustrates the importance of pili (fimbriae) in the invasion of pathogenic bacteria, and their spread along the small intestines (Hakalehto *et al.* 2000). The type I fimbrial antigens were more effectively expressed in aerobic than in anaerobic conditions in the PMEU (Hakalehto *et al.* 2007). This could reflect either the search for oxidative conditions *e.g.* in the small capillary veins or the epithelia, or the more competitive mode of the unspecific binding by the type I fimbriae in the anaerobiosis of the gut. However, this expression of the fimbriae onto the bacterial cell surfaces, as appendiges, is most active during the exponential growth phase (Hakalehto 2000). In a PMEU culture at time point 3.5 hours most cells had these fimbriae on their surfaces according to electron microscopy, whereas after 7 hours they had almost completely disappeared once the culture had reached the stationary phase (Hakalehto 1999; Hakalehto 2000). It is noteworthy that using the peptides derived from the fimbrial sequences seemed to serve as a universal reagent for detecting members of the *Enterobacteriaceae* (Hakalehto 1999). The Nissle 1917 strain has been found to be a more efficient biofilm forming strain than the enteropathogenic, enterotoxigenic and enterohaemorrhagic *E. coli* strains, being thus able to outcompete the latter during the establishment of the biofilm structure (see below) (Hancock *et al.* 2010).

Klebsiella pneumoniae has been shown to possess type 1 and type 3 fimbriae, the latter of which strongly promote the biofilm formation (Schroll *et al.* 2010). Surprisingly enough they were found to be down-regulated in biofilm forming cells. *In vivo*, the type 3 fimbriae seem to attach predominantly to the Type V collagen (Tarkkanen *et al.* 1990). Since the type 1 fimbriae seemed not to contribute to biofilm formation during the adhesion onto catheters and other medical devices they were deduced not to play a role

in the spread of such contaminations. However, the homogenous pattern of the type 1 fimbrial expression of the PMEU cultures suggests that this type of fimbrial antigen is taking part in the fast spread and initial attachment of the pathogenic strain of at least *Salmonella* sp (Hakalehto *et al.* 2000). In fact, in a later study it was found that both type 1 and type 3 fimbriae take part in the biofilm formation on urethral catheters (Stahlhut *et al.* 2012). In case of an effective probiotic strain, *E. Coli* Nissle 1917 it was found that F1C fimbriae were necessary for biofilm formation on an inert surface (Lasaro *et al.* 2009). This mechanism might be less developed, or relevant, in case of *Klebsiella* sp., which mainly belong to the normal intestinal flora. In case of *Vibrio* sp., besides the *V. cholera* MSHA (Watrick *et al.* 1999), also other species like *V. vulnifiens* (Paranjpye & Strom 2005) and *V. parahaemolyticus* (Shime-Hattori *et al.* 2006) were shown to possess a biofilm forming type IV fimbriae.

Regardless of its unspecific nature as adhesin, the type 1 fimbria is considered to be one of the major two surface located virulence factors of *K. pneumoniae*, the other being the capsular polysaccharide. The genes coding for the type 1 fimbriae in *K. pneumonia* were resembling the corresponding genes of *Salmonella typhimurium*, *E. coli* and *Serratia marcescens* although they were coding of the synthesis of fimbrial proteins somewhat varying in size and in other characteristics (Clegg *et al.* 1987). This close relatedness of the members of the *Enterobacteriaceae* was detected also in case of the sequences of flagellar N-terminal parts (Hakalehto *et al.* 1997). In this study the N-terminus of the flagellin from *Enterobacter aerogenes* was closely resembling that of *E. coli*. In fact, *E. aerogenes* is nowadays considered to be more closely related to *Klebsiella aerogenes* than *Enterobacter cloacae*, ever though *K. aerogenes* is non-motile, *i.e.* not containing flagellae (Janda & Abbott 2006).

With regard to the type 1 fimbriae, it seems possible that different molecular characteristics of different enterobacterial species could influence their pathogenesis (Madison *et al.* 1994; Duncan *et al.* 2005). In case of *Klebsiella* sp., a novel fimbrial adhesin besides the known type 1, type 3, and KPF-28, and a non-fimbrial CF29K was found (Przondo-Mordarska *et al.* 2003). This new adhesin resembles the P fimbriae of *E. coli*. All the various adhesins are believed to have a role in the adherence of *Klebsiella* sp. to respiratory and urinary tracts, as well as to the intestinal epithelium. It has been stated that in case of *K. pneumoniae* the type 1 fimbriae would not influence the colonization of the intestines or the lungs, whereas it plays a significant role in the urinary tract infections (UTI) (Struve *et al.* 2008). It was found that the genetic element responsible for fimbrial phase variation was in operation in the UTI. A causative agent of pyelonephrites was designated as a

strain of *E.coli* by rapid analysis of the volatiles produced by this causative agent in the PMEU Scentrion® (Pesola *et al*. 2012). In this type of analysis a direct cultivation in such media as colilert® in the PMEU could give a fast double confirmation (Hakalehto *et al*. 2013b).

With respect to the pathogenecity of *K. pneumoniae*, their adhesion to host cells has been quantified by an immunoassay (Ofek 1995). In this essay, the attachment of bacterial cells on most peritoneal macrophages was investigated. The macrophage-bacterium aggregates were fixed either with methanol, or by using heated glutaraldehyde solutions. This treatment stabilized the structures for the recognition with specific active sites of the antibody molecules. The dilution of the primary rabbit antibody was 1:2000, and that of the second anti-rabbit peroxidase labeled antibody (anti-rabbit) 1:5000. In case of the primary rabbit antiserum against *Pectinatus* sp. flagellar and other surface antigens, the dilutions of primary antibodies varied from 1:200 000 to 1: 2 500 000 depending on the specific strain immunologically screened in the experiment (Hakalehto & Finne 1990). In these experimentations, the polyclonal primary antibodies reacted with so called superantigens and they detected various cell surface components, such as:

- flagellae
- fimbriae
- lipopolysaccharides (LPS)
- outer membrane proteins

According to the LPS antigens the gram-negatives are classified as smooth (S) and rough (R) strains. With the phenotypically "roughest" strain (DMS 20466) extract no O-side chain existed. This strain was aggregating in the beginning of the stationary phase. The LPS of these beverages contaminating obligate anaerobes contain unique fatty acids in the lipid A of portion of the LPS molecule (Helander *et al.* 1983). As endotoxin, the *Pectinatus* LPS is at least as potent toxin as the *E. coli* LPS preparate (Helander *et al.* 1984). The N-termini of the *Pectinatus* flagella proteins somewhat differed from the corresponding structures of the *Enterobacteriaceae* (Hakalehto *et al.* 1997). The protein patterns of the *Pectinatus* sp. were characterized after extraction with mild hydrochloric acid (0.05 M), which peeled off the outer membranes of the gram negative cells into vesicles (Hakalehto *et al.* 1984). The antibody preparates combined with immunoluminometric detection provided a very sensitive method for the detection of *Pectinatus* sp. as industrial countaminants (Hakalehto 2000). In the case of the industrial screening of

aerosol contamination, immunoluminometric signal of 0 counts/min was obtained from the bottle washing machine, whereas 430 counts/min were calculated in the air by the sticker machine close to the bottling line. Even tough, the species is an obligate anaerobic bacterium, it was shown to spread into the product via the air flow, in the aerosol particles. The ultrasensitive detection of *Pectinatus* sp. inside the factory and by its production lines with luminometric detection, is reflecting the options for the screening of *Klebsiella* sp. and other gram-negative, endotoxin-containing bacteria. Then it should be kept in mind that the agents of pulmonary disease transmission could be air-borne, not usually originating from the intestines. It has been recently documented that the unique capsular polysaccharides with lacking LPS O-specific side chain were the characteristic surface traits of a couple of two carbapenem resistant *K. pneumoniae* outbreak isolates (Kubler-Kielb *et al.* 2013).

Bacterial strains of the genuses *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus* and *Hafnia* are generally considered as members of the human normal flora (Tissari & Anttila 2011). They seldom cause diseases to healthy individuals. In Finland the first carbapee producing multiresistant *K. pneumoniae* strains have been found in 2009. In case of klebsiellas some capsule types (K1 and K2) are considered as the most virulent ones, and new type of fimbriae detected in most CAZ-S/SHV-X type ESBL (Extended Spectrum beta-lactamase) strains. However, as mentioned above, most *Klebsiella* strains can be considered as non-pathogenic. The relatively low numbers of *Klebsiella* infections inside gut compared to the e.g. *K. pneumoniae* pulmonary pathogenesis implies to the essential role of *Klebsiella* sp as commensal, or even beneficial member of the intestinal microbiota.

In case of the pathogenic intrusion into the body system, one potential route could be the “leaky gut” (Maes & Leunis 2008, Jaakkola & Hakalehto 2012). Normally the intestinal epithelial cells in the intestinal, respiratory and urogenital tract consist of tightly packed cells (Wilson *et al.* 2011). These cells are attached to each other by protein structures called tight junctions and desmosomes, which normally prevent the microbial cells from invasing the underlying surfaces of the inner body system called endothelium. There the thin blood or lymphatic veins could offer the bacteria an entry inside the body. Additionally they may offer an access to oxygen for facultatively anaerobic pathogens like *Salmonella* sp. (Hakalehto *et al*. 2007). The *Salmonella* cells have been shown to express type 1 fimbriae more readily in the presence of oxygen, even though the anaerobic and aerobic growth rates of the bacteria were proven identical in the PMEU conditions.

One potential explanation of the establishment of the pilus/fimbrial structures for attachment could be the need of a bacterium to overcome the repulsions between the negative surfaces of both the host and the microbial cells (Wilson *et al.* 2011). In fact, the flushing by gas bubbles in the PMEU equipment could also serve in lowering this repulsion between the bacterial cells which have been detected to be able to grow in 100 fold densities in the PMEU (Pitkänen *et al*. 2009).

The research on the pilus (fimbriae) structures has led to deeper understanding on the mechanisms how these structures of the *Enterobacteriaceae* and also other opportunistically pathogenic groups of bacteria attach to the mucosal membranes of e.g. the urinary tract. For example, the type 1 and 3 fimbriae of *E. coli* were shown to assemble and export first the tip of the filament adhesive structure by the usher protein dimer (Fim D)(Wilson *et al*. 2011). The extruded fimbrial proteins were prevented from folding by special proteins called chaperones (Fim C). The assembly of type 1, 2, 3 and 5 pili by gram-negative bacteria occur via the chaperone-usher system. Especially in case of urinary tract infecting *E. coli*, the requirement for these filaments for infections has initiated a research of potential antibiotics called pilicides. Production of antidotes against pilus structures could offer a lead to new antibiotics. Corresponding to the flagellar N-termini, the eubacterial fimbrial N-termini seem to consist of closely related amino acid sequences (Hakalehto *et al*. 1997, Hakalehto 2000). Their occurrence as antigens is a reflection of ”war of peptides” between the host and the colonizing bacteria. For example, frogs are producing magainin peptides in order to destroy the wound infecting bacterial cells by drilling holes into their surfaces (Samgina *et al*. 2012). The effect of these peptides was comparable to some most effective antibiotics according to the PMEU studies. Even more effective antimicrobial substances were the defensing of human origin (HBD-2), which according to the PMEU results started to exhibit influences onto the *E. coli* cells at concentrations 100-1000 lower than the magains (Hakalehto 2011a). In fact, it was recently documented that these frog skin peptides were able to neutralize *in vitro* the endotoxin, proinflammatory lipopolysaccharide (LPS) complex, from two different gram-negative bacterial pathogens, human pathogen *E. coli* (0111:B4) and frog pathogen *K. pneumoniae* (Schadich *et al.* 2013).

10. ANTIBIOTIC RESISTANCE AND *KLEBSIELLA* SP.

In the 16th of March number, 2013, the New Scientist magazine cites the director of CDC (US Center for Disease Control), Tom Frieden. He was in the opinion that there still is time to stop the CRE (Carbapen Resistant *Enterobactericeae*) (MacKenzie 2013). However, already 4% of all *Enterobactericeae* infections and 10% of all *Klebsiella* infections were resistant to carbapens), the latter percentage in Greece was alarming 68% already in 2011.

The efflux mechanism in resistant *Enterobacter aerogenes* and *K. pneumoniae* strains seem to contribute to the development of the multidrug resistance phenotype (Chevalier *et al.* 2004). This mechanism has been found to be operational with respect to structurally or functionally unrelated antibiotics, quinolones, tetracyclines, and chloramphenicol. *Klebsiella* spp. account for 8% of all nosocomial infections in the Western countries, which makes it belong to the eight most common infective agents in the hospitals (Podschun & Ullmann 1998). It is noteworthy, that although the intestines could serve as a source of contaminating strains for urinary tract or lung infections, the gut infections are relatively scarce in proposition. Klebsiellas were also found as septic blood contaminants in the pediatric unit in the Kuopio University Hospital (Hakalehto *et al.* 2009; Pesola *et al.* 2011).

The occurrence of antibiotic strains could be screened by a fast PMEU analysis (Hakalehto 2011b). A septic *Enterobacter cloaceae* strain originating from the medical devices was found to be sensitive to netilmicin, but not to other tested antibiotics. It is considered to be of a high likelihood to have transfer of genetic elements in mixed intestinal microbial communities, which carry the resistance genes (Hennequin *et al.* 2012).

CONCLUSION

In human digestive tract, a balanced microbial community is a prerequisite for our health, effective food uptake, energy metabolism, and well-being. Numerous strains of *Klebsiella* sp. belong to the core part of this alimentary population, which interacts with the host body. These gram-negative bacteria seldom produce intestinal pathogens, even though the gut may save as an occasional reservoir to pathogenic strains mostly belonging to species *K. pneumoniae*. This species is a potential causative agent of pulmonary, urinary

tract and septic infections. However, regardless of this taxonomic relatedness with some true pathogens. the klebsiellas are essential micro-organisms for maintaining dualistic facultative balance in the duodenum, as well as the Bacteriological Intestinal Balance (BIB). More research is warranted in order to achieve deeper understanding on the molecular communication between various *Klebsiella* species and other intestinal strains. This is important in order to facilitate improved protection against gastrointestinal and other diseases, and in prevention of the spread of antibiotic resistance traits. The PMEU equipment offers a tool for this research and simulations in the field of gastrointestinal microbiology.

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