Chapter 3

Mixed Strain Fermentation and Metabolomics for Solving Issues of Bioproduction

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ABSTRACT

In the research of mixed microbial cultures, the numbers and identifications of individual strains are often fully or partially unknown. Their metabolic capabilities are also partially unpredictable, especially if the joint potential is to be understood. In these kinds of situations, deeper insight into the variable microbial communities cannot be obtained by genetic analysis only. Even more critical than the taxonomic aspect is usually the functional metabolic outcome of the mixed flora in question. The results from such studies as NMR (Nucleic Magnetic Resonance) give a precise view from versatile angles into the biochemical activities during the multiparametric metabolic responses of the microflora as a whole.

Originally, metabonomics was mainly used for the pathophysiological research of various microbes or for recording the genetic or biochemical modifications of mixed microflora. This approach offers a tool for monitoring changes in microscopic or otherwise confined ecosystems or multiple locations from which representative specimens are difficult to obtain. In microbiological studies, the research group can attain overall views on variable populations and their alterations in time and space.

1. Introduction to Metabonomics

Metabonomics was defined in an experimental paradigm by the groups of Everett and Nicholson as “the quantitative measurement of the multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification” (Lindon et al., 2000). A little later metabolomics was defined in an observational paradigm as “a comprehensive analysis in which all the metabolites of a biological system are identified and quantified” (Fiehn, 2002). Both terms, metabonomics and metabolomics, are used interchangeably now to describe metabolic profiling experiments in a variety of settings including biology, drug discovery, medicine, microbiology, natural product research, toxicology, and environmental sciences.

Metabonomics experiments are typically conducted in a diagnostic fashion i.e. an intervention of some sort is given to a biological system and the response of the system to the intervention is measured. A typical example might be that a patient with diabetes is given a drug and the metabolic response of the patient to the drug is measured in that patient’s blood plasma or urine across time (Everett et al., 2019).

A more recent development is the development of prognostic approaches where pre-intervention metabolite profiles are used to predict the outcome of an intervention before it occurs. This field was started with the discovery of pharmacometabonomics, which is defined as “the prediction of the outcome (for example, efficacy or toxicity) of a drug or xenobiotic intervention in an individual based on a mathematical model of pre-intervention metabolite signatures” (Clayton et al., 2006). This field has developed significantly over the past 10 years and now the field of predictive metabonomics (Everett, 2015) can be used to predict the outcomes of various interventions beyond drug treatment, such as environmental change, diet change or just the passage of time. In the latter case, predictive metabonomics can be used to predict disease onset in individuals, based on their current metabolite profiles, years in advance. A good example is the prediction of pulmonary dysfunction as a complication following cardiac surgery using serum metabolite profiles (Maltesen et al., 2016).

Metabonomics experiments can be conducted on a wide variety of biological samples including the biological fluids mentioned above, intact biological tissues and cell extracts. The metabolites in these samples are typically detected using two main technologies: nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), the latter typically hyphenated to a separation technology such as ultra-performance liquid chromatography (UPLC). Mass spectrometry has the advantage of cheaper instruments and generally higher sensitivity than NMR spectroscopy but suffers from history effects in the chromatography and in the mass spectrometer due to the direct contact of the samples with the instrument, and ion suppression and enhancement effects which do not occur with NMR spectroscopy. NMR spectroscopy is ideally suited to automated analysis of large batches of samples with no significant batch effects and essentially full quantification of the metabolites (Everett et al., 2019).

Metabonomics experiments are typically conducted by measuring the concentrations of metabolites in a biological sample before and after some sort of intervention. However, many different sorts of experiments are possible. In longitudinal metabonomics, the levels of metabolites are measure across an extended time period, so that metabolite trajectories in a metabolic hyperspace can be measured. It is also possible to measure metabolic entropy or the disorder in metabolite concentrations caused by an intervention and finally metabolic networks, correlations and dependencies can also be measured; these are powerful approaches but much less often utilized (Everett et al., 2019).

A key task in a metabolic profiling experiment is to identify the signals being detected by, usually, MS or NMR methods. This is a difficult task, as compared to the 4 bases to be identified in a gene sequencing/genomics experiment, the number of metabolites present in a typical biological sample could easily run into thousands, many of which are currently unidentified. The Metabolomics Society has issued guidelines for assigning confidence levels in metabolite identification and these are currently being updated (Sumner et al., 2007). For the unambiguous identification of a metabolite present in a complex mixture, generally, two orthogonal pieces of data compared to an authentic reference standard are required e.g. the retention time and accurate mass of a metabolite detected in a high resolution UPLC-MS experiment. If this level of identification is not possible, then a putative metabolite is referred to as 'annotated' rather than fully identified (Sumner et al., 2014). The identification of metabolites by NMR or MS methods has been the subject of recent reviews (Dona et al., 2016; Watson, 2013).

Figure 1 below shows an interesting example of metabolism of several metabolites in a mouse urine sample by *in vitro* fermentation by contaminating bacteria. In particular, there is complete conversion of the metabolite hippurate to benzoic acid.

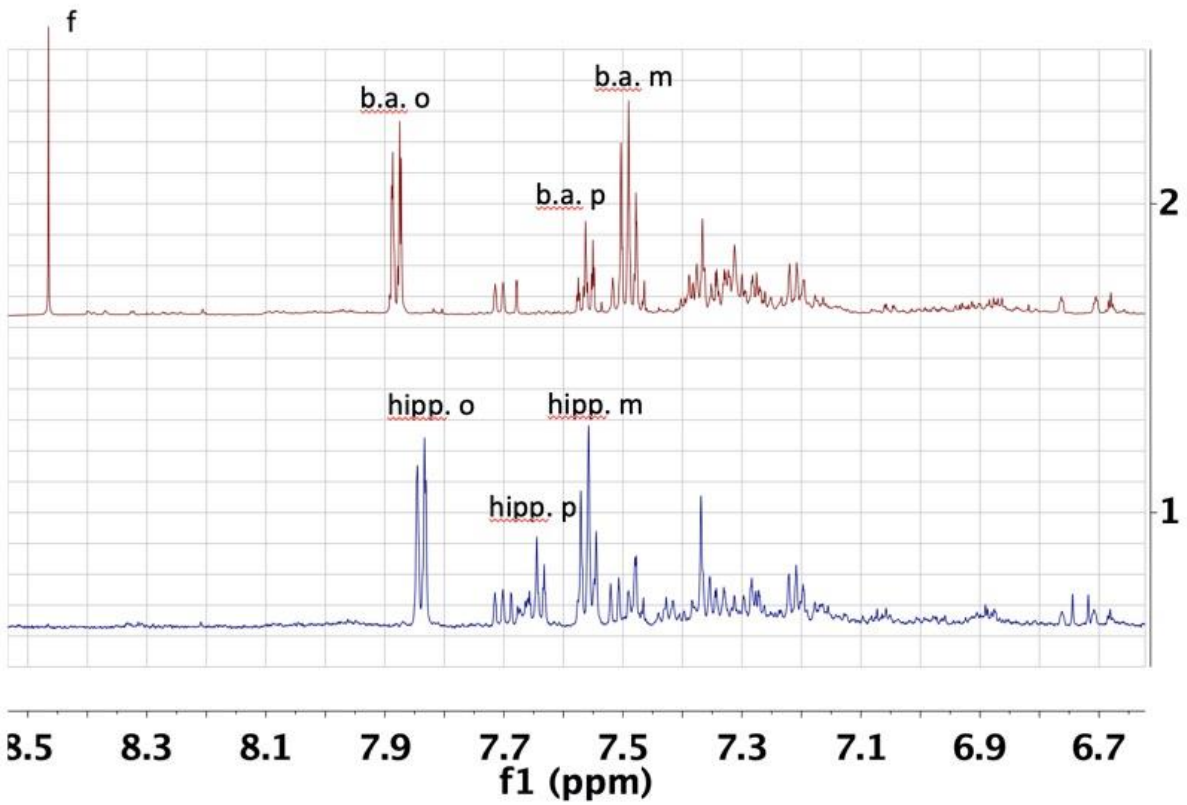

Figure 1. An expansion of the 600 MHz ^{1}H NMR spectra of the urine of a male, 30-week-old, FMO5 knockout mouse: 1) before bacterial fermentation and 2) after bacterial fermentation after leaving the sample at ambient temperature for several days. The bacterial fermentation caused many metabolic transformations including that of hippuric acid (hipp) to benzoic acid (b.a.) and glycine (3.57 ppm, not shown) and the formation of formate (f). The lower spectrum 1) prior to fermentation shows many signals including those from the ortho (o), para (p) and meta (m) protons of hippuric acid, whereas post-fermentation, spectrum 2) at top, shows corresponding signals from the ortho, para and meta protons of benzoic acid.

The application of metabolic profiling to the understanding of mixed bacterial cultures is an exciting area of research with metabolic profiles providing a completely different window on the mixture of bacteria in a culture and their functional capability, compare with genetic analyses (Barkal et al., 2016; Chevrette and Handelsman, 2021; Zhang and Zhu, 2022).

2. Metabolic investigations of human intestinal column and digestion

2.1. Microbial communities strive for balances

Since the development of science and technology is a continuum of preceding works and thinking and experimentation, the course of future work is dependent on the angle of the scientific community to look at their science and its future prospects. The history of microbiology has been many times directed by the investigations on pure strains and clinical applications. However, in “natural” conditions, the microbial populations are usually versatile. This versatility is often the precondition for balance, resilience and interaction with plants, animals and the ecosystem functions. – Correspondingly, the biochemical composition of various environments, such as soils, sediments, biofilms, processes, foods, chyme etc. are enormously complex phenomena.

However, in the microbiological culture or community development there are certain main lines and directions, which bring order into the complexity, or reflect the current domination of specific metabolic alleys. These signposts in the biochemical sense have led to the development of metabolomics. Such scientific approach has paved wat for the mixed strain fermentations.

How is the production environment shaped? In the mixed cultivations Bacteriological Intestinal Balance is an example of the paramount role of the regulation of a mixed microbial community on its structure and function. The metabolic associations between various microbial species or strains are causative functions where the produced biochemicals by some strain(s) constitute substrates for the following one(s) and they also influence on the corresponding metabolic pathways. These successive reactions are regulated by genomes of individual strains together. The phenomenon called co-metabolism is an example of the effectivity of the microbial culture alone (Clark and Rhea, 2023). This implies to the bioremediation capabilities of the microbial community together being more versatile and effective than those of any individual strains alone. In various environments the metabolic pathways are often forming networks, whose net outcome is dependent not only on the speed of individual reactions, their side products, further processing of cascading intermediate or end products, but also on the environmental condition in the surrounding milieu or niche. The microbial strains may also shape this milieu to better correspond their growth requirements. The modifying of their surroundings is often a joint operation of several strains.

For example, in the small intestines we have observed the highly important interaction between the two major groups of enterobacterial strains. These groups are 1. the mixed acid fermenting strains, such as *Escherichia coli*, and 2. the 2,3 -butanediol producing strains, such as *Klebsiella* / *Enterobacter* species. In fact, there is a dualistic balance prevailing between those groups in the duodenal tract in the normal individuals (Hakalehto et al., 2008). There the *papilla vateri* duct and port secrete, besides bile substances and pancreatic enzymes, also pancreatic bicarbonates, which adjust the pH onto favorable level for nutrient uptake. The bacterial BIB then further adjusts and balances the pH onto a level which is beneficial for the entire intestinal community. This sensitive sequence of reactions is further described

below. By regulating for their part, the pH and other conditions in the duodenum, and along the intestinal tract, the enterobacterial strains together keep up the intestinal balance which is called Bacteriological Intestinal Balance (Hakalehto, 2011, 2012, 2013, 2015b). Besides the pH regulation, also other parameters of the chyme are adjusted. Also, other bacterial strains have proven to adjust their metabolic according to the prevailing enterobacterial balance (Hakalehto et al., 2010).

In a study with biliary isolates, we could demonstrate that *Bacillus* sp. and staphylococcal strains adopted with the BIB to determine their position in the intestinal ecosystem (Hakalehto et al., 2010). When these strains were added to the balanced enterobacterial coculture, the BIB prevailed. Although the concentrations of mixed-acid fermenters and the 2,3 -butanediol producing strains decreased, they remained identical in proportion to each other. In other words, the additional species or strains did not shake the balance but adopted to it or integrated themselves according to it into the existing community or population. There “unit operations” of various strains could be followed up by the metabonomic approach. At the same time, they made the microbial community more versatile, stable and resilient. Besides the intestines, the effects of microbiome on the body functions and on itself, on its own structure and function, are dependent on the gas emissions of the microbial strains (Hakalehto 2015b).

One example of the contribution of metabonomics on human health and disease is the transformation and making adjustment to the phenotype by *Vibrio cholerae* O1 biovar El Tor occurring in the 1960s as the dominating new pathogenic agent of the species (Meehan and Markel, 2009; Son et al., 2011; Monir et al., 2022). This variant was distributed during a few decades to become the most common cause of the cholera disease, being the so called seventh pandemic of this pathogen (1961 - present). The most essential new feature of the emerging strain is the replacement of mixed acid fermentation by the 2,3 -butanediol pathway as the main metabolic route for utilizing glucose (Hakalehto, 2015c). It also is distinguished from the classical type by the production of hemolysins. This metamorphosis of ages old bacterial pathogen is an example of the modifications of microbial communities when the strains belonging to them acquire entirely new characteristics. Namely, the fimbriae Types (in the classical strains TcpA, Type B, Type C) have been somewhat modified in the El Tor strains (Hall et al. 1988) which has an impact of attachment properties of both pathogenic and environmental variants. The El Tor’s dominance as a pathogen could be, according to many studies, derived from its improved attachment and distribution in the aquatic environment (Hakalehto, 2015c).

2.2. Implications of Bacteriological Intestinal Balance (BIB)

Intestinal microbiome is in a constant interplay with human body forming an important microecosystem. Foundation of the intestinal microbiome of an individual is laid during early childhood. This process is influenced by e.g. birth method, antibiotics, breast vs. formula feeding, and introduction of solid foods. The early colonization of the intestinal microbiome could be relatively stable, even despite occasional or repeated antibiotic treatments (Pesola and Hakalehto, 2011). However, antibiotic resistance development in the early childhood could constitute considerable risks, especially when new resistant strains are spreading, or the community pressure is otherwise increasing (Medernach and Logan, 2018).

Healthy intestinal conditions are characterized by steady physicochemical situation with suitable microbiological constitution. Based on his fundamental research utilizing Portable Microbe Enrichment

Unit (PMEU) Adj. Prof. Elias Hakalehto has introduced a concept of “Bacterial Intestinal Balance” (BIB) that is formed by different intestinal microbes interacting with each other (Hakalehto et al., 2008) (Figs. 2-3). In healthy gut, for example enterobacterial species capable of mixed acid fermentation, like *Escherichia coli* (Hakalehto, 2011), and 2,3-butanediol producing species, like *Enterobacter*, or *Klebsiella* (Hakalehto, 2013), grow together maintaining a suitable equilibrium resulting in optimal intestinal conditions. When the pH is initially lowered by the former group, this activates 2,3 -butanediol pathway leading to the moderation of pH change.

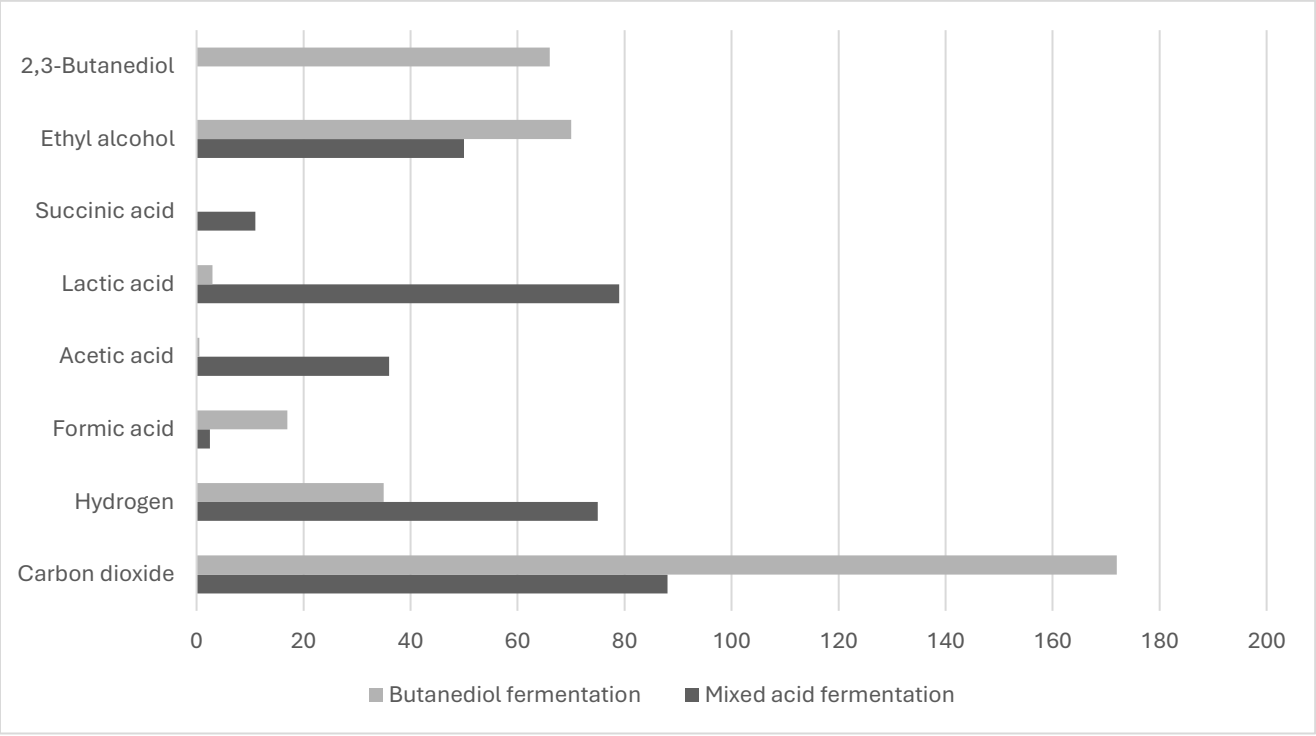

Fig. 2. Fermentation products (in moles) when of glucose (100 moles) is fermented via mixed acid fermentation and via 2,3 -butanediol route, e.g. by *Escherichia coli* and *Klebsiella* sp., respectively. (Based on original data from Stanier et al. [1970]).

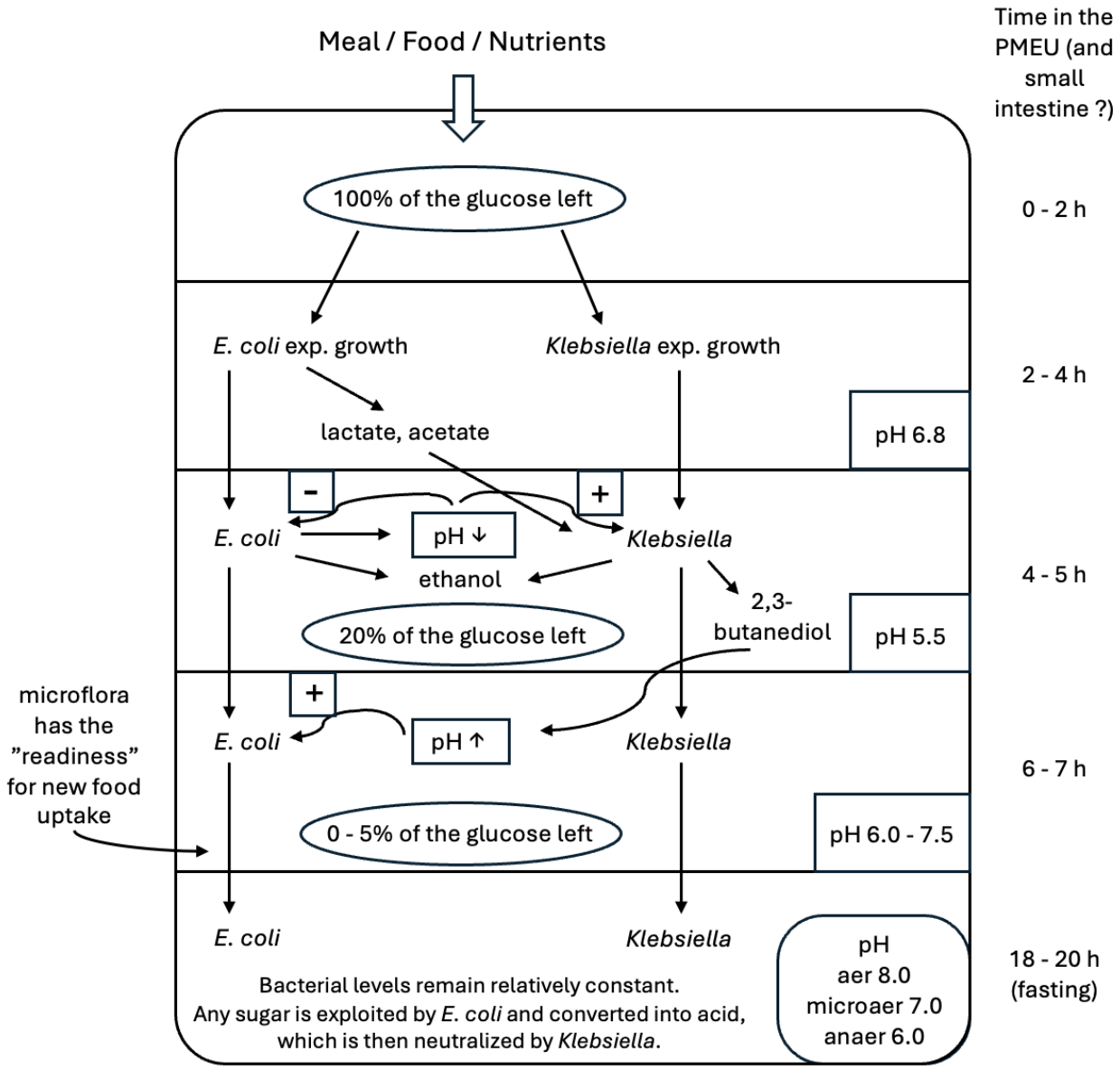

Fig. 3. Maintenance of Bacterial Intestinal Balance in small intestine simulated in Portable Microbe Enrichment Unit (PMEU) in aerobic, microaerobic or anaerobic conditions. The mixed acid fermentation pattern is accomplished by *Escherichia coli*, and more 2,3-butanediol and ethanol producing bacteria are represented by *Klebsiella mobilis*. Modified from Hakalehto et al., 2008.

The "harmony" of the BIB may be disturbed by e.g. health issues or antibiotics, or otherwise challenged by dietary changes and medications. Beneficial microbiological constitution of the BIB could be promoted by pre- and probiotics (Hakalehto and Jaakkola, 2013).

If the healthy microbiome and BIB get disordered for some reason, gut metabolism may be altered with intestinal symptoms or other disorders. These sometimes reflect the small intestinal bacterial overgrowth (SIBO) syndrome or irritative bowel syndrome (IBS). Later they can also lead to intestinal, often very multifactorial diseases, such as lactose-intolerance, coeliac disease, or more serious inflammatory bowel diseases (Crohn's disease, ulcerative colitis).

The health of the intestinal microbiome can be monitored at taxonomic, metagenomic and metabonomic levels which show natural interdependence. Taxonomic analysis reveals the structure of the microbiome. Metagenomics can show functional capacities of the intestinal ecosystem. Metabonomic approach reveals the ultimate functional output of the intestinal microbiome (Wilmanski et al., 2021). Intestinal microbes are involved in the production of vitamins, and the utilization of dietary nutrients. As a result of microbial metabolism, different gases are produced. All these functions are extremely important for human wellbeing. 2,3-butanediol, for example, has been shown to influence on lipid metabolism in mice (Veeravalli et al., 2022).

Changes of the intestinal microbiome and metabolic pathways of the involving microbial strains is currently under active investigation. Corbin et al. (2023) showed that the activity of intestinal microbiome can be enhanced by Microbiome Enhancer Diet (MBD) that is rich in fiber and resistant starch, provide larger food particles (whole nuts instead of nut butter, for example) and minimal use of processed foods. Due to higher energy consumption by intestinal microbes host metabolizable energy was lower on the MBD compared to subjects using Western diet.

By methods like Shotgun Metagenomic Sequencing it is possible to get information on microbial composition of the microbiome and microbe-derived genes involved in e.g. butyrate production, polysaccharide utilization and secondary bile acid production (Elgarten et al., 2022). In the ongoing Finnish - English EDIMGAS trial our group is investigating the effects of supplementation of beef meat for 2 weeks to intestinal microbiome (Fig. 4). The intestinal microbial metabolism of fecal, urine and serum samples in 12 female individuals is monitored over a 112-day time course prior to and after the red meat supplementation. In this experimentation, intestinal microbiome is investigated from fecal samples by both cultivation methods and molecular techniques, antimicrobial antibodies from serum samples by immunological methods and both endogenous and microbiota-derived metabolism from urine samples by mass spectrometry and NMR spectroscopy (Everett et al., 2019; Hakalehto, 2012, 2020).

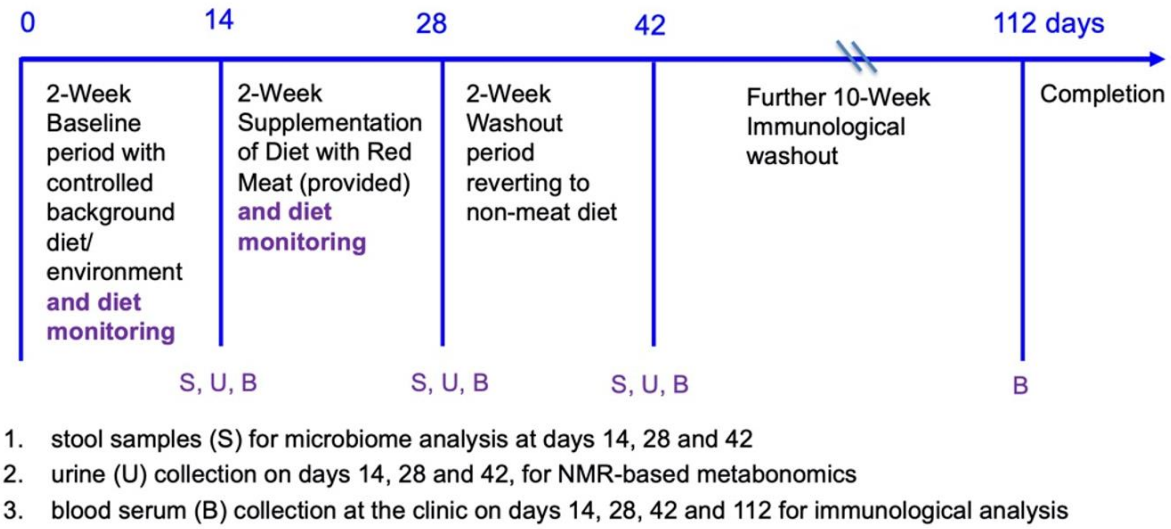

Fig. 4. Schematic study design of the Finnish – English EDIMGAS trial (2024) with indication of sampling points. The purpose of the study is to investigate the microbiome effects and metabonomics after meat supplementation.

3. Insights into metabonomics on industrial and environmental cultures

3.1. Metabolic mainstream in the production of mixed microbial cultures

The roots of metabonomics are in the pathophysiology or genetic regulation and expression. Similar phenomena as described above in relation to human health, is often readily establishing and maintaining the balances in the mixed cultures that are active in various natural surroundings or industrial process that involve highly diversified communities. Within these communities, individual strains may be used for producing several products, also by metabolically engineering these strains (Förster and Gescher, 2014). During the Finnoflag biorefinery projects we have noticed the advantages of using the more versatile microbial cultures instead of pure cultures, as biocatalysts. This approach provided record level productivity of lactate and mannitol in the biorefining of lake bottom cellulosic fiber sediments (Hakalehto et al., 2022a, 2022b).

In the meat industries we have used mixed rumen microbiomes to produce mannitol, which is a sugar alcohol that has potential uses as medical excipient, low-calorie sweetener, diuretic substance, food additive, and for many other purposes (Hakalehto et al., 2016). The analysis of mannitol production was performed by NMR (Nucleic Magnetic Resonance) (Laatikainen et al. 2016). This method is precisely differentiating various metabolites which makes it useful tool for metabonomics (Fig 5.). It can be used for the qualitative and quantitative analysis of biorefinery products. We applied this procedure in the meat, sugar, food and forest industries to produce mannitol which was purifiable by simple chemical methods on the basis of the NMR results (Fig 6.).

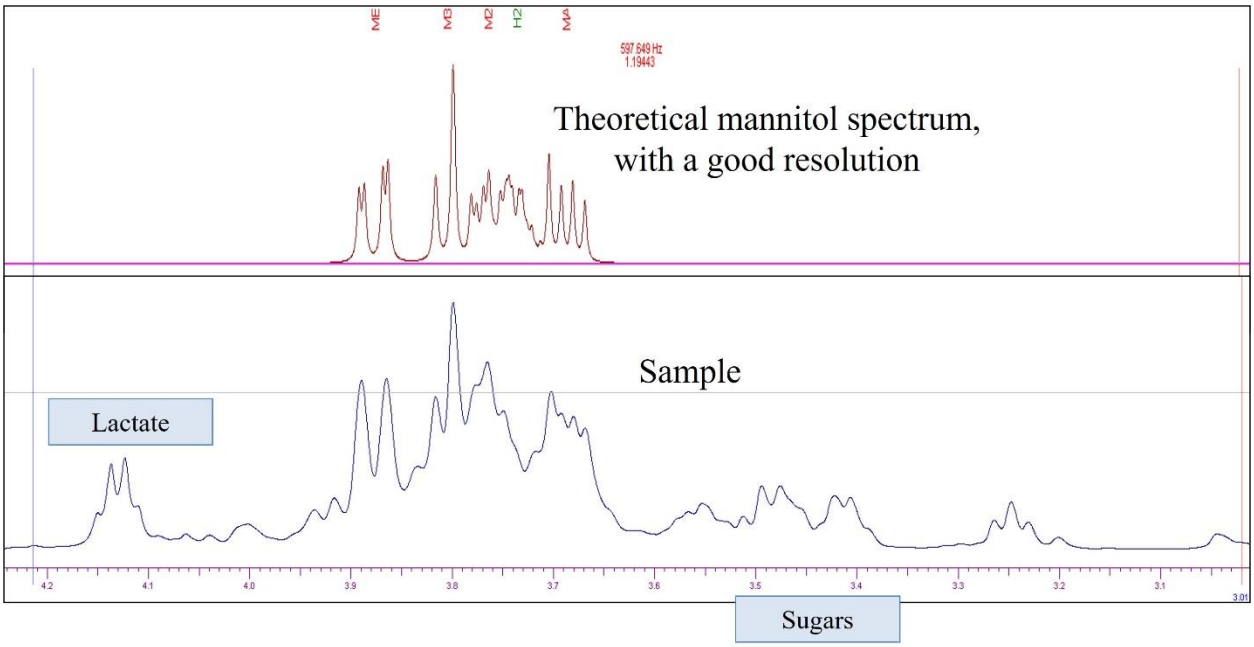

Figure 5. Location of Mannitol peaks in the NMR graph. Spiking can be used to identify a component in complex samples – and a more sophisticated deconvolution analysis is needed for the quantitative analysis.

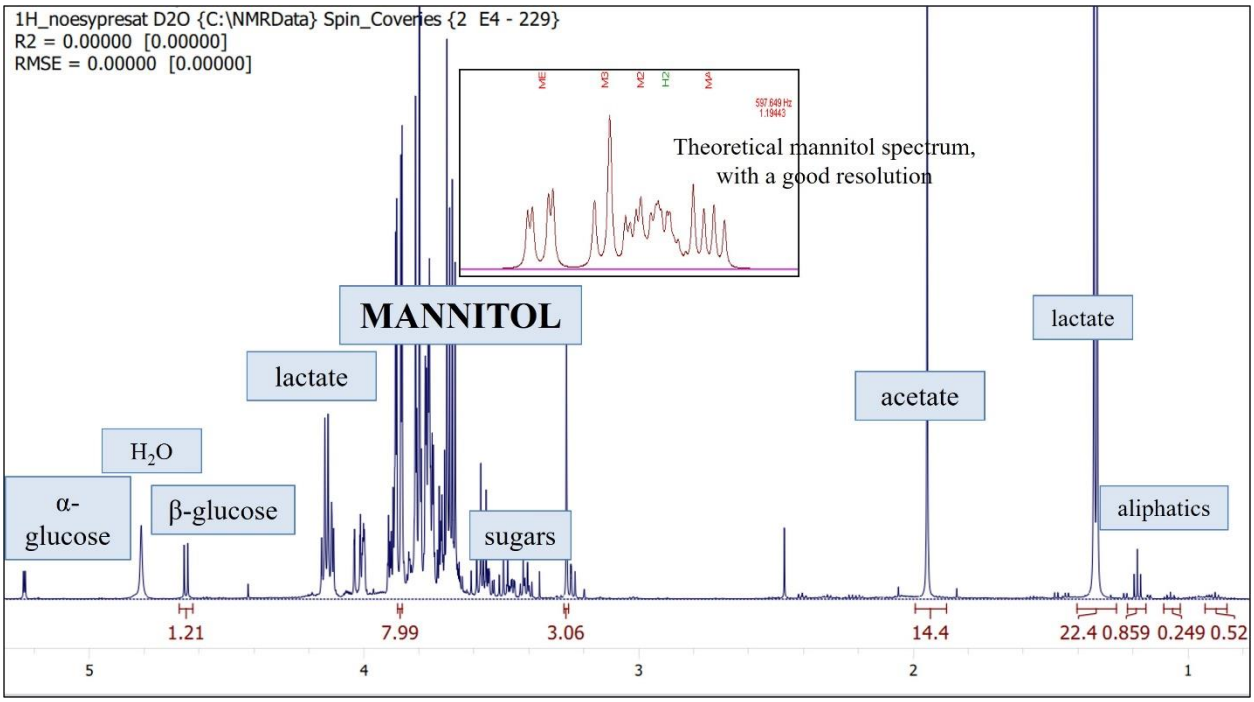

Figure 6. The quantitative measurement of mannitol as a metabolic of mixed microbial community produced 80 mg/ml as a result. The highest fermentations yields were up to 10% (w/v) of mannitol into the broth.

3.2. Ecosystem services by microbial strains

One of the most well-known methods for environmental cleansing is the removal by degradation of the petroleum and other hydro-carbon contaminations from the soil and waters. Some 35 years ago the oil tanker accident of Exxon Valdez in Alaska spiked the attention on problem hydro-carbon contaminations in the environment (Leahy and Colwell, 1990). This kind of ocean disaster was repeated in a massively larger scale in case of the Deep Water Horizon oil drilling platform leakage in 2010 (Hakalehto and Dahlquist, 2018) The latter incidence actually initiated the wider use of the ecosystem services (or engineering). This accident was supposed to spoil the waters, fisheries, tourism and ocean related activities as well as the marine ecosystem of Mexican Gulf. It was voted as the worst ecocatastrophe of the year by the international editors. Fortunately, after some time it came evident that the natural microbial mixed cultures had eliminated for a large part the threatening spillage. Consequently, the use of microbial products for recalcification and bioremediation purposes was remarkably revived. The total oil discharge in the Exxon Valdez case was approx. 42 million liters and 650 million liters of released to the Mexican Gulf by the Deepwater Horizon accident.

In the aftermath of above-mentioned accidents new research was initiated. Oil degradation was studied with different micro-organisms as communities (Röling et al., 2022) Numerical analyses was carried out using denaturing gradient gel electrophoresis (DGGE) profiles of PCD-amplified 16S rRNA sequences. In this study different amounts of nitrogen and phosphorous were added. Then it was observed that the degradation efficiency was more related to the nutrient conditions than to composition of the mixed microbial population. As a conclusion, it could be postulated that addition of nutrient will enhance natural microbial strains in their degradation activities.

3.2.1. Biotechnological treatments in metal removal, collection and reuse

A quotation from the environmental report of the Outokumpu Corporation (year 2007): “As examples of new applications of biotechnology in metal industries are e.g. the new research programs for the exploitation of peat and bacterial strains for the purification of mine waters and the usage of bioleaching for the separation of metals.” In an application proposal initiative from Montana USA, the metals and radionuclides would be controlled by fermentative microbes via their reductive reactions or chelates formation (Apel et al., 2006).

Microbiological recovery of metals is often called as bioleaching. This method is particularly effective when used for the enrichment of metals from very low concentrations. In 1957 copper was leached by *Asidithiobasillus ferrooxidans*, which represents a group of thermoacidophilic archaeal strains. These bacteria survive in pH 1 and tolerate the temperature increase caused by the oxidation of sulfur in ores (Paulino et al., 2001). The corresponding new field of research was then designated as biohydrometallurgy. These investigations led to the establishment of the largest open mine in Europe, in Talvivaara, Sotkamo, Finland. For example, nickel was produced more than 30 000 tons (https://www.savonsanomat.fi/paakirjoitus-mielipide/5773986) in year 2022. Other metal sulfides obtained from the mine included zinc, copper and cobalt derivatives. All these were recovered by mixed thermoacidophilic strains. When the sulfides are oxidized by the micro-orgasms the reaction is elevating temperatures of the leachate which enables the operations also in wintertime.

The use of mixed microbial cultures for the collection of metals has been successful also from the waste materials. Many of the bacteria used for the purpose are so-called extremophiles which are highly thermotolerant and withstand also other stressing conditions, such as low pH (Perlman, 2007).

3.2.2. Distinction between anoxygenic phototrophic microbes and photosynthetic plants (and microbes)

The red sulfur bacteria can grow on inorganic minerals with CO2 as sole carbon source, but only when sources are illuminated (van Niel, 1956). These organisms also depend on supply of H2S which is oxidize via sulfur to sulfate, and the amount of growth is strictly proportional to the quantity of H2S available. This reaction could make it possible for bacterial biofilm to get formed in places where no organic substances are readily available. The biochemical oxidation of sulfur is potentially bringing about the formation of organic layers in this case. In fact, the formation of multi-sectorial microbial communities is leading to the metabonomic versatility of these microbiomes. Their constitution and growth could then be followed by screening the joint metabolic products of corresponding ecosystems. Series of micrographs could demonstrate the formation of biofilms out of swarming cultures (Hakalehto, 2015a).

A quotation from the chapter above by C. B. van Niel in the book "The Microbe's Contribution to Biology" by A. J. Kluyver and C. B. van Niel (pp. 78-79) (1956):

"The results of quantitative experiments with the red sulfur bacteria can be conveniently expressed by simple, though only approximate, chemical equations. For young cultures, in which the available H_2S has been principally oxidized to intercellularly deposited sulfur, the equation takes the form:

$2H_2S + CO_2 \xrightarrow{light} 2S + H_2O + (CH_2O);$

for old cultures, in which the sulfur has been further oxidized, it becomes:

$H_2S + 2H_2O + 2CO_2 \xrightarrow{light} H_2SO_4 + 2(CH_2O).$

It should be mentioned that the CO_2 utilized is actually converted into bacterial cells; the symbol (CH_2O) in the above equations, representing organic matter on the reduction level of carbohydrate, is merely a close approximation used for the sake of simplicity.

These equations show that here, as in all other cases, the biochemical events involve the oxidation of one reactant and the reduction of another. Furthermore, comparison with the equation for green-plant photosynthesis reveals that it, too, falls into line, especially if it is written in the form:

$2H_2O + CO_2 \xrightarrow{light} O_2 + H_2O + (CH_2O).$

The striking similarity of the three equations, together with the recognition that the fundamental characteristics of biochemical processes are transhydrogenation reactions, thus logically suggested that the essence of photosynthesis would be a photochemically operated hydrogen-transfer mechanism in which CO_2, the hydrogen acceptor, is reduced to $(CH_2O) + H_2O$, with the simultaneous oxidation of a hydrogen donor, the latter being H_2S or sulfur in the case of the red sulfur bacteria, and H_2O in the case of green plants. A marked specificity of the respective organisms for particular hydrogen donors, a feature frequently encountered in many nonphotosynthetic organisms, could then be invoked to account for the fact that the red sulfur bacteria require H_2S instead of H_2O as reducing agent for CO_2 assimilation."

On the basis of considerations above, it is characteristic for the biological production of primary organisms that their activities accumulate organic materials which could then be utilized by the heterotrophs. In addition to the two groups described above, there are so-called chemoautotrophic bacteria which comprise several groups: methanogens, halophiles, sulfur oxidizers and reducers, utilizers of anaerobic ammonium by oxidation, and the organisms thriving in high temperatures and low pH. One example of these so-called chemolithotrophs is *Sulfolobus* sp. which is a genus of strict thermophiles and acidophiles. These extremophilic organisms are anticipated to become most feasible producer organisms in future biotechnology (Quehenberger, et al. 2017). This group of bacteria can accommodate themselves to dark conditions (Fig 7.).

Figure 7. Elias Hakalehto investigating the biological surface growth on the walls of an underground cave by the sea shore of Helsinki. Photo: Finnoflag Oy.

3.2.3. Plant-microbe associations

For long it has been supposed that microbiological nitrogen fixation is highly effective only in symbiotic mode. In other words, root nodules of pea plants or alder trees would be among the few proficient binders of atmospheric nitrogen. In this case, however, the Finnish forests could not grow much since they would be soon deprived from soil nitrogen. Also, the nitrogen fixing cyanobacteria of the watery rice fields have been forgotten in the ingrained attitude taught at schools and universities. – Indeed, in

the Finnoflag biotechnology procedures we have supplemented the residual organic fractions with the nitrogen fixing bacteria, and by so doing the production of various plant species could be accelerated. For example, the growth of organically cultivated Chinese cabbage was boosted by 50% in two months (Hakalehto, 2016).

In the plant production, the metabolic distinction between C3 and C4 or CAM crops is significant since the latter ones fix CO2 more efficiently, thus having much higher efficiency from sun to chemicals. For example, all grasses are belonging to the more producible C4 group of plants (Dahlquist, 2016). Other commercially important C4 crops are sugar canes, sorghum, maize etc. With respect to the plant cultivation, it is not a worthy that bacteria and other microbes can be beneficial if they improve the soil and make the nutrients useable for the plants. However, pathogenic microbes may hamper the production. Overly chemicalized or otherwise intensified agriculture could destroy the soil quality and its resilience. Mixed microbial cultures are the answer for these problems which relate also to food adequacy. Correspondingly, all strengthening of the soil ecosystem by organic soil improvement by beneficial micro-organisms will improve soil texture and quality for plant nutrition, and consequently increase human food production if managed ecologically and by maintaining versatility of the soil and entire ecosystem.

According to Dahlquist (2017), plant crops are also the sources of all synthetic drugs. Someone has stated that a major part of modern medications originates from the Amazonas rainforests. Therefore, it is crucial to maintain the biodiversity of our planet (Fig 8.).

Figure 8. Mojave desert in California is one of the hottest places on the Earth. It is favorable environment for the thermophiles and other so-called extremophiles. However, the desert soil often contains as many microbial species as the rainforest soil. (Photograph by Jukka-Pekka Hakalehto)

A quotation of Professor Erik Dahlquist regarding waste denitrification (see the reference above): “Nitrogen removal is a very energy intensive process consuming a lot of energy for aeration.” According to several Finnoflag biorefinery field trials, and the greenhouse experiments of the EU project “BioResque” (2023- 2024) with Finnoflag Oy and Suomen Ekosovellus Oy as the active partners, upgrading of cellulosic side streams by autonomous nitrogen fixing and other soil modulating bacteria could facilitate the recycling of the said biomass (Hakalehto et al., 2024). This effort could produce novel recipes for organic fertilizers (Kivelä and Hakalehto, 2022).

Conclusion

Metabonomic studies of mixed microbial communities and their products are valuable tools and testing methods for various applications where the composition of the flora and its metabolic capabilities are complicated or rapidly changing. In biorefineries, this approach could provide fast and comparable information during process runs, laying a foundation for process control and adjustment. It also gives ideas for surveying various surroundings, such as agricultural soil, desert sand, sea bottom rocks and sediments, and intestinal biofilms, for instance. Since the micro-organisms typically occur and are distributed from versatile and changeable compositions and combinations, the metabonomic approach is a sensitive means for monitoring and descriptive analysis. This advancement will be invaluable for future microbial biotechnology and process science.

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