Norges miljø- og
biovitenskapelige
universitet

Master thesis 2017 60 credits

Department of Biosciences
Supervisor : Siri Fjellheim

The Perceived Taste of Lactic Acid-Fermentation in Protein Hydrolysates

A screening of the sensory properties of lactic Acid Bacteria (LAB) Implemented in hydrolysate products produced with co-products from the food industry (poultry-, fish- and dairy)

Magnus Rein
Master in Molecular Biology and Evolution

Acknowledgements

The work presented in this thesis was performed from August 2017 (including preliminary work in June) to May 2018 at Nofima AS (Ås), commodity and process department and Food Safety and Quality department. Supervisors at Nofima have been Diana Lindberg and Lars Axelsson. Administrative Supervisor at NMBU has been Siri Fjellheim. The project is funded by Nofima AS, with internal resources and with general financial support from "forskningsrådet".

I would first like to express my most sincere gratitude to my two Nofima supervisors, Diana Lindberg and Lars Axelsson for good advices, guidance and supervision and support throughout the course of this master thesis. Their positive attitude throughout the study was truly inspiring and working under their supervision has been very educational. I want to thank everyone at Nofima AS with a special thanks to Signe M. Drømtorp and Kenneth A. Kristoffersen who has given advice, disciplinary expertise and guidance throughout the experimental phase. I also want to thank Mats Carlhøg and Nofima's internal sensory panel for their disciplinary expertise and bravery related to a projective mapping of food-samples produced in a master thesis. I would also like to express my gratitude to my NMBU supervisor Siri Fjellheim for being available and for accepting this thesis, giving me the opportunity to study at Nofima AS. Furthermore, I want to extent a thanks to Mari Linnea Rud and Aurélien Godard, each of which have provided information used in this thesis. I also want to thank Hilde Haver for providing continuously sterile equipment throughout the experimental phase. I also want to thank my office and coffee companion Eirik E. haugen for providing good company and for lending me a second pair of eyes when needed. I Further want to express my gratitude to Nofima AS (Ås); Never was experimental expenses a limiting factor, whereof there certainly were many. Lastly, I want to thank all the employees at Nofima for providing a superior platform from which I have been able to further my educational progression far beyond any prior expectations.3

Norges Miljø- og Biovitenskapelige Universitet

Ås, 14.05.2018

_____

Magnus Rein

Abstract

The use of enzymatic protein hydrolysis is today common practise within the food industry to extract additional high quality nutritional components, however the method is rather limitedly used towards products for later human consumption. The most limiting factors prohibiting a successful implementation of hydrolysates as a sustainable protein food source is the challenging sensory properties perceived by such products, whereof bitterness is a major challenge in hydrolysates from fish and whey. Small peptides with hydrophobic amino acids in key positions are often related to the perception of Bitter flavours. At the same time lactic acid bacteria (LAB) are used to refine flavours in different food products, with the capabilities to metabolize certain sized peptides. The industrial use of LAB raised the question; can LAB be used as a food-modifier of protein hydrolysates, and furthermore can they provide the perception of improved flavours?

This study started with the initial production of nine differentiated protein hydrolysates using three different enzymes (Alcalase 2.4L, Corolase 2TS and Flavourzyme) to hydrolyse co-products from the poultry-, fish- and dairy industry. Each hydrolysate acted as a complex nitrogen sources in a sugar reduced media and was implemented for screening for growth capacity from 47 LAB using a Bioscreen-C system. Growth rate and max growth was used to select candidates for further studies. The fermented hydrolysate products of Four LAB was characterized by Size exclusion chromatography (SEC) and compared to the corresponding non-fermented sample. Further experiment was carried out on two LAB strains through a projective mapping using an internal semi trained panel to identify different sensory characteristics of the perception to flavour from the unfermented and fermented hydrolysates.

Our results provide a glimpse into the possibilities of enzymatic protein hydrolysis coupled with LAB fermentation and may indicate that favourable flavour formation is rather strain specific and relative to the choice of co-product. Furthermore, the amount and significance in alteration of the peptide concentrations of different peptide-size fractions by LAB strains in the fermented product seems to be dependent on the utilized enzyme for each hydrolysate. Here, Flavourzyme produced hydrolysates with the largest relative change of peptide fractions seen by LAB.

This study highlights the importance of several key aspects in relation to the production of hydrolysates and the implementation of LAB for flavour development in hydrolysate products.

Sammendrag

Bruken av enzymatisk protein hydrolyse er i dag vanlig praksis innen næringsmiddelindustrien for å videre utvinne høyverdige næringsmiddelkomponenter. Imidligtid er bruken av metoden relativt begrenset for produkter som senere går til menneskelig konsum. De mest begrensende faktorene som motvirker en vellykket implementering av hydrolysater som en bærekraftig proteinmatskilde, er de utfordrende sensoriske egenskapene som oppfattes av slike produkter, hvorav bitterhet er en stor utfordring i hydrolysater fra fisk og myse. Små peptider med hydrofobe aminosyrer i nøkkelposisjoner er ofte relatert til oppfatningen av bittre smaker. Samtidig brukes melkesyrebakterier ("lactic acid bacteria" LAB) industrielt til å påvirke smak i forskjellige matvarer, med evnen til å metabolisere bestemte peptider. Den industrielle bruken av LAB hevet spørsmålet; kan LAB brukes som matmodifikator av proteinhydrolysater, og kan de også gi oppfatning av forbedrede smaker?

Dette studiet startet med produksjonen av ni differentierte proteinhydrolysater ved hjelp av tre forskjellige enzymer (Alcalase, Corolase 2TS og Flavourzyme) for å hydrolysere samproduktene fra fjærfe-, fisk- og meieri industrien. Produserte hydrolysatprodukter fungerte som en kompleks nitrogenkilde i et sukkerredusert medium og ble implementert i en "screening" for vekstkapasitet fra 47 LAB ved bruk av et Bioscreen-C system. Vekst rate og maksimal vekst ble brukt til å velge LAB kandidater for videre studier. De fermenterte hydrolysatproduktene fra Fire LAB ble analysert ved bruk av størrelseseksklusjonskromatografi (SEC) og sammenlignet med den tilsvarende ikke-fermenterte prøve. Ytterligere eksperiment ble utført på to LAB-stammer gjennom en projektiv kartlegging ved bruk av et internt semi-opplært dommerpanel for å identifisere forskjellige sensoriske egenskaper rundt oppfatningen til smak fra de ufermenterte og fermenterte hydrolysater.

Dette studiet gir et innblikk i mulighetene for enzymatisk proteinhydrolyse kombinert med LAB-fermentering. resultatene kan tyde på at gunstig smakdannelse med bruk av LAB er relativt spesifikk i forhold til valget av samprodukt. Videre synes mengden og signifikansen ved endring av peptidkonsentrasjonene av forskjellige peptidstørrelsesfraksjoner med LAB i det fermenterte produktet å være avhengig av det anvendte enzymet for hvert hydrolysat. Her produserte Flavourzyme hydrolysater den største relative forandringen av peptidfraksjonene sett av LAB. Dette studiet understreker viktigheten av flere aspekter i forbindelse med produksjon og bruk av hydrolysater med implementering av LAB som matmodifikator for smaksutvikling.

Abbreviations

A	Alcalase
A/C/F	Alcalase, Corolase and Flavourzyme
ACN	Acetonitrile
APT	All Purpose Tween
ATP	Adenosine triphosphate
BC	Backbone and skin from Cod
BHI	Brain heart infusion
BSA	Bovine serum albumin
C	Corolase
DH	Degree of Hydrolysis
dH_2O	Distilled Water
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
F	Flavourzyme
FPLC	Fast protein liquid chromatography
GFC	Gel filtration column
HPLC	High performance liquid chromatography
IS (strain)	Strain Isolate
LAB	Lactic Acid Bacteria
MAPT	Modified All Purpose Tween
MDCR	Mechanical deboned chicken residue
MF (strain)	Nofima Ås strain collection registry
MOPS	4-Morpholinepropanesulfonic acid
MRS	De Man, Rogosa and Sharpe
MVF	Multiple Variable Finder
MW	Molecular Weight
OD	Optical Density
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pH	Potential of hydrogen
PSS	Polymer Standards Service
RM	Raw material
RNA	Ribonucleic acid
rRNA	Ribosomal Ribonucleic acid
RT	Retention Time and Real Time
SDS	Sodium dodecyl sulphate
SMAPT	Screening Modified All Purpose Tween

SMOPH	Screening Modified Only Protein Hydrolysate
TBE	Tris/Borate/EDTA buffer
TBE	EDTA
TCC	Thermostatted Column Compartment
TKN	Total Kjeldahl Nitrogen
UV/Vis	Ultraviolet-visible
WP	Whey protein
^{o}B	Degree of Brix (refraction)
^{o}C	Degree Celsius
2D	Two dimensional
3D	Three dimensional

Table of content

1. Introduction

The modern industrialized food-industry is responsible for almost all food production and manufactured premade-food products in today's society (more prevalent in the western world). The industrialization has had several advantages with one of them being accessibility. Providing food for many individuals demands a system that can process large amounts of product fast and cheap. Possessing food in big-batch has an economical value, however with it comes also disadvantages. Waste-production or by-products is a large part of the industry and have persisted since the start of the era. Aesthetics, expiration dates and "high quality" products has further fed into the waste-production statics. In 2013, roughly 231 000 ton (20-25%) of all food-production resulted as food-waste in Norway (Helgesen H., 2013). This number was even higher in the USA with as much as 40% of all food production (Hall et al. 2009). In perspective this means that one third of all food-waste produced in the US and Europa could eliminate hunger for the roughly 842 million people who suffers on a day to day basis (Helgesen H., 2013). Furthermore, our food-greed and overproduction have huge implication on the global warming and in turn the environment, as microbial decomposition of food on landfills exhaust large amounts of methane and carbon dioxide (Hall et al. 2009). Food-waste occur in all sectors of food-handling, from the farmer, in the production-line, the food-trade and lastly by the consumer (Helgesen H., 2009). As it has become more and more obvious that the food-trends seen globally is not sustainable, an effort is being made to reduce the waste-production dramatically. In Europa the EU-commission have requested a reduction in food-waste (food-waste which can be further exploited) by 50% within 2020 and to reduce the gross production of food-waste on a general basis (Helgesen H., 2009). Food-waste or food-residues are categorized into; (1) human consumption, (2) potential for human consumption and (3) not for human consumption.

A large portion of the waste production contains of animal by-products which still harbours a great amount of high quality proteins. Proteins is an important nutritional source and should constitutes 10-20% of the acquired energy obtained through food in the human diet (Arsky et al. 2016). It is therefore important that food-products reflect this requirement in terms of protein content.

By utilization of enzymes and hydrolysis to recover more proteins from a given product, it is possible to produce a wide selection of food ingredients and products which would be available in a wide range of applications (Safari et al. 2009). Processing food-residues by enzymatic protein hydrolysis is one method of extracting proteins of high quality, from otherwise wasted materials. Enzymatic protein hydrolysis may be

utilized in many aspects towards animalistic proteins and animal carcass to obtain high quality proteins, whereas the resulting proteins can then be further utilized and/or added as supplements in other food products (Pasupuleti & Braun., 2010)

Waste-materials of many different food-products are today subjected to a hydrolysis reaction in order to recover as much proteins as possible. The most commonly used protein hydrolysates in biotechnological applications originates from Bovine milk (whey and casein), meat (organs, bone and binding-tissue), fish materials, but also from plant sources like soy (Pasupuleti & Braun., 2010).

Flavour is an important criterion when producing hydrolysates or any food product applied for human consumption. Many hydrolysates may be suitable and economical protein-sources for animal feed or microbiological applications but falls short when applied for human consumption due to taste (bitterness) (Safari et al. (2009)). Flavour in some ways dictates the use of a given hydrolysate. Today, hydrolysates are utilized to mask a unwanted sensory attribute or as a flavour-enhancer in other food products (Pasupuleti & Braun 2010; Safari et al. 2009).

Other methods of Flavour-enhancing are in many cases performed by microbial activity and fermentation processes, especially from bacteria of the lactic acid bacteria (LAB) group. LAB can be implemented as a starter-culture in the curing process of many different food products, such as dairy (cheese, yoghurt and sour-milk), meat (fermented sausages), fish, cereals (bread and beverages), fruits (malolactic fermentation in wine) and in vegetables (sauerkraut and kimchi)) (Calo-Mata et al. 2008). LAB affect the flavour of a given food by utilizing peptides of a certain length present in the food material and metabolizing the carbohydrates available (Holzapfel & Wood., 1995). The bacteria's energy is obtained by carbohydrate-metabolism which results in lactic acid and possibly other end-products like ethanol, acetic acid or CO_2. The production of lactic acid in the food-product gives a pH reduction and the undissociated lactic acid itself prevents the growth of unwanted microorganism and thus acts as a food-preservation method (Pontonio et al. 2017) (see also below).

Since the implementation of protein hydrolysates and LAB (fermentation) in food-products to affect the resulting products expiration date, texture, taste, smell and content, it has been of interest for the producer to acquire knowledge to improve the curing-processes and the resulting products. Increased knowledge of the enzymatic hydrolysis process in regard to the given product, enzyme and the hydrolysis time, gives room for process-optimization (increase yield, producing specific products, repeatable and reliable products in terms of content) and may result in hydrolysate-products applicable for a variety of food-products (protein supplement, flavour masking or flavour-enhancer) (Pastipuleti & Braun 2010). Producing

good fermented products with refined flavour with high reproducibility and consistency (similar batch after batch) requires detailed understanding of the starter culture (bacteria) and its growth requirement. Repeatable fermentation processes with specific results are achieved by adding single or multi-strain starter-cultures (LAB) with known concentration and incubated under specific conditions. In some instances where defined cultures are not used, it is still common practice to use the remainders from previous product-batch as start-culture in following batches to increase the conformity of the product-line.

A possible use of LAB-strains is to improve the peptide-profile of a given protein hydrolysate with unfavourable sensory attributes, which eventually leads to flavours which is perceived as palatable and allows for human consumption. In this respect, improving the result of hydrolysis may be achieved by a secondary processing step involving LAB fermentation.

1.1 Lactic acid bacteria (LAB)

The lactic acid bacteria's (LAB) has from historic times played an important role as a food-preservation method together with salting and drying, although LAB also have other favourable effects like modifications to taste and consistency towards a more refined flavour (Holzapfel & Wood., 1995; Axelsson.,2004). Many LAB are known for their probiotic effects and is commercially used as such (Schleifer et al. 2009). Over generations humans have learned to improve the fermentation methods of foods to more accurately get the desired result from a specific strain or food-material (Axelsson.,2004). The definition of fermentation in this instance however, is not in thread with the scientific meaning, but rather that a food is fermented if it "has been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification of the food" (Campbell-Platt., 1987). In certain cases, fermented foods contain more vitamins and pre-digested proteins allowing for a more effective uptake of nutrients compared to the unfermented product (Axelsson.,2004). In addition to the nutritional benefits, food preservation has also impacted the human diet and health positively. Many LAB have an inhibitory effect on human pathogens and on other bacterial growth from non-lactic acid producing bacteria, which is achieved mainly by reducing the pH in the food-material to a more acidic environment (Axelsson.,2004). The bactericidal and bacteriostatic effect of lactic acid is summarized in detail by the review paper from Theron M.M. & Lues J.F.R (2007) on organic acids and meat preservation. Lactic acid and other organic acids exists in two states; undissociated and dissociated state in a pH dependant equilibrium, whereas the version of uncharged or undissociated state poses the antimicrobial activity with increased effect at low pH. The uncharged molecule of Lactic acid is thought to be able to cross the cell membrane and enter

cytosolic space where to pH is considerably higher. The change of pH shifts the equilibrium and the molecule starts to dissociate and release charged anions and proton. The accumulation of anions and protons are found to be toxic and inhibit metabolic reactions. Further mechanical disruption is proposed to be membrane disruption, stress on intracellular pH and homeostasis. Strains that inherent types of traits that enables the effect of other pathogenic strains are thought to be probiotic if they are able colonize the intestinal tract and outcompete pathogens or give other advantageous health-benefits that precedes the mere nutritional value of the bacteria and its fermented products (Klein et al. 1998).

When using LAB for food preservation or biochemical modification it is important to have sufficient information about that specific strain and its properties. Some strains may show LAB characteristics under certain environmental conditions, shown by *Acinomyces israelii* (Holzapfel & Wood., 1995). Some strains like *Listeria monocytogenes* and some of its relatives (does not belong to the LAB group) have also shown traits which is indicative characteristics of the LAB, meaning that the definition of LAB is somewhat vague (Axelsson.,2004). Even LAB strains of the *lactobacilli* genus have shown to act as opportunistic pathogens if reaching to high concentrations (Holzapfel & Wood., 1995). However, in most cases the patients affected suffered from underlying conditions, which potentially weakened the immune defence. In this regard, all food-grade bacteria should be recognized and listed on the QPS (qualified presumption of safety) report of status; recommended biological agents for safety risk assessment (2011 and 2013), carried out by EFSA. This safety evaluations are frequently updated to consist the bacterial agents currently accepted for use in food modification or in food preservation (EFSA., 2013; Renata et al. 2011; Qualified presumption of safety (QPS) report performed by the European Food Safety Authority (EFSA)). Any bacteria-strain found on this list is assumed to be of food-grade and therefore presumed to be safe for consumption. However, any strain subjected for food modification must be used with great caution (Axelsson.,2004) Investigating the occurrence of potential antibiotic-resistance in LAB-strains implemented for human consumption is especially important (Korhonen et al 2008).

The common LAB we reconcile in today's food industry are Gram-positive, catalase-negative, non-respiring, non-spore forming cocci/rod shaped bacteria. Producing lactic acid as one of the main fermentation products of carbohydrates (Schleifer & Ludwig., 1995; Holzapfel & Wood., 1995; Axelsson, 2004). According to the current taxonomic classification, they belong to the phylum *Firmicutes*, class *Bacilli*, and order *Lactobacillales.*

The principal genera, encompassing the main food-grade LAB are; *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella*. The genus *Bifidobacterium* is historically also considered to belong to the LAB group. However, although *Bifidobacterium* species essentially fit the general description above, they have a different sugar metabolism and belong to the phylum *Actinobacteria*, the second major branch of Gram-positive bacteria (Holzapfel & Wood., 1995; Axelsson., 2004). As the term LAB is quite vague and generalized and includes a broad group of bacteria, the scope of this text will aim on the food-grade strains from mainly the *Lactobacillus* genus but also a few species from *Lactococcus, Leuconostoc, Pediococcus, Carnobacterium* and *Weissella*.

Before DNA sequencing became more widely utilized in phylogenetic-studies, morphological and phenotypic traits greatly influenced the classification. Since then, many studies have been published on the phylogeny of LAB. The paper of Sun et al. (2015), with focus on the genus *Lactobacillus*, shows an example of comprehensive work on the theme where whole-genome sequencing was employed. The phylogeny (maximum likelihood) was based on the DNA sequence of 73 core genes across 213 strains.

Growth requirements

Most LAB have particular growth requirements, whereas the presence of carbohydrates, essential amino acids (whereas methionine/cysteine, histidine, Valine generally essential for LAB) and vitamins may be required (Holzapfel & Wood., 1995; Axelsson., 2004; Teusink & Molenaar., 2017)). This requirement to a rich nutrient niche may indicate a dependency on other species and indeed a symbiotic relation for survival which has led to many metabolic-pathway-mutations and subsequently modifications in different LAB strains (Kuratsu et al. 2010). Metabolic modification is of great interest both in a food industry perspective, but also in a medical aspect, as an effective approach to producing desired enzymes or end-products may be achieved by over-exciting a given metabolic pathway of a specific LAB (Kuratsu et al. 2010). Many LAB strains are facultatively anaerobic or strictly anaerobic, but oxygen is generally well tolerated. Some LAB utilize oxygen or even prefer the presence of some oxygen under certain conditions (Salvetti et al. 2012; Holzapfel & Wood., 1995; Axelsson., 2004). The temperature requirements depend on each specific strain, whereas many LAB strains are known to prefer temperatures at 30^{o}C and at 37^{o}C depending on their original habitat. Although temperatures above 30^{o}C are preferable for many LAB, lower temperatures can maintain growth by LAB.

The lactic acid production by carbohydrate-metabolism is what mainly defines a LAB and the fermentation pathways is classified as either homolactic or heterolactic (Holzapfel & Wood., 1995; Axelsson.,2004). Homolactic LAB produce lactic acid as the main end-product of carbohydrate metabolism, although both D(-) and L(+) conformations of lactic acid may be produced, whereas a heterolactic LAB may produce significant amounts of other products; mainly ethanol, acetate and CO_2 (Holzapfel & Wood., 1995; Axelsson., 2004). The homolactic strains typically achieve energy production through the Embden-Meyerhof-Parnas (EMP) pathway for glycolysis (Holzapfel & Wood., 1995; Axelsson.,2004) thus producing theoretically two molecules of lactic acid for each available glucose molecule. However, homofermentative LAB may also be able to produce other products besides lactic acid under certain environmental conditions or if grown in a special way (Holzapfel & Wood., 1995; Kuratsu et al. 2010), meaning that a homolactic strain may not be solely homofermentative in nature (Holzapfel & Wood., 1995). Most LAB are, in fact, facultatively heterofermentative, i.e. homofermentative typically on hexoses, but heterofermentative on other sugars, e.g. pentoses. (Axelsson., 2004). Heterofermentative LAB mainly uses the 6P-gluconate pathway, also known as the phosphoketolase pathway (Holzapfel & Wood., 1995; Pokusaeva et al. 2011; Axelsson., 2004). The lower part of glycolysis/EMP pathway, i.e. the metabolism of 3-carbon compounds to pyruvate and finally lactic acid, is common for both homo- and heterolactic fermentation.

Peptide transporter systems in LAB

Peptide transport is utilized by many microorganisms to fuel its biosynthesis but also for signalling and gene-regulation (Doeven et al. 2005). In general, LAB is dependent on the presence of pre-formed amino acids as their capabilities to synthesize amino acids are highly limited (Axelsson L. & Ahrné S., 2000). Many LAB require the presence of free amino acids or peptides of a certain length to be able to transfer them into the cell for utilization. Peptide transport is an important part of any microorganism's nitrogen uptake; however, it is energy dependant, and the cost of transport may vary for different peptides (composition and size) (Doeven et al. 2005; Holzapfel & Wood., 1995). Furthermore, LAB is auxotrophic for several amino acids (may vary from 4-14 amino acids) and in some cases also in need of some essential vitamins (Chopin A., 1993). Previous studies on LAB and the use of De Man, Rogosa and Sharpe (MRS) media may indicate that components found in the MRS media may help improve the growth-performance in other growth media. For instance, nicotinic acid, biotin, folic acid, pyridoxal, adenine and uracil may help stimulate

growth of LAB (Horn et al. 2005). Many LAB-strains have proteases, which assist in acquiring the essential amino acids through proteolytic activity of the food product, outside of the bacterial cell (Mills & Thomas., 1981).

The peptide transport system in LAB consist of several genetic variations of the systems. For instance, the ATP-binding Cassette transporter complex (ABC-transporter) is a superfamily of proteins with some subunits being transmembrane proteins (Kunji et al. 1996). These complexes allow for the active transport of di-, tri- and oligopeptides across a cell-membrane. It is this protein-complex that essentially orchestrates the peptide-transport system in LAB, including the ion-linked transporter channels. Today there are many peptide transporter systems currently characterized, although not all completely understood. For instance, the function of two different transporter systems may be close to identical, but the underlying sequence may only share 20-30% sequence similarity in a multiple sequence alignment (Berntsson et al. 2009).

The *Lactococcus lactis* MG1363 strain shows the presence of three different peptide transporter systems; (I) the ion-linked transport "DtpT", (II) the ABC transporter "Dpp" and (III) the ABC transporter "Opp". Both Opp and Dpp has a (oligo)peptide-binding protein affiliated (OppA and DppA), which is anchored to the membrane and are flexible in nature (Doeven et al. 2005). OppA and DppA are also known as substrate binding proteins (SBP) and helps in the delivery of di-, tri- and oligopeptides to their respective cognate systems and in turn decides the selectivity of each system (Doeven et al. 2005; Berntsson et al. 2009). The Opp and Dpp system is assumed to acts in quite similar fashion where the transmembrane protein (OppB, OppC, DppB and DppC) forms the translocation pore which is fuelled by the two homologues nucleotide-binding proteins found in each system (OppD, OppF, DppD and DppF found in the cytosolic space) (Doeven et al. 2005; Berntsson et al. 2009; Tynkkynen et al. 1993). Cleavage is achieved through the "Venus's flytrap mechanism" (Mao et al. 1982). This mechanism can cleave the peptide-chains by changing state from an "open" conformation to a "closed"-state. the equilibrium between open- and closed conformation is moved towards a closed state with the binding of a peptide or oligopeptide. It is then associated with the translocation pore and carried into the cell. After entry to cytosolic space, peptidases act on the peptides and branched amino acids present and subjected to a hydrolysis process resulting in the cleavage of peptides in to single amino acids (Mao et al. 1982). Gene regulation of this system is controlled by a protein called *CodY* and works as a negative feedback on gene expression in the presence of branched amino acids (Doeven et al. 2005). Similar systems of the Opp and Dpp (also referred to as genetic organization *"OppABCDF"* or *"DppABCDF"* or similar*)* are found in other species and relatives with different affinity to substrates (Doeven et al. 2005).

In general, *DtpT* and *dpp* are shown to be responsible for the uptake of di- and tripeptides while the latter *Opp* system catalysing the uptake of oligopeptides (Doeven et al. 2005), however as mentioned, the selectivity of each system varies (Doeven et al. 2005). The peptide transporter system *Opp* and *Dpp* is transcribed by the *OppABCDF* and *DppABCDF* genes respectively and consist in general of five subunits each (Doeven et al. 2005). Further evidence of other transporter systems has been identified in *Lactococcus lactis* although they are either not affiliated with peptide transport or repressed and therefore has no known function (Doeven et al. 2005). These genes may be explained by gene duplication which is not uncommon in LAB strains (Sun et al. 2015).

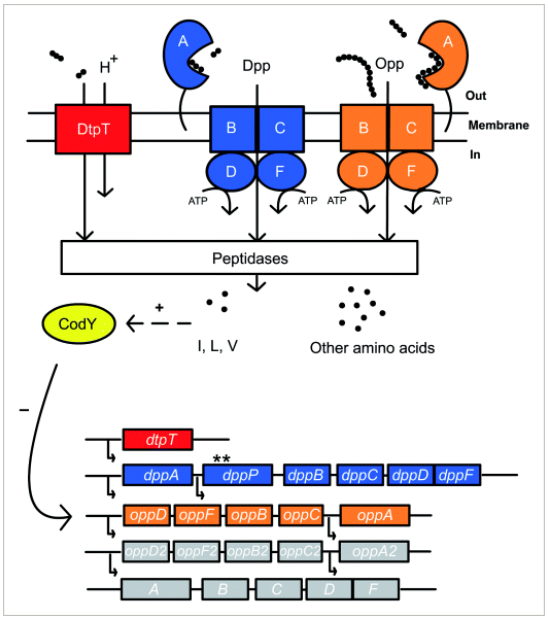


Figure 1: *shows the schematic outlay of the peptide transporter system in Lactococcus lactis MG 1363 with its function, regulation and genetic organisation. The peptide transporter system found in Lc. lactis is comprised by three membrane-bound protein complexes which together orchestra the uptake of di-, tri- and oligopeptides. The ion-linked transporter DtpT and ABC transporter Dpp progress in the uptake of di- and tripeptides, whereas the last ABC transporter Opp facilitates the uptake of oligopeptides of 4-35 amino acid residues. Each ABC transporter is associated with a (oligo)peptide-binding protein (DppA and OppA). These specialized proteins are flexible and anchored to the membrane via lipid modifications in N-terminal of Cysteine residues. The (oligo)peptide-binding proteins are found close in space to the ABC transporter and proceeds by feeding the cognate protein complex with peptides. Peptide transport is regulated by the presence of branched amino acids (I, L and V) in the cytoplasm of the bacteria by CodY. The figure is taken from Doeven M.K., Kok J. & poolman B., 2005., specificity and selectivity determinants of peptide transport in Lactococcus lactis and other microorganisms.*

LAB are known to grow on milk and are commercially used in cheese and yoghurt cultivation but also in meat curing (Safari et al. 2009). Milk consists of a mixture of four different milk-proteins (α_{s1}-, α_{s2}-, β- k-casein) which constitutes 80% of the basis protein in bovine milk (Schmidt., 1982). In free solution casein is a largely flexible branched molecule which allows for more easy access and cleavage of residues by proteolytic activity (Ludwig., Schleifer. & Whitman., 2009). Other substrates from meat and plants also supports growth by LAB as mentioned earlier. The best characterized proteolytic system is found in *Lactococcus lactis* and is thought to be quite similar in function to the once seen in Lactobacilli (Doeven et

al. 2005; Kunji et al. 1996). *Lactobacillus* strains may for this reason be suitable candidates for fermentations and food applications in products of this nature, like for instance as modifiers of protein hydrolysates.

1.2 Enzymes and Proteases

The mechanisms involved in the degradation of food-materials into nutritional components are largely performed by enzymes. Enzymes are specialized structural proteins that accelerates a given chemical reaction and dependant on mode of action, they can facilitate both catabolic and metabolic reactions toward proteins. Enzymes help in the regulation of chemical-reactions and pathways and is continuously recycled and reused to perform the same reaction multiple times (Tymoczko et al. 2015). Most enzymes are specific towards its substrate and the substrates binding site. The catalytic site or active site is where a given substrate undergoes a chemical reaction and the binding site facilitates temporary binding and keeps the substrate in position (Lesk., 2016). The enzyme recognizes a sequence or stretch of the amino acid-chain, and cleavage occur between the specific amino acids recognized (Lesk., 2016)

Peptidases or proteolytic enzymes (proteases) plays an important role in protein degradation, where they facilitate a hydrolytic cleavage of the peptide-chain at a specific site, resulting in shorter peptide chains and free amino acids (Liggieri et al. 2009). Binding of the substrate to the active site of a protein or peptide causes a shift in the charge of the functional groups (within the substrate), which in turn lowers the energy requirement. The cleavage occurs between the carboxylic and amine group of two neighbouring amino acid residue (due to the electrostatic potential) in the presence of H_2O (Baker & numatal., 2013). Enzymes which performs a hydrolytic reaction to achieve cleavage are often referred to as hydrolases, however for simplicity the general term “protease” is used to also include this group.

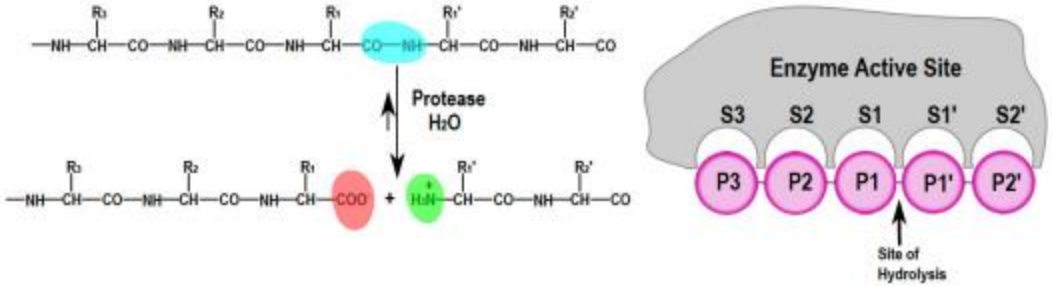


Figure 1.2: shows protease mediated hydrolysis and cleavage of a given peptide bond. The figure illustrates the enzymes active site and site of hydrolysis using a hypothetical enzyme. The presence of H2O facilitates the enzymatic activity and results in two peptides of shorter length. The illutrations are taken from the article written by Baker P.J. & Numata K., (2013) on Polymerization of Peptide polymers for biomaterial applications

Enzymes are further affected by the surrounding pH, whereas the exact pH for optimal efficiency may vary from enzyme to enzyme, although many enzymes are prevalent to higher efficiency at neutral pH (Baker & Numata., 2013). The effect of enzymes and their hydrolytic effect is specific to substrate and will only cleave a peptide chain at the recognized site (shown in the example of figure 1.2). The recognized site may vary in sequence length and so enzymes may have different effect and cleavage capabilities (Baker & Numata., 2013). Hydrolysis may also be performed with acid/alkaline solutions (Pasupuleti & Braun., 2010).

Enzyme activity is positively affected by increasing temperatures, until optimal temperature is passed. Any further increases would ultimately reduce activity or result in degradation of the enzyme itself (Lesk., 2016). Enzymes are produced in specific cells and transported or secreted from the cell to exert its effect either inside or outside the cell. This allows for the extraction, isolation and production of proteases for hydrolytic reactions (hydrolysis) by using recombinant DNA technology or the organisms natural occurring production (Azarkan et al. 2003). Enzyme technology is today applied in many production aspects. One example is in soap and detergents used in washing powders (cloths and dishes) which contain proteases and peptidases to help with the degradation of proteins (Vojcic et al. 2015).

Proteases are generally divided into two groups depending on their mode of cleavage. The enzymes may be considered as endo- or exopeptidases where the cleavage occurs at the ends (exo) of an amino acid chain or between bonds imbedded deeper into the residue-chain (endo). Endopeptidases are usually less specific towards its substrate and the catalysis of a given peptide-bond. Endopeptidases are sometimes referred to as proteinases due to their ability to fragmentize polypeptides and proteins to shorter peptides

and oligopeptides (Hauge., 2009). On the other side, exopeptidases prefer shorter protein-fragments and oligopeptides as substrates and cleaves off amino acids from the ends of the peptide chain (Hauge., 2009)).

Enzyme-products sold commercially contain a mixture of exo- and endopeptidases and nature of the cleavage of proteins into smaller peptides is reflected by the enzyme composition and the specificity of each individual enzyme. Typically, the choice of raw-product relative to the utilized enzyme(s) is of high importance. The enzyme composition dictates the perceived effect on a given raw material and the quality of the resulting product relies on the enzyme product used and its inherent capabilities (Pasupuleti & Braun., 2010).

1.3 Enzymatic protein hydrolysis

Hydrolysis which from the Greek expression means "to release by water" is a method used to cleave molecules. Protein hydrolysis is a method used to degrade proteins into smaller peptides and free amino acids (Uio., 2011). The cleavage of the peptide chain occurs when H^+ and OH^- interact with the carboxylic group of a specific amino acid, allowing for degradation of bigger peptide chains. The binding of H^+ reduce the binding affinity between its neighbouring amino acid which later results in peptide cleavage (the neighbouring amino acid may interact with the hydroxylic group) (Uio., 2011). Hydrolytic cleavage by acids and bases is non-specific and require the presence of H_2O, whereas enzymatic hydrolysis is more specific towards substrate and amino acid sequence.

Several methods of hydrolysis are industrially used for commercial production of hydrolysates; acid/alkaline hydrolysis, enzymatic hydrolysis and hydrolysis coupled with fermentation processes. However, for the purpose of this study, only enzymatic protein hydrolysis will be described in more detail.

Enzymatic hydrolysis is often performed under neutral (pH) conditions, giving a gentle reaction-process in respects to the resulting product (Pasupuleti. & Braun., 2010). Due to the gentle process the enzymatic protein hydrolysis doesn't affect the functional group of the amino acids and hence serve to maintain their biological activity which is advantageous for biotechnological and microbiological applications where essential amino acids may be a criterion (Bucci & Unlu., 2000: Pasupuleti. & Braun., 2010). The choice of enzyme relative to the product being hydrolysed is of high importance, whereas enzymes which is site-specific towards hydrophobic amino acid-chains results in higher degree of hydrolysis (DH), when applied to proteins with high hydrophobic content and vice versa (Adler-Nissen 1986).

The use of Enzymes are favourable catalysts in a hydrolysis reaction to increase the degradation efficiency of proteins. The use of enzymes rather than acids and bases is favourable due to the more specific mode of cleavage perceived by enzymes but also due to economic aspects. Furthermore, the corresponding instrumental requirements needed for inorganic catalysts are in general more extensive and economically costly. Furthermore, the use and specificity of proteolytic enzymes allows for a controlled hydrolysis and for products to be tailored to suit specification and requirements toward the content of a given hydrolysate.

Several methods of hydrolysis are industrially used for commercial production of hydrolysates; acid/alkaline hydrolysis, enzymatic hydrolysis and hydrolysis coupled with fermentation processes. However, for the purpose of this study, only enzymatic protein hydrolysis will be described in more detail. The rate of a reaction increases with the use of enzymes and catalysts, whereas enzymes are organic and catalysts inorganic molecules. Enzymes are globular molecules with high MW, whereas catalyst are small and simpler molecules. The reaction rate is typically several times faster with the use of enzyme compared to the use of an inorganic catalysts. Furthermore, enzymes are specific in their mode of cleavage, whereof inorganic catalyst are not. Inorganic catalysts are more prone to high temperature and pressure, whereas enzymes prefer mild conditions, physiological pH and temperature (Diffen., 2018)

Enzymes are added to a hydrolysis-reaction to increase the degradation efficiency of proteins which subsequently also increase the resulting yield. The choice of enzyme relative the product being hydrolysed is of high importance, as it dictates the efficiency and the resulting end-product, whereas enzymes which is site-specific towards hydrophobic amino acid-chains results in higher degree of hydrolysis (DH), when applied to proteins with high hydrophobic content and vice versa (Adler-Nissen 1986). Furthermore, the use and specificity of proteolytic enzymes allows for a controlled degree of hydrolysis (DH) and for products to be tailored to suit specification and requirements toward the content of a given hydrolysate.

A wide selection of enzymes is today commonly used for enzymatic hydrolysis (e.g. pancreatin, trypsin, pepsin, papain bromelain and bacterial and fungal proteases), whereas the enzymes may be utilized alone, as a mixture or in a sequential manner to achieve different end-products (Pasupuleti & Braun., 2010). A typical enzymatic hydrolysis-reaction performed in the laboratory consists of a desired raw-material which is diluted (1:2) with H_2O, heated to 37-65^{o}C and/or pH adjusted to 3.5-9.0 (optimized conditions for most Proteases). Protease(s) are added when the temperature is optimal for the specific enzyme-activity (typically 1% w/w concentration of enzyme to raw-material ratio). The timespan of the hydrolysis in a manufacturing relation is often restricted to 1-4 hours due to bacteriological activity (Pasupuleti & Braun., 2010).

Inactivation of enzyme is achieved by heat treatment of the sample, but also acts for microbial inactivation. After completed hydrolysis, the product is separated by centrifugation into the three main products (define to separate the aqueous-phase and lipid-phase from the solid-phase) (several other methods are available but will not be discussed). Further purification by filtration (micro, ultra or nanofiltration, reverse osmosis, column chromatography and ion exchange) can be performed (Pasupuleti & Braun., 2010). The Lipid-phase is further separated from the aqueous-phase by phase-separation (laboratory) or centrifugation (industry) and may be manufactured into a separate product (Lipids are unwanted in the finished protein-product as it may lead to further oxidation) (Silzyte et al. 2010). Not all lipids are desirable, for instance; lipids from salmon are of high quality whereas lipids from chicken is less desirable.

After a completed hydrolysis the retrieved product consists of a Solid-, aqueous- and lipid-phase, whereas high fraction of the aqueous and lipid-phase are often desired.

The solid-phase or sediments from the hydrolysis reaction consists of less degradable proteins (relative to the enzyme used) and other materials which could not be broken down (Pasupuleti & Braun., 2010). The sediment phase may be sold for production of animal-feed products. In a laboratory setting the centrifuged and filtrated aqueous-phase is sterilized by pasteurization or other forms of treatment and later freeze-dried/lyophilized (reduce the water activity), which reduce the bacterial activity. The finished product consists of a powder or paste with a high peptone content with various applications (Pasupuleti & Braun., 2010)

1.4 Proteins and peptides

Proteins are important molecules found in all living organisms and serve important roles necessary to sustain life. Proteins perform vital tasks involved in meta- and catabolism, they provide motoric movement and mechanisms for transport of molecules inn and between cells and are involved in DNA repair (Stuart et al. 1996). Proteins have several different functions whereas structural integrity, signalling pathways and catalytic functions are some examples (Alberts et al. 2014). Protein synthesis by ribosomes found in the cytosol of eukaryotic cells progress as a linear polymer-chain of amino acids bound by a condensation reaction between the carboxylic (COO-) and amine group (NH+) of the neighbouring residue (peptide-chain). In the most general sense of the definition, a protein consists of 50 or more residues, anything less is considered as polypeptide or peptide (<30 residues). Peptides are further subdivided into di, tri and oligo-peptides. Furthermore, peptides are of <35 residues in a solution is often referred to as peptone (Aspmo et al. 2005) The first and the last amino acid of the peptide-chain are often referred to as N- and C-terminal due to them maintaining their free amino-group and carboxyl-group respectively. Furthermore, amino acids have two stereoisomer conformations (L and D) whereas the amino acids incorporated in living organisms are of the L-conformation (Clark et al. 2012).

The 3D-structure of a protein is achieved after completed synthesis when the polypeptide-chain is released from the ribosome and transported. The primary structure of a protein referees to the sequence of amino acids whereas β-sheets and α-helix' are secondary structures of a protein. The tertiary structure is achieved through hydrogen-bonds between the different secondary structures (β-sheets, α-helix' and loops) (Whitaker et al. 2003; Lesk., 2016). In solution, the tertiary structure of proteins and the final 3D-structure is achieved through hydrogen-bonds, salt-bridges and Van der Waals force between the secondary structures (Clark et al. 2012; Lesk., 2016).

Proteins provide a good source for essential amino acids and through evolution different species have learned to recognize nutritional beneficial molecules like proteins. Essentially all amino acid has a distinct taste, and the flavour of multiple residues in a peptide-chain are synthesised into new combined flavours. For instance; branched amino acids (Leu, Ile and Val) contributes to malty, fruity and sweet flavours; aromatic amino acids (Phe, Tyr and Trp) contributes to floral, chemical and faecal flavours; Aspartic acid contributes to buttery flavours and the sulphuric acids (Met and Cys) contributes to flavours that resembles boiled cabbage, meat and garlic (umami) (Ardö Y., (2006)). The flavour of short peptides (di, tri and oligopeptides (3-5 residues)) with hydrophobic amino acids in key positions are often characterized as

bitter in nature. A high content of short peptides may affect the flavour of a given product in a big manner. However, in a biotechnological aspect, taste and flavour is not necessarily a good nor precise method to recognize peptide components. It is rather a useful as a tool to investigate how the given peptides are perceived by humans.

1.5 High Performance Liquid chromatography - Size Exclusion chromatography (UHPLC-SEC)

Liquid chromatography like Fast protein liquid chromatography (FPLC) and UHPLC are methods used to analyse and characterize biomolecules and small chemical compounds respectively. UHPLC is performed with high pressure in order to analyse small chemical compounds whereas FPLC is rather used to purify DNA and larger molecules.

A typical HPLC instrument setup is composed of; solvents, control module, degasser, pump, injector & autosampler, Thermostatted column compartment (TCC) and a wavelength detector. Injection of the sample is performed at specific volumes and performed in most cases automatically giving high precision across multiple samples and high reproducibility (Rønningen., 2017). The nature of the samples and the end-goal of the analysis will dictate the choice in separation-column (Biosep™-Sec-S., 2017). The stationary phase utilized in UHPLC/HPLC often consists of Silica particles densely packed (pKa 3.8-4.2) which may interact with polar ionized components and result in “tailing” (Crawford Scientific 2013).

Several methods of UHPLC/HPLC is applied in biotechnology studies; reverse-phase chromatography (RP), Aqueous/organic normal-phase chromatography (ANP/ONP), Hydrophilic interaction chromatography (HILIC), absorption chromatography and ion-exchange chromatography. The most common method is RP-chromatography using different sized Carbon-chain columns, with a non-polar stationary phase and a polar mobile-phase (methanol (CH_3OH), acetonitrile (ACN) or H_2O) (Crawford Scientific 2013). separation occurs based on retention by hydrophobic interactions with increasing detergent concentrations at a decreasing polarity in the stationary-phase (Harris., 2010). The method of UHPLC proceeds through the pre-selected arrangement of samples by injecting the designated sample-volume into the system. the pump-system controls the usage of solvents and mixtures, hence being vital for the instruments processing capabilities (Rønningen., 2017). In the use of binary solvents, two methods are utilized (high and low pressure). At low pressure the solvents need to be premixed. The use of only one solvent is often coupled with a wash-

injection evenly spaced between samples thought-out the analyses to reduce retention and to avoid reduced separation efficiency (Rønningen., 2017). The UHPLC method is quite robust and allows the separation and detection of a given samples components. UHPLC is coupled with a column which facilitates the separation of the samples components based on size or electric-charge. The separation is achieved through interactions between mobile-phase and stationary phase which creates retention, or by molecular size through a silica-based gel-filtration column (GFC) (Harris., 2010). Obliviousness towards the sample content may lead to interactions and bonding of components in the sample and the column which may destroy the column. Degassing of the samples while processed through the instrument is done to avoid gas to enter the column and interfering with the pressure or the detection (Harris., 2010). Knowledge of the sample-content is important when using UHPLC as previously mentioned. This is parts due to the column being vulnerable to salt precipitation and high pH but also in some cases due to specific components in a sample being problematic for certain columns (Rønningen., 2017; Biosep™-Sec-S., 2017).

Size Exclusion Chromatography

The separation principle of SEC is based on the molecular (Harris., 2010). The nature of the porous silica particles used in the column dictates how the separation proceeds. The silica particles and their pore size and/or depth may vary. Pores may be shallow or continue through the silica particles. Silica particles of continuing pores gives a separation based on size, with smaller molecules being retained longer than bigger sized molecules (due to the increased travel path created by the pores) (Barth.,1996). The separation will give a gradient of components of different sizes with the biggest proteins and peptides being retrieved first, followed by oligo, tri and dipeptides and lastly amino acids. A typical run progress over 35 min and followed spatially by washing-injections. Separation using columns in tandem may be used to increase the separation effectiveness and get clear and more defined peaks of each compound (Barth.,1996). The SEC method may give a margin of error in relation to the separation of peptide sizes due to the relative molecular size measured and the comparative use of a standard curve and calibration. (compounds of pre-identified molecular weight may skew the actual size of the protein when calculated using the formula $(Mw = \frac{\sum \mathrm{Ai\ x\ Mi}}{\sum \mathrm{Ai}})$ (with Molecular weight (Mw), detector signal (Ai) and molar mass (Mi)). This is due to the occurrence of passing unfolded proteins which may be perceived as larger molecules compared to the actuality.

Detection occurs after separation where the detection of measured signal is proportional to the sample-concentration. The components are measured by spectroscopy and identified by the characteristic emission of electromagnetic radiation detected from each material or component (Perkampus., 2012). UV-detection is the common choice in SEC analysis, however several other methods of detection exist.

1.6 Projective mapping analysis (Napping)

Flavour and texture are important aspects to consider in food products, especially when applied for commercial purposes. Through evolution and adaptation many species have adapted sensory organs. A taste or flavour is in some way the chemical entity of a food product of which a composition of nutrients all together collaborates to the entirety of the sensated flavours (Nelson et al. 2002). Sensory organs have been of huge importance in the persistence of individual species throughout time and history, due to the advantages of recognizing nutrients with high nutritional value simply by recognizing distinct flavours perceived in the food-product and furthermore, to distinguish foods from toxins (Nelson et al. 2002). This is also a part of the reason why multiple species prefer the same or in other cases, different food-products and nutritional content. For instance; it is shown that fat- and meat- content in pet food for dogs and cats are distinct formulas tailored to suit the preference of the respective animal. In general taste is divided into five categories; sweet, bitter, salt, sour and umami (meaning “good taste”, and is recognized as a meaty or broth-like flavour). Flavour and taste is sensed through pores in the different papilla-structures found in the oral cavity and on the tongue. Each papilla contains the taste-receptors (taste buds) which is spatially located on the tongue surface. Flavours are perceived through two different types of receptors (type 1 and type 2, corresponding to the sensation of sweet and bitter taste respectively) (Hervé., 2012). Several versions and genetic variations of each type (1 and 2) are today characterized and identified and it is the combination, arrangement and dimerization of the two individual receptors which contributes to the perceptions of different flavours (Hervé., 2012). Sensation towards taste in a combination of sensing the visual, olfactive (taste perception), sapictive (taste perception), trigeminal (hot and cold sensation) and mechanical (texture) aspects of the perceived food (Hervé., 2012).

Many amino acids have a sweet or delicious (umami) sensation to humans as well as to other animals and is likely due to the L-amino acids being the building-blocks of proteins and other relevant biological molecules, making L-amino acids essential to all known life (Nelson et al. 2002; Temussi P.A., 2011). The essential amino acids and peptides are perceivably recognized by two G-protein-coupled receptors which

is tuned to recognize and respond to the presence of L-amino acids (most of the 20 standard amino acids), giving a corresponding sensation to taste of the given amino acid, peptide or protein (although not tuned towards the D-enantiomers) (Nelson et al. 2002). The difference in taste of the two conformations of a given amino acid may not deviate much. For instance, the L and D form of serine are both perceived as sweet with the L-enantiomer perhaps tasting more umami (Kawai et al. 2012).

Napping

Analysing the perceived flavour of a given product may be affected by several biases, whereas some examples may be how the product was perceived by others or having detailed background information about the product in question. When performing a Napping- analysis it is important to reduce the factors of biases to a minimum. Napping® is a rapid sensory profiling technique using a 2D-statistical model to estimate the similarity or differences in a sample set. Whereas, each dimension helps to explain a given percentage of the total similarity across multiple samples based on the total data input (sample size and repeats will affect significance in data and subsequently the similarity (%) each of the dimension helps to explain) (Pagès., 2003). In practice this means that two samples which is located closely in space on a 2D-plot would exert more similarities compared to samples spatially separated. The choice of fundamental rules as a basis for a napping analysis will further affect the outcome greatly. This would entail assessment of a framework or guidelines/words chosen to explain the perceived taste. The significance (%) of each dimension would reflect the accuracy and precision of the results and their perceived taste. Napping analysis may be conducted by untrained, semi-trained and expert-panels and the maximum sample size should reflect their experience. The number of samples should therefore not exceed 15 if using an untrained panel or 20-30 when using semi- or expert panels (Pagès.,2003). Data is collected by tasting samples with different content, at the same temperature and isolated from environmental disturbance (several steps may be applied to reduce sample biases although not mentioned here in great detail). The data is collected by measuring the distance in placement of the samples according to similarity or difference with a discriminatory word explaining the samples attributes (appearance, aroma flavour or texture) (Pagès.,2003). Samples are randomized by identification. However, samples of the same product or processing-method are often analysed together.

After all considerations are made towards the experiment-setup and the potential biases, the resulting graph-plots and discriminating attributes will help elucidate the similarities and differences of the tested samples and how they are perceived.

2 Aim of the study

In the food-industry, economical and repeatable processes for food-production is highly sought out. New and improved ways of reducing by-products and increasing the percentage of utilized raw material at an affordable price is important aspects to take into consideration in an industrial setting.

There are several ways to further utilize raw materials inn and from the food industry today. Implementation of co-products from the food production as ingredients in animal-feed or as other products like glue and concrete are among the common practises. Improving the extraction process would mean an increase of the total raw-product that proceeds towards human consumption and commercial purposes.

Enzymatic protein hydrolysis is a commercially available method to extract proteins and peptides from raw-materials where the biological activity of the extracted residues is maintained. However, producing hydrolysates alters the amounts of peptides and free amino acids relative to the bigger proteins and in turn the flavour-profile of the hydrolysate product.

Di-, tri-, and shorter oligo-peptides are thought to affect taste in a negative manner when hydrophobic amino acids are found in key positions. In this study nine different peptide compositions were produced by using three different enzymes on three different raw materials using enzymatic protein hydrolysis. LAB are auxotrophic for a variation of amino acids, e.g. some hydrophobic amino acids like Valine and Methionine are often essential for growth. By implementing different LAB to the hydrolysates, it is hypothesized that the di, tri- and short oligo-peptides will be taken up differently by the LAB peptide-transporter systems in addition to other factors like production of lactic acid and by products like CO_2, which may affect the flavour-profiles in different manners.

This thesis will try to elucidate the effect on taste and flavour by implementation of LAB in protein hydrolysate from different animal raw-material, processed by different enzymes. the best performing LAB strains was used to ferment the hydrolysates for SEC- and Napping analysis. Obtaining information about the peptide size fractions and the changes of each hydrolysate, as well as a projective mapping of the perceived flavours for two LAB strains, we expect to be able to more accurately explain what factors affects the perception of taste the most. Answering these questions will hopefully elucidate the importance of key aspects in relation to the use of enzymatic hydrolysis of co-products and the implementation of LAB for later commercial purposes.

3 Materials

Table 3.1 *Bacterial strains retrieved from Nofima Ås stock collection*

Sample ID	Strain ID	Other designation / Culture collection/ reference
MF 9	*Lactobacillus plantarum*	NC8 / CCUG 61730 / Axelsson et al. (2012)
MF 110	*Lactobacillus reuteri*	DSM 20016 (Type strain)
MF 150	*Lactobacillus amylovorus*	NRRL B-4542 / Gold et al. (1992)
MF 1127	*Lactobacillus sakei*	LS25 / McLeod et al. (2013)
MF 1964	*Lactobacillus brevis*	DSM 20054 (Type strain)
MF 1965	*Lactobacillus alimentarius*	DSM 20249 (Type strain)
MF 1974	*Leuconstoc paramesenteroides*	DSM 20193 (Type strain)
MF 1978	*Lactobacillus dextrinicus*	DSM 20335 (Type strain)
MF 1979	*Pediococcus pentosaceus*	DSM 20336 (Type strain)
MF 1980	*Lactobacillus helveticus*	ATCC 15009 (Type strain)
MF 2033	*Lactobacillus delbrueckii*	ATCC 12315 (Type strain)
MF 2035	*Lactobacillus casei*	ATCC 393 (Type strain)
MF 2357	*Lactobacillus salivarius*	DSM 20555 (Type strain)
MF2576	*Lactobacillus coryniformis*	DSM 20001 (Type strain)
MF2900	*Lactobacillus paracacei*	DSM 5622 (Type strain)
MF 2983	*Lactobacillus sanfransiscensis*	Classification uncertain**
MF 3579	*Lactococcus lactis*	MG1363 / Gasson (1983)
MF 5214	*Carnobacterium divergens*	Lab01 / Ringø et al. (2001
MF 02996	*Leuconostoc mesenteroides*	DSM 20343 (Type strain)
MF 6580	*Pediococcus clausenii*	DSM 14800 (Type strain)
MF 6581	*Weissella confusa*	DSM 20194

Table 3.2 *Bacterial strains retrieved from Nofima Bergen. Identification are based on 400-800bp of the V4-V5 region of the 16S rRNA gene. Identification in nBLAST (NCBI) above 98% was considered as potential hit when using parameters; Excluding models (XM/XP) & uncultured/environmental sample sequences with limits to sequences from type material and mega-blast with scoring parameters (match/mismatch) 4/-5, Existence:12 and extension 8. Strain identification was not conclusive (due to the size of the analysed fragment 200-500bp) but rather in approximation towards a group of species within a genus.*

Sample ID	Strain ID	% Basepair match (bp)	Isolated from
IS 61	Carnobacterium divergens Carnobacterium inhibens subsp. gilichinskyi	(100%) 398/398bp (99%) 396/398bp	*Potentilla crantzii*
IS 64	L. paracasei L. casei	(99%) 600/601bp (99%) 599/601bp	*Silene Suecica*
IS 79	L. paracasei L. casei	(99%) 546/548bp (99%) 545/548bp	*Fucales (fucoids)*
IS 93	*L. plantarum* *L. paraplantarum* *L. pentosus*	(98%) 546/556bp	Canned/conserved summer Capelin *(Mallotus villosus)*
IS 118.1	Not identified	--	Summer Capelin *(Mallotus villosus)*
IS 118.2	Not identified	--	Summer Capelin *(Mallotus villosus)*
IS 118.3	Not identified	--	Summer Capelin *(Mallotus villosus)*
IS 118.4	*L. fuchuensis* *L. fuchuensis* *L. sakei*	(100%) 394/394bp (100%) 394/394bp 100%) 394/394bp	Isolated from: Summer Capelin *(Mallotus villosus)*
IS 118.b	*L. paracasei* *L. casei*	(99%) 749/753bp (98%) 739/753bp	Summer Capelin *(Mallotus villosus)*
IS 145	*L. paracasei* *L.casei*	(99%) 673/677bp (99%) 666/672bp	Summer Capelin *(Mallotus villosus)*

IS 185	*L. paracasei* *L.casei*	(99%)761/765bp (98%) 751/765bp	Summer Capelin *(Mallotus villosus)*
IS 196.1	*L. sakei subsp. sakei*	(99%) 760/761bp	Summer Capelin *(Mallotus villosus)*
IS 196.2	*L. sakei subsp. sakei*	(99%) 553/553bp	Isolated from: Summer Capelin *(Mallotus villosus)*
IS 196.3	*L. sakei subsp. sakei*	(99%) 390/390bp	Summer Capelin *(Mallotus villosus)*
IS 196.4	*L. sakei subsp. sakei*	(99%) 760/761bp	Summer Capelin *(Mallotus villosus)*
IS 200	*Carnobacterium maltaromaticum* *Carnobacterium inhibens subsp gilichinskyi*	(100%) 380/380bp (99%) 378/380bp	*Gentiana sino-ornata,*
IS 204	*L. paracasei*	(99%) 395/396bp	Winter Capelin *(Mallotus villosus)*
IS 269	*Carnobacterium inhibens subsp. Gilichinskyi Carnobacterium Maltaromaticum*	(99%) 394/396bp (100%) 396/396bp	Winter Capelin *(Mallotus villosus)*
IS 352	*L. paracasei* *L. casei*	(99%) 396/399bp (99%) 396/399bp	*Silene suecoca*
IS 357	*L. pentosus* *L. plantarum* *L. paraplantarum*	(99%) 758/760bp (99%) 758/760bp (99%) 756/760bp	Alchemilla alpina
IS 361	*L. paracasei* *L. casei* *L. zeae*	(99%) 746/749bp (98%) 736/749bp (98%) 735/749bp	*Anthyllis vulneraria*
IS 366	*L. plantarum* *L. pentosus* *L. paraplantarum*	(100%) 403/403bp (100%) 403/403bp (100%) 403/403bp	*Veronica fruticans*
IS 371	*L. pentosus* *L. plantarum*	(99%) 689/690 (99%) 688/690	*Ranunculus glacialis*

	L.paraplantarum *L. plajomi* *L. xianfangensis*	(99%) 687/690 (99%) 685/690 (99%) 683/690	
IS 376	*L. paracasei* *L. casei*	(99%) 748/751bp (98%) 738/751bp	*Dryas octopetala*
IS 380	*L. plantarum* *L. Paraplantarum* *L. pentosus* *L. fabifermentans*	(100%) 396/396bp	*Taraxacum officinale*
IS 384	*L. plantarum* *L. pentosus*	(99%) 750/751bp (99%) 750/751bp	*Pinguicula vulgaris*

Table 3.3 *Equipment and instruments*

Equipment and instruments	Details	Supplier	Procedure (method)
3730xl DNA analyzer	Genetic analyzer	Applied biosystems HITACHI	Method 4.7
Accu-jet® pro pipette controller	pipette	BRAND®	Method 4.1
ANRITSU HD-1250 K	Thermometer	ANRITSU METER CO. LTD	Method 4.1
Avanti™J-301	centrifuge	Beckman Coulter Inc.	Method 4.1-4.4.3
Bergman Mettler MX5	microbalance scale	Mettler Toledo	Method 4.4.1-4.4.4
Beta 1-8 LDplus-system	Freeze-dryer	Martin Christ Freeze Dryers	Method 4.2
Bioscreen-C	Real-time bacterial Growth analyser	Labsystems Bioscreen-C	Method 4.8
BIOSEP™-SEC-S (00H-2145-K0) 5µm SEC-s2000 145 Å LC column 300 x 7.8 mm	SEC/GFC column	BioSep™	Method 4.10

Certoclav-Trich-Autoclave	Certoclav sterilizer. Gambatt (CV-EL 12L). A-4050	CertoClav GmbH	Method 4.4.1
DeltaRange® AG 204	scale	Mettler Toledo	Method 4.1
DeltaRange® PG5002-S	scale	Mettler Toledo	Method 4.1, 4.3
Dionex UltiMate 3000 UHPLC⁺ focused system	Pump	Thermo Fisher Scientific	Method 4.10
Dionex UltiMate 3000 UHPLC⁺ focused system	Autosampler	Thermo Fisher Scientific	Method 4.10
Dionex UltiMate 3000 UHPLC⁺ focused system	Wavelength detector	Thermo Fisher Scientific	Method 4.10
Electrophoresis gel box	Electrophoresis equipment	Bio Rad	Method 4.7.1
Gel Doc ™ EZ Imager	Electrophoresis equipment	Bio Rad	Method 4.7.1
Heraeus Megafuge 8	centrifuge	Thermo scientific	Method 4.7
Heraeus Multifuge 4KR Centrifuge	centrifuge	Thermo Electron Corporation	Method 4.1
HERAguard ECO	Sterile-bench	Thermos Scientific	Method 4.4-4.4.4
IBS INTEGRA BIOSIENCES Flameboy	Sterilizing tool	VWR	Method 4.4-4.4.4
JULABO 5 heating Circulator	Water-heater	JULABO	Method 4.1
KERVEL KPH heat plate	Heat-plate	KERVEL KPH	Method 4.1
Menumaster® commercial DEC18E2	microwave	Menumaster®	Method 4.1
Mettler PE 360	scale	Mettler Toledo	Method 4.4-4.4.4
Millievac-Mini XF54 230 50	Vacuum pump	Merck KGaA	Method 4.4-4.4.4
pHenomenal™ pH 1000 H	pH-meter	VWR	Method 4.10.1
PHM210 Standard pH Meter	pH-meter	MeterLab®	Method 4.4-4.4.4
PowerPac™ Basic	Electrophoresis equipment	Bio Rad	Method 4.7.1

Radleys Overhead stirrer RS50	Overhead stirrer (300rpm)	Radleys	Method 4.1
Radleys PFTE Turbine propeller	Propel	Radleys	Method 4.1
Reactor Ready ™ Lab Reactor (2000mL)	Reactor-core	Radleys	Method 4.1
Reichert AR200 Digital Refractometer	Refractometer	BIO LAB	Method 4.1
SHARP R-5000E	microwave oven	OSTA.EE	Method 4.7-4.7.1
SI-SLEEVE (Art. No. 60043806)	Rubber-tubing	SEEBERGER®300	Method 4.1
SPECTROstar^{nano}	Protein assay tool	BMG LABTECH	Method 4.3
Squibb separatory funnel	Phase-separation tool	Thermo Scientific	Method 4.1
Stepper™ Adjustable repeater pipette (411 Socorex Swiss)	pipette	Socorex Swiss	General use
Ultrospec 3000	Spectrometer	Pharmacia Biotech	Method 4.8
Veriti 96 well thermal cycler	PCR-equipment	Applied Biosystems	Method 4.7

- Thermo Fisher, Nunc and Merck has merged, and so the use of the brands and the suppliers are may not be accurate in this relation.

Table 3.4 Disposable and Miscellaneous equipment

Disposable and miscellaneous Equipment:	Details	Supplier
CLINGFILM	Hydrolysis equipment	TORO®
CO_2 Gen ™	2.5L atmosphere generation systems	Thermo Scientific
Corning® Falcon test tube with snap cap	14 ml polystyrene round bottom	Sigma-Aldrich, Merck
CryoTube ™ Vials	2,5 ml	Fisher Scientific
Disposable Cuvettes	Spectrometer	Brand®
Ecostep™ bioproof™ syringes	500-5000µl	Socorex Swiss
Eppendorf tubes	2.5 ml	Eppendorf

Facial tissue/extra soft 20x195cm 2 ply	General use	VWR North America
Flacon Tubes	15- and 50 ml	Fisher Scientific
Inoculating loops	2-, 10µl	VWR
Luer Lock FINE-JECT® 0.8 x 55 mm TW/LB needles	2,5-, 5ml syringe and needle	BD Plastipak™ HENKE SASS WOLF
M 68-50 clear APET DELIBEGER LID 50-100CL REKT M68	Hydrolysis equipment Tupperware box Tupperware lid	Faerch Plast MASKE AS
MicroAmp Optical 96 well reactionplate	PCR-plate	Applied biosystem
Millex®-GS MF-Milliepore™	Sterile filter units with 0,22µm MCE membrane	Merck Millipore Ltd.
Millex®-HV Milliepore ™ filter	hydrophilic PVDF 45µm	Durapore® Merck KGaA
Multiply® polypropylene 96-well (PP) PCR plate	PCR-plate	SARSTEDT AG & Co
Nalgene ™ Rapid-Flow ™ filter	Sterile disposable PVDF 0,2µm filter units with PES-membrane (150- and 500ml)	Thermo Scientific ™
Nitrile Gloves	General use	VWR North America
Nunc® Microwell™ 96 well Polystyrene plates	PCR-plate	Nunc® Merck
Pipette tips	10-, 20-, 100-, 300-, 1000 and 1200µl barrier tips	SARSTEDT AG & Co Thermo Fisher Scientific
Serological pipette	2-,10-, 25 ml	Fisher Scientific
Wiping paper Plus Combi	General use	TORK®
X100 Bioscreen-C sterile honeycomb plate	Bioscreen 100 well plate	Thermo Scientific
Xylem- TEP 10 Technical buffer solutions 108704	pH 7.00 and 4.01 (used for calibration)	WTW

- Thermo Fisher and Merck has merged, as well as products from Thermo Fisher Scientific, Nunc and Millipore, and so in this relation the use of the brands and suppliers are inconsistently used depending on product.

Table 3.5 Software and programs

Software and programs	details	Supplier	Procedure
BMG LABTECH data analysis software	2015	BMG LABSTECH	Method 4.3
Excel	2016	Microsoft	General use
Image Lab™	V 4.1	Bio Rad	Method 4.7.1
Minor Variant Finder (MVF)	V 1.0, 2015	Applied Biosystems by Thermo Fisher Scientific	Method 4.7
nBLAST	--	NCBI	Method 4.7
Norden Lab Professional	Bioscreen-C edition	Norden Logic	Method 4.8
PSS WinGPC® UniChrom	V 8.00 build 994	Polymer Standards service GmbH	Method 4.13

Table 3.6 Reagents and Solutions

Reagents and Solutions	Details	Supplier
Acetonitrile (ACN)	CH_3CN	Sigma Aldrich
Adenine	$C_5H_5N_5$	Sigma Aldrich
Ampicillin	100mg/ml	Sigma Aldrich
Big Dye buffer 5x	Reagents for 16S rRNA sequencing	Thermo Fisher scientific
Big Dye v 1.1	Reagents for 16S rRNA sequencing	Thermo Fisher scientific
Bio-Rad DC™ Protein Assay Reagent A and B	Bio-Rad DC™ Protein Assay kit	Bio-Rad
Biotin	$C_{10}H_{16}N_2O_3S$	Sigma chemical company
BSA (bovine serum albumin)	Std.-curve (protein assay)	Thermo Fisher Scientific
Dihydrate sodium citrate*	Substitute for sodium citrate	Merck
Dipotassium hydrogen phosphate	K_2HPO_4	Merck
Erythromycin	10mg/ml	Sigma Aldrich

Ethanol (~70%)	CH_3CH_2OH, Diluted with dH_2O	--
ExoSAP-IT ™	Reagents for 16S rRNA sequencing	Thermo Fisher scientific
Folic acid	$C_{19}H_{19}N_7O_6$	Sigma Aldrich
GelRed nucleic acid stain 10,000X in water	Gel-dye	Sigma Aldrich
Glucose – D (+)-monohydrate*	1M sterile stock solution glucose (500ml) = 108,9926g dissolved into 500ml end volume)	Merck
Glycerol (>99%)	$HOCH_2CH(OH)CH_2OH$	--
Heptahydrate ferrous sulphate*	Substitute for Ferrous sulphate	Merck
Magnesium sulphate	$MgSO_4$	Merck
Manganese (II) chloride	$MnCl_2$	Oxoid
Molecular Biology Agarose	--	Oxoid
MOPS	$C_7H_{14}NNaO_4S$ (Sodium salt)	Oxoid
Nicotinic acid (niacin)	$C_6H_6NO_2$	Sigma Aldrich
Nucleic acid free water	--	--
Orange G	Dye (electrophoresis):	Sigma Aldrich
Pantothenic acid	$HOCH_2C(CH_3)_2CH(OH)CONHCH_2CH_2CO_2$	Sigma Aldrich
phosphate buffered saline (1X PBS)	NaCl : 8 g/l KCl : 0.2 g/l Na_2HPO_4 : 1.42 g/l KH_2PO_4 : 0.24 g/l	Oxoid
Platinum ™ Hot Start PCR 2X Master Mix	Reagents for 16S rRNA sequencing	Thermo fisher Invitrogen ™
Polysorbate 80	Tween 80	Sigma Aldrich
Pyridoxal HCl	$C_8H_9NO_3 \bullet HCl$	Sigma Aldrich
Riboflavin (B2)	$C_{17}H_{20}N_4O_6$	Sigma Aldrich
SAM solution	Reagents for 16S rRNA sequencing	Thermo Fisher scientific
Sodium chloride	NaCl	Merck

Sodium dihydrogen phosphate monohydrate	NaH_2PO_4	Sigma Aldrich
Thiamine hydrochloride	$HC_{12}H_{17}ON_4SCl_2$	Sigma Aldrich
Trifluoracetic acid (TFA)	CF_3COOH	Sigma Aldrich
Tris/Borate/EDTA (5X TBE-buffer)	(1.1M Tris; 900mM Borate; 25mM EDTA; pH 8.3)	Sigma Aldrich
Uracil	$C_4H_4N_2O_2$	Sigma Aldrich
Xanthine	$C_5H_4N_4O_2$	Sigma Aldrich
X-terminator Solution	Reagents for 16S rRNA sequencing	Thermo Fisher scientific

- Sigma-Aldrich and Merck has merged, as well as products from Thermo Fisher scientific and so in this relation the use of the brands and suppliers are inconsistently used depending on product.

* Hydrated version of the reagent was used and so the volume is adjusted accordingly based on MW of the additional H_2O.

Table 3.7 Enzymes

Enzymes (Proteases)	Origin	Peptidases (type)	Supplier
Alcalase 2.4 L (A)	*Bacillus licheniformis*	Subtilisin (Endo) Glutamyl endopeptidase (Endo) Extracellular neutral metallo (Endo) Aminopeptidase (Exo)	Novozymes
Corolase 2TS (C)	*Thermoproteolyticus*	Thermolysin (Exo) Extracellular neutral metallo (Endo)	AB Enzyme
Flavourzyme 1000L (F)	*Aspergillus oryzae*	Leucine Aminopeptidase (Exo) Dipeptidyl-peptidase (Exo) Neutral Protease (Endo) Alkaline protease (Endo) Carboxypeptidase (Exo) Aspartic Protease (Endo)	Novozymes

Table 3.8 Raw by-products

Raw-Product (animal by product)	Origin	Supplier
Mechanically deboned chicken residue	Poultry (MDCR)	*Nortura Hærland (Norway)*
Cod (back-bone and skins)	Cod (BC)	*Sjømat AS (Norway)*
Whey protein (α-, β-, k- Casein)	Casein dairy product (WP)	*Barentz ApS (Danmark)*

Table 3.9 Primers and DNA ladder (16S rRNA sequencing): Shows the *primers used for sequencing by 16S rRNA, by the "Microwave-method" using protocol given by Invitrogen ™ Platinum ™, Hot Start PCR. Sequencing was performed twice. One trial using both forward and reverse primers from Nadkarni et al (2002), and a second trial with the forward primer replaced with the forward primer described by Lane D.J (1991) (performed on samples with poor Identification at first sequencing).*

Primers and ladders	Details	Supplier/Reference
Forward Primer	5«-TCCTACGGGAGGCAGCAGT-3«	Nadkarni et al. (2002)
Reverse Primer	5«-GGACTACCAGGGTATCTAATCCTGTT-3«	Nadkarni et al. (2002)
Forward Primer	5«-AGAGTTTGATCMTGGCTCAG-3«	Lane D J. (1991)
DNA molecular Weight Marker VI	version 07 (0.15-2.1 kbp), Ref: 11062590001	Roche

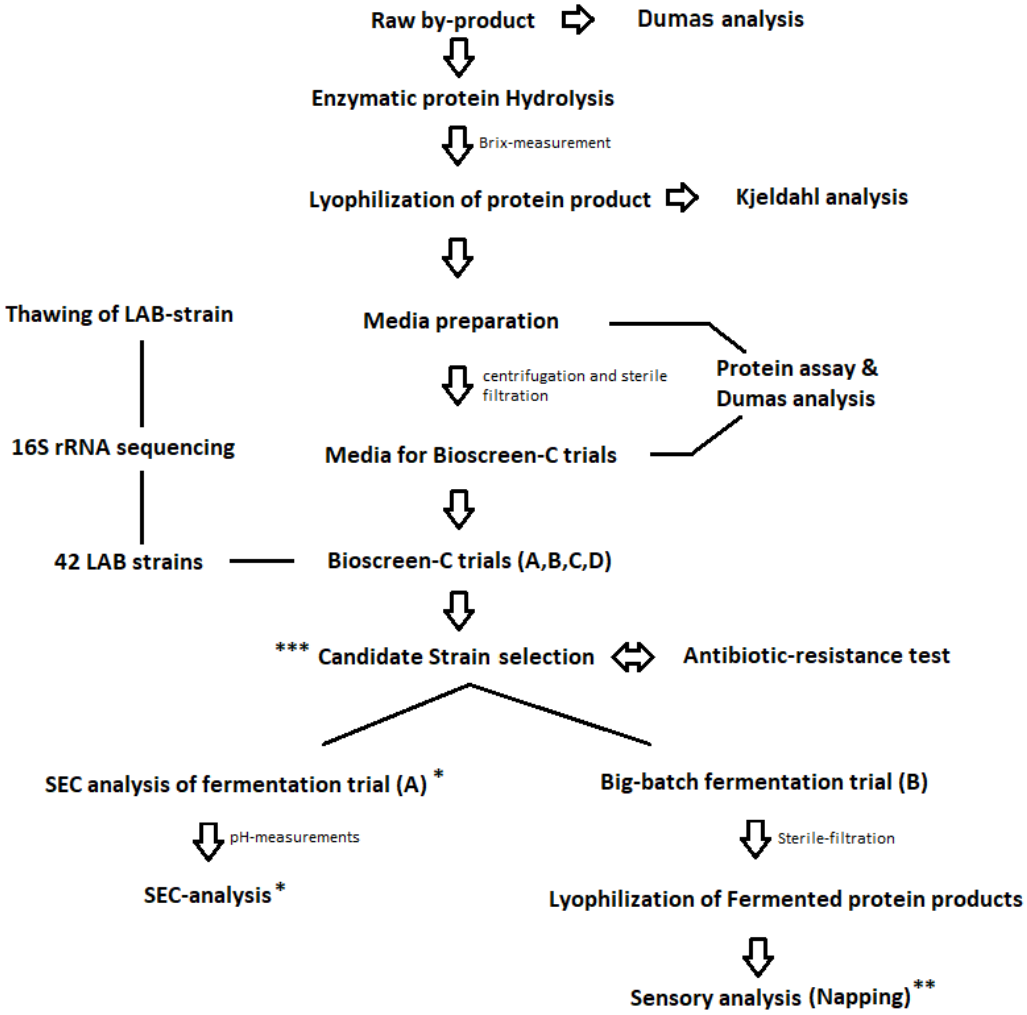
Table 3.10 Commercial Growth medium (agar and broth)

Description	Details	Supplier
MRS	Broth and agar	Oxoid
BHI	Broth and agar	Oxoid
Bacteriological peptone	Complex nitrogen source (MAPT)	Oxoid
Yeast extract	Complex nitrogen source (MAPT)	Oxoid

4 Methods

The figure 4.0 below shows the setup of the performed experiments in this study. The organisation of the different experimental-parts is in thread with the actual progression throughout this study. Detailed descriptions of each part are found in the corresponding methods.

Figure 4.0: Shows the experimental-setup and progression-order of the performed experiments.



* SEC: Size Exclusion Chromatography

** Napping (projective mapping): method for screening of taste and flavours.

*** candidate strains: the top six LAB strains showing good growth performance relative to the nine Screening modified All-Purpose tween media (SMAPT)

4.1 Enzymatic protein hydrolysis of animal co-product

Most LAB needs free amino acids, dipeptides and oligopeptides of 4-35 residues for its biosynthesis and many are auxotrophic for several different amino acids (Chopin A., 1993; Mills O.E. & Thomas T.D., 1981). Enzymatic protein hydrolysis was used to break down animal by-product of chicken, cod and whey to produce protein-powders containing the necessary nutrients like peptides and amino acids (complex nitrogen source). The hydrolysis experiments were continued until 18 protein hydrolysates was produced (two parallels of each enzyme and animal by-product).

Each hydrolysis was performed on 500g by-product with a protein content of 15-20%, added to 1000ml dH_2O, with an enzyme concentration of 1% w/w. three different raw-materials was hydrolysed; mechanical deboned chicken residue (MDCR), Cod backbone and skins (BC), and whey protein (WP) using three different enzymes; Alcalase (A), Corolase 2TS (C) and Flavourzyme (F). Both by-products of mechanically MDCR and BC had presumably 15-20% protein content, whereas the whey-protein (WP) contained 80% proteins (casein-protein powder mix). To be able to use a WP product with a similar protein content as that of MDCR and BC, 125g of whey powder was mixed with 375 ml dH_2O resulting in 500g of product with ~20% protein content. A temperature of 50^{o}C were applied to the product in each enzymatic hydrolysis in order to get a more effective enzymatic process, increase the fluidity of the lipid-phase, but also to reduce growth of any potential bacteria's already in the product. A continued stirring of 300rpm was applied to the product to allow more enzyme to facilitate peptide chain cleavage. All samples were processed in accordance with normal practise of food production with food safety maintained. All equipment was autoclaved before use, and glass-equipment and other multi-use equipment was cleaned with 70% EtOH before use.

Procedure:

- The food packaging from the by-product was cleaned with 70% EtOH before opened.
- 500g of product were added to the hydrolysis reactor together with 990ml dH_2O under stirring at 300 rpm and the content heated by a continuous feed of hot water (51,4 ^{o}C) in the outer shell of the reactor.
- When the reaction content had reached 50^{o}C, the enzyme was added to the reactor. 5ml of dH_2O was mixed with the enzyme, and an addition 5ml dH_2O was used to rinse the enzyme beaker and applied to the reactor.

- Roughly 15ml Samples (for FT-IR, SEC and brix) was extracted (using a serological pipette tip with 30 cm rubber-tube extension) from the core with intervals (minutes) of; 0.5, 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50, 60 and 80.
- Samples was heat-treated in a microwave at full power for 8-15 seconds, until bubbles or disturbance in the surface of the sample was observed. Microwaved samples were then placed 15 min on water-bath at 95-100^{o}C.
- After a complete hydrolysis, the entire content of the reactor was heat-treated by microwave for 2 mins, followed by water-bath at 95-100^{o}C for 15min.
- The weight of cooled samples was measured, and samples together with the end-product was centrifuged, at 4400rpm at 25^{o}C for 15 min.
- After centrifugation, FT-IR and SEC samples was taken from the samples extracted throughout the hydrolysis. A syringe was used to extract only the aqueous-phase of each sample and then filtrated using a 0,45µm Millipore-HV filter.
- 0.5-1 ml of the clear aqueous-phase from each sample was extracted into one Eppendorf tube and one SEC vial. Vials was sealed off using a clamp with caps.
- Brix was measured using 100 µl from each sample applied on a calibrated brix-meter (calibration was done using dH_2O) (dH_2O was used to clean the lens between measurements).
- the remaining hydrolysate end-product produced was then separated from the solids by gently pouring the liquid phase from each centrifuge-container into a squib-separatory funnel, leaving the solids behind (weight measured).
- the aqueous- and lipid-phase was separated in the squib-separatory funnel, and the weight of each phase was measured.
- A sample of 45 ml solids and 14 ml lipid phase was taken for storage at -40^{o}C.
- The aqueous phase was vacuum-filtrated using Seitz® T-Series Depth Filter; T2600 Sheets 400x400 (PALL Corporations).
- After filtration, the aqueous-phase was measured, and two 45 ml samples was taken for storage. The remaining content was measured and split into four Tupperware boxes for storage.
- FT-IR-, SEC-, solid-, lipid-, aqueous-samples and finished products was stored at -40^{o}C. (FT-IR and SEC analysis from the hydrolysis process is not presented in this study, nor used in any downstream analysis').

4.2 Lyophilization of hydrolysis end-products

Lyophilization also known as freeze-drying is a technique in which water is removed from a sample while still being in a frozen state. This is achieved by applying vacuum in a cooled system, allowing H_2O in solid state to go directly to a gaseous-state, which then is removed from the system. This method helps to prevent samples from degradation or peptides to interact while the samples are drying. Lyophilization was utilized to removed H_2O to make dry samples of each protein hydrolysate. The duplicates were mixed together by crushing the hydrolysates into fine powder. Hydrolysates powders was later tested for protein content by Dumas method and Kjeldahl-analysis (see under 4.3) and used as protein source in broth media for bacterial growth.

Procedure:

- Each Tupperware box with hydrolysates was perforated to allow gas exchange.
- replicate series of each hydrolysis product (Tupperware boxes) was placed at -40^{o}C, packed into Styrofoam containers
- The samples were then placed in a Beta 1-8 LDplus-system (Martin Christ Freeze Dryers), precooled to -50^{o}C and lyophilization was initialized when the vacuum was applied.
- Samples was left to freeze dry for 14 days.
- Dried samples were deemed to be completely dried when the product was crisp and porous.
- Dried samples with the same Id (duplicates) was pooled together and homogenized, using a mortar

4.3 Dumas method, Kjeldahl-analysis and Lowry Protein Assay

Kjeldahl analysis (protein content)

To estimate the nitrogen content of the hydrolysates produced (see method 4.1), roughly 4 grams from each of the protein hydrolysates was sent for Kjeldahl-analysis at BioLab, Nofima Bergen (results validated and approved by Laboratory leader Arne Brodin Nofima Bergen).

Total Nitrogen Analysis (Dumas method)

The total nitrogen content was estimated using the Dumas method in order to validate that no losses of protein occurred though out the centrifugation and filtration process (performed in method 4.4.2). This was done for each media sample with except BC-samples and the actual analysis was performed by Irene E. Eriksen Dahl at the institute of Jordfag, dept. Soil sciences at the Norwegian University of life sciences (NMBU), using a Leco Truspec analyser performed on SMAPT media and not on the hydrolysate powder directly, which was performed separately later. The Dumas method was first developed by Bremmer & Mulvaney, 1982. The method estimates total nitrogen content by the reduction of NOx to N2 through interactions with copper. The concentration of dinitrogen-gas is then measured by thermo-conductivity which gives a total percentage in nitrogen in each sample. On request from Nofima Ås. To estimate the crude protein content from the Dumas method, the results were converted using the Kjeldahl conversion factor of 6.25 for MDCR and BC and 6.38 for WP series.

$$Protein\ content\ RM(g) = Tot\ RM(g) \times \%TKN\ of\ RM \quad (I)$$

$$Protein\ yield\ (lyophilized\ samples)(g) = Tot\ Lyophilized\ sample\ (g)\ \times \%TKN\ of\ sample. \quad (II)$$

$$Protein\ yield\ (\%) = \frac{Protein\ yield\ (lyophilezed\ sample)}{Protein\ content\ RM\ (g)} \times 100 \quad (III)$$

Bio-Rad protein-Assay Microplate protocol (Lowry protein assay)

All SMAPT-media was centrifuged and sterile-filtrated to produce "clear" media usable for Bioscreen-C trails. Due to the centrifugation and sterilization by filtration steps, some concerns to whether loss of protein content occurred or not was raised. The Bio-Rad protein assay method was used to evaluate whether a loss of proteins occurred or not. This was done by measuring the protein content of a sample before centrifugation and filtration and compared it to the same sample of the finished media at 750nm. For each of the nine SMAPT media, three dilutions were made in duplicate series (10^{-1}, 10^{-2} and 10^{-3}).

Method:

- Two series of seven standard solutions of Bovine Serum albumin (BSA) with known concentration was made; 125, 250, 500, 750, 1000, 1500, 2000 µg/ml.
- 5µl of each standard series dilution was pipetted into wells in a 96-well microplate.
- Each dilution sample was mixed with 25 µl of reagent A
- 200 µl of reagent B was then applied to each well, and the content was gently homogenized by agitation (bubbles removed)
- After 15 minutes, the plate was read by a SPECTROstar^{nano}.

Preparations of samples prior to analysis:

- Samples of each SMAPT-media (before and after centrifugation and filtration. See procedure 4.4.2, last step) was measured to ~20g and dried at 60 ^{o}C for 48 hours.
- The percentage weight-loss was estimated by subtracting weight remaining water and the sample-loss was estimated by comparing filtrated samples to the non-filtrated samples after drying (samples was normalized to the same amount, e.g. measured weight divided on the total weight and multiplied by 20)
- Samples was crushed into a fine powder and homogenized.
- Samples was sent to NMBU where 2x 200mg of each sample was combusted in a Leco Truspec analyzer (duplicate series).

In order to estimate the yield from the protein hydrolysis, samples of the raw-material and the hydrolysed products was sent for Dumas analysis. The analysis was performed on each of the nine protein hydrolysates (MDCR_A/C/F, BC_A/C/F, WP_A/C/F) after freeze-drying as well as to the raw-materials (MDCR, BC and WP).

4.4 Preparation of Basis- and Screening media

Most, if not all LAB requires nutrient-rich media to support growth. However different strains of LAB may prefer certain growth media over others. Today several media recipes are available and known to support growth of LAB, such as; MRS (Oxoid), All Purpose Tween (APT) (Evans & Niven., 1951) and Brain Heart Infusion (BHI) (Oxoid). Modified All Purpose Tween (MAPT), is a modified version of APT and used as basis medium in this study. Protein-hydrolysates (see method 4.1) was used as a substitution for complex nitrogen-source in MAPT-media to produce additionally nine modified media called Screening Modified All Purpose Tween (SMAPT). To promote growth further a vitamin-solution (see method 4.4.4) was added, with components known to promote growth (Aspmo et al. 2005) reagents that was hydrated are marked with "*" and the volume were adjusted accordingly.

4.4.1 Modified All Purpose Tween

The MAPT medium was used as basis media and contain (g L^{-1}): *glucose 25mM (4.954g glucose or 5.5g D-glucose monohydrate), bacteriological peptone (oxoid) (12.5), yeast extract (Oxoid) (7.5), *sodium citrate (5.00) (dihydrate sodium citrate 5.61), sodium chloride (5.0), dipotassium hydrogen phosphate (1.0), manganese (II) chloride (0.14), magnesium sulfate (0.8), *ferrous sulfate (0.04) (heptahydrate ferrous sulfate 0.073), MOPS sodium salt (23.1), thiamine hydrochloride (0.001). Reagents marked with "*" that was hydrated, and therefore amount (g) is adapted accordingly.

Procedure for 1L media:

- All reagents were dissolved in 0.8 L dH_2O and pH-adjusted to 7.00 using HCl- and NaOH-solution (pH meter was calibrated using technical buffer; pH 7.00 and pH 4.01 from WTW).
- 0.2 ml/l Tween 80 were then added, and volume adjusted to 0.9 L with dH_2O.
- Media were sterilized by autoclavation at 121 ^{o}C for 15min.
- 25 mL of glucose were added from a 1M sterile stock solution giving 25mM end-concentration.
- Volume were adjusted at room temperature by adding sterilized dH_2O up to 1 L.
- MAPT media was filtrated using Nalgene ™ 0,2µm rapid-flow sterile-filter

4.4.2 Screening Modified All Purpose Tween

The SMAPT media contained the same amounts (see method 4.4.1) (g L^{-1}) of glucose, dihydrate sodium citrate, sodium chloride, dipotassium hydrogen phosphate, manganese (II) chloride, magnesium sulphate, heptahydrate ferrous sulphate and MOPS sodium salt. Based on the protein-hydrolysate made earlier, nine different SMAPT media were made. The protein hydrolysates ranged from ~67-87% protein content in the hydrolysate products. Each SMAPT had approximately 20 g/L protein-hydrolysate content which were normalized, based on their respective protein percentage (estimated from
Kjeldahl results, performed at Nofima Bergen, see table 5.2.1, with the corresponding measured hydrolysate (g) in table 4.4.1). 2X-SMAPT stock-solution was made by dissolving all reagents needed for 1litre media (excluding the complex nitrogen source (hydrolysates)) in 500ml. To make a finished SMAPT media with protein-hydrolysates, two equal parts of dH_2O containing 40g/l protein-hydrolysate and 2X SMAPT stock-solution was mixed.

Preparation of 500ml of 2X SMAPT stock solution:

- All reagents were dissolved in 400ml dH_2O and pH-adjusted to 7.0 using 1 M and 10 M stock solutions of NaOH- and HCl (pH meter was calibrated using technical buffer; 7.00 and 4.01 from WTW).
- 0.2ml Tween 80 was added and sterile filtrated with 0.2µm Nalgene Rapid Flow ™ filter.
- 25ml of 1M glucose stock-solution (25mM concentration in 1liter finished media), 10ml vitamin solution (see method 4.4.4) was added before the final volume were adjusted to 500ml with dH_2O.

Preparation of 50ml SMAPT-media with protein hydrolysates

- Nine media, each containing one of the hydrolysates (table 4.4.1) was prepared by dissolving the calculated amount of protein-hydrolysates into a total of 25ml with dH_2O and mixed with 25ml prepared 2X-SMAPT stock-solution (example: 50ml of MDCR_A contained ~1.22g (24.39g*0.05ml)).
- The 50ml of dissolved SMAPT was centrifuged at 10 000xg for 10 min before each media were sterile-filtrated using 0.2µm Nalgene ™ Rapid-flow filters.
- Prepared media was kept at +4^{o}C until use.

Table 4.4.1: *Shows the amount needed from each hydrolysate to get 20 g/L final protein concentration in SMAPT media. The amounts listed are based on the results from table 5.2.1 which lists the % total Kjeldahl protein acquired by the use of the different enzymes throughout the hydrolyses'.*

Sample ID	Weight (g) for 20 g/l protein-concentration
MDCR_A	24.39
MDCR_C	23.95
MDCR_F	27.51
BC_A	22.83
BC_C	22.80
BC_F	23.34
WP_A	27.32
WP_C	26.67
WP_F	29.67

4.4.3 Screening Modified Only Protein Hydrolysate

SMOPH media was made to check if growth could be sustained with only the nutrients present in the nine protein-hydrolysates products (see *Table 4.4.1).* Each SMOPH-media was made with 20 g/l protein content. Glucose was added to improve growth, and was tested at concentrations of 0-, 6- and 12,5 mM (6mM concentration was made with 6ml/L of 1M glucose stock-solution).

Procedure (1L SMOPH):

- Each of the nine protein-hydrolysates was mixed (according to table 4.4.1) with 900 ml dH_2O and 6ml glucose 1M stock-solution.
- The pH of each medium was adjusted to be in between 6.8-7.0 (only whey protein had to be adjusted).
- The final volume was adjusted to 1L
- Each SMOPH media was centrifuged at 14.000 rpm for 15 min, and the supernatant centrifuged a second time at 14.000 rpm for an additional 5 min.
- Centrifuged samples were filtrated using Nalgene ™ 0,2µm rapid-flow 500ml sterile-filter (on average, 2-3 filters per litre media)
- Media was stored at +4^{o}C until use.

4.4.4 Vitamin solution (100X stock-solution)

Vitamin solution was added to the 2X-SMAPT stock-solution to ensure that all factors needed to promote growth was present. The solution contained (mg l^{-1}): adenine, (1000), Uracil, (1000), Xanthine, (1000), thiamine hydrochloride, (100), nicotinic acid (niacin), (100), pantothenic acid, (100), riboflavin, (50), pyridoxal HCl, (50), folic acid, (10) and biotin, (1). The vitamin solution was stored at -20 ^{o}C in 10ml containers until use. For one litre SMAPT-media, 10 ml of vitamin solution was used.

Procedure:

All reagents were measured and dissolved in dH_2O with exception to adenine, uracil and xanthine. Adenine was dissolved in a 200µl 1M HCl, while uracil and Xanthine was dissolved in the same amount of 1M NaOH and added to the mixture. All reagents dissolved fully when the solution was adjusted to pH 7.00. The vitamin solution was filtrated using 0,2µm Nalgene ™ Rapid-Flow filter and stored at -20^{o}C.

4.5 Freeze-stock of bacterial strains

Freeze stock of each strain was made using 200µl glycerol mixed with 800 µl bacterial suspension and stored at -20^{o}C. The bacterial suspensions were made by isolating one colony of each specific strain on MRS- or BHI-agar and transferring them over to liquid MRS-media (broth). All incubations were done at 30- and 37^{o}C for 24-48h under anaerobic conditions (CO_2-atmosphere).

Freeze stock was made from all viable strains retrieved from Nofima in Bergen, verified to be LAB (see method 4.7) (strains marked as "IS") (table 3.2). Further additions from the strain stock library was done, found at Nofima in ÅS (strains Marked as "MF") (table 3.1).

4.6 Thawing of bacterial freeze-stock and subcultures

Before each Bioscreen-C trial (see method 4.8 Bioscreen-C trials) all strains used was grown in subcultures, using both MAPT- (25mM glucose) and MRS-broth (control growth), however only strains from MAPT was taken for inoculation in the Bioscreen-C system.

4.7 Sequencing by 16S rRNA using the "microwave-method"

Strains recovered from Bergen, Nofima (see table 3.2, only viable strains are included) was isolated from widely different habitats. Strains from Bergen was however not identified to a genus or family but suspected to be of LAB due to phenotypical traits shown by growth trials (done at Nofima Bergen). The isolated strains were sequenced using 16S rRNA and the results from sequencing is listed in table A5.1. The isolates from Bergen was included in this study to investigate if any strains had inherent capabilities that allowed improved growth.

Many LAB are very closely linked phylogenetically and therefore identification to specie based on a segment of the 16S rRNA (250-800bp) are not always possible. Sequencing by 16S rRNA was expected to give identification as to what genus and group of species each bacterium belonged to. Sequencing was performed on all viable bacterial samples retrieved from NOFIMA Bergen. Lysis of bacterial cells directly from colonies on a plate were performed by a modification of a protocol for yeast cells (OpenWetWare., 2016)

The PCR protocol used is given by Invitrogen ™ Platinum ™ Hot Start PCR. Primers used was 27F- and Mangala forwards/backwards primers (see table 3.6). All samples were kept on ice or stored at 4^{o}C.

Procedure:

All related tables are listed at the bottom of the procedure.

- Subculture of each bacteria was cultivated for 24-48h at 30^{o}C on MRS-agar in an anaerobic environment (each strain was cultivated in duplicates).
- One colony was sampled by a brief contact with an inoculation loop/rod and placed in a PCR-tube in duplicate series. For each bacterium a total of 4 replicates was prepared, and isolated from 4 separate colonies.
- Samples was dried at +99^{o}C for 30 mins
- The sample was then lysed in a microwave for 60 seconds to release the DNA.
- 24 µl Master mix 1 (MM1) was applied to each sample (shown in table 4.7.1)
- PCR was set until the next day for all samples with the setup shown in table 4.7.2
- All DNA-samples was checked with gel-electrophoresis to ensure the presence of RNA and no smears (for gel electrophoresis, see method 4.7.1).
- The remaining PCR products was then diluted 1:1 with nucleic acid free water.

- Each product was cleaned for nucleotides and access primers using 2 µl ExoSap-IT reagent mixed with 5 µl of the diluted PCR product
- PCR setup for ExoSap-IT was set to one cycle at 37^{o}C for 30 minutes, followed by one cycle of 80^{o}C for 15 min, followed by and stored at 4^{o}C.
- After a completed ExoSap-IT PCR, DNA-samples was placed in a MicroAmp Optical 96 well reaction plate and applied 10 µl of Master mix 2 (MM2) (shown in table 4.7.3)
- PCR setup was set to 96^{o}C for 15 secs followed by 60^{o}C for 4 min and cycled 25 times, followed by and stored at 4^{o}C.
- After complete PCR with Big dye, each product was precipitated using 55µl master mix 3 (table 4.7.4) after a short centrifugation at 1000rpm for 1 min.
- Each sample was sealed with a lid and vortexed for 30 min at 1500rpm
- Each 96-well plate was centrifuged at 2500 rpm for 2 min using Heraeus Megafuge 8 (Thermo scientific)
- After centrifugation each plate was sequenced using ABI 3130xl Genetic analyzer (protocol: BDx_50_1_1_E_short and analysing protocol; BD_1_1) (Applied biosystems).
- Results was analysed using applied Biosystems Minor Variant Finder (MVF)
- Sequences with likely profile hit was trimmed and the remaining sequence was identified using standard nucleotide BLAST from NCBI with scoring parameters for gap and match/mismatch; 4/-5 and gap-cost; 12 existences, 8 extensions.

Table 4.7.1: *Shows the content of Master Mix 1 for samples used for the Invitrogen ™ Platinum ™ Hot Start PCR protocol.*

Product Name	Content (µl)
dH20 (nucleic acid free water)	11,5
Invitrogen ™ Platinum ™ Hot Start PCR 2X Master Mix	12,5
Forward primer *	0,5
Reverse primer *	0,5
Template	N/D

* primers from table 3.6 Two sequencing sessions was performed with the respective forward primer being the alteration between sequencings sessions.

- N/D; Not Detected (preferably few copies of the template)

Table 4.7.2: *Shows the PCR protocol used for 16S rRNA sequencing by Invitrogen ™ Platinum ™ Hot Start 16S PCR protocol.*

Step	Temperature	Time
Initial denaturation	94˚C	2 min
Denature (25-30 cycles)	94˚C	30 secs
Anneal (25-30 cycles)	~55˚C	30 secs
Extend (25-30 cycles)	72˚C	1min/kb
Hold	4C	Indefinitely

Table 4.7.3: *Shows the reagents of Master mix 2, using Big dye from life technologies.*

Product name	µl per PCR reaction
Big Dye buffer 5x	1,5
Big Dye v 1.1	1
Sequencing primer 3.2µM	1,0*
dH_2O	5,5
Pre-sequencing product	1
Total (µl)	10

* primers from table 3.6 Two sequencing sessions was performed with the respective forward primer being the alteration between sequencings sessions. 0.5 µl was used from both primer set respectively

Table 4.7.4: *Shows the reagents of Master mix 3, using* X-terminator-solution and SAM-solution (life technologies).

Product name	Content (µl per PCR reaction)
X-terminator solution	10
µl SAM solution	45

4.7.1 Electrophoresis

Electrophoresis was utilized to validate that the right sized fragment of the 16s rRNA was collected from prior PCR (see method 4.7) and to ensure that smears of PCR product was not further analysed by sequencing.

Procedure:

- 0.7% agarose gel was made by mixing 2.1 g molecular Biology agarose with 300ml 5X TBE-buffer and stained using Gel Red Nucleic acid stain 10.000X in water (6µl GelRed stain was mixed with 60ml agarose-gel).
- Each PCR product from method 4.7 was applied to a well on the 0.7% agarose gel containing staining dye.
- 3 µl Orange-G was mixed with 5 µl from each sample in a new 96 well-plate (bubbles were avoided and removed if occurred).
- Gel-electrophoresis was applied at 100 volts for 30 mins. A total of 7.5 µl was applied from each sample (0.5X TBE was used as buffer under the electrophoresis) (DNA ladder was applied to the first and last well of each gel).

4.8 Bioscreen-C trials

The Bioscreen-C system measure change in turbidity of a given medium. This is done by a spectrophotometry in the range of 280-750nm wavelength. In the case of measuring bacteria growth, a wavelength of 600nm is preferred as it can interfere with the bacterial cell without having a harmful effect often related to the UV/Vis spectra. Furthermore, this wavelength does not greatly interfere with the media itself, giving a more precise measure of cell growth. This allows for Real-time growth and measurements by optical density and gives an indication to generation time, and growth speed. The use of spectrophotometric method allows for measurements of bacterial growth by an increase in optical density (OD) over time.

For this study, each run was prepared with two plates of 100 wells. In a typical experiment run, the experiment setup consisted of six bacteria's, screened against nine different growth media. For each medium two blanks were made (for plate-setup see picture A7.0 in appendix 7). MAPT was used as basis media with bacteriological peptone (Oxoid) and yeast extract (Oxoid) as protein source, whereas SMAPT refers to the nine protein-hydrolysate products listed in table 4.4.1.

Experiment:

- Trial A: incubation in Bioscreen-C was done at 30^{o}C with 25mM glucose concentration SMAPT and MAPT media. Performed on 42 strains. Each strain was screened towards 10 mediums in triplicate series.
- Trial B: incubation in Bioscreen-C was done at 37^{o}C with 25mM glucose concentration SMAPT and MAPT media. Performed with nine strains on 10 mediums in triplicate series.
- Trial C: incubation in Bioscreen-C was done at 30 ^{o}C with 0mM- and 12.5mM glucose concentration SMAPT- and MAPT-media. Performed with six strains on 10 mediums in triplicate series.
- Trial D: incubation in bioscreen-C was done at 30^{o}C on media consisting of SMOPH with 0- and 12,5mM glucose concentration. Subculture was centrifuged, and pellet separated from the broth (MRS). Pellet was resuspended in PBS and centrifuged again at 1000 rpm. Supernatant was removed, and this step was repeated twice. Washed pellet was resuspended in 1ml PBS and measured to ~0.05 OD by spectrophotometry. Performed with six strains on nine mediums in triplicate series. Note that wrong wavelength when measuring OD of inoculum. Samples were measured at 440nm and later control measurement of ~50 % of the used inoculations at 600nm gave values with a range of 0.018-0.025 OD_{600}.

Procedure:

- Subcultures of each strain was incubated for 48 hours at 30°C in MAPT media prior to experiment run.
- 10µL bacteria suspension from each strain was diluted with 990 µL and measured by spectrophotometry (MAPT was used as Blank). The measured values were then used to prepare inoculation suspension for each strain with a bacteria-concentration of 0.050 OD (+/- 0.002 OD). All suspensions were control-measured before inoculation. To prevent more growth, all strains were kept on ice.
- Triplicate series + blanks of 245 µL from each SMAPT media was applied to the respective wells. For each media three sets of triplicates were made (one series for each strain, per plate) (see picture A7.0).
- Each triplicate-series was then inoculated with 5 µL of the normalized bacteria suspension (0,05 OD).
- The bioscreen-C instrument was set to 30- or 37°C, for 48 hours with 20 minutes measuring intervals. Shaking was applied for 10 seconds at low intensity before each measurement was done at 600nm wavelength.

Data Evaluation: After completed run, triplicates for each strain and each hydrolysate were grouped (excluding series that deviated greatly from the trend). The average values for each measurement was plotted against time in a "line-plot" in Excel. The Max growth, Lag phase and μ_{MAX} values for each strain (from trial A) was produced from these graphs.

μ_{MAX}-values

μ_{MAX}-values was obtained using the same formula as Horn et al. 2005. Briefly a timeframe of 1-4 hours of the exponential-phase was used to determine μ_{MAX} for each strain on all the 11 growth media.

$$\mu_{MAX} = \frac{\Delta Ln(OD_{600})}{\Delta t} \quad \text{(IV)}$$

The max cell density (OD_{MAX})

OD_{MAX} was determined as the OD max-value close to where the growth curves reach stationary-phase.

Lag-phase

Lag-phase was determined by dragging a line through the exponential phase of the curve, and an additional line through the Lag-phase of each specific graph. Where the lines crossed was considered to be the time-point of which lag-phase was finished. Generation time was calculated but not listed in this paper).

$$Generation\ time\ = \frac{LN2}{\mu_{MAX}} \quad \text{(VI)}$$

Selection of Candidate strains

Selection of potential candidates for further Bioscreen-C trials (B, C, D) was based solely on the Max OD and μ_{MAX} values (acquired from trial A) compared to the achieved growth on MAPT (MRS was included as a second guideline if MAPT showed to be ill suited for a specific strain).

4.9 Antibiotic resistance test

Antibiotic resistance is an unwanted trait in any bacterial strain used for food-processing. All strains used in this study was found on the list Qualified Presumption of Safety (QPS) (EFSA., 2013), however as a safety precaution, a small-scale antibiotic-resistance screening was carried out. Strains (from Bioscreen-C Trial D) was screened for resistance against; Ampicillin and Erythromycin (antibiotics was mixed into MRS-agar at low concentrations). Antibiotics and their respective start-concentrations are listed in table 3.3 in materials.

Procedure:

- MRS-agar was mixed and sterilized after the supplier's recommendations.
- 60mL of media was mixed with 6-, 9-, 15- and 30 µl ampicillin and poured into separate petri-dishes, giving an end concentration of 10-, 15-, 25-, 50 µg/ml of ampicillin.
- The same was done with 15- and 30 µl erythromycin giving an end concentration of 2.5- and 5.0 µg/ml respectively.
- A positive control was also made, using no antibiotics, only MRS.
- Bacterial suspensions from liquid-subcultures (using MRS) was inoculated onto each plate and dried off before anaerobic incubation at 30^{o}C and 37^{o}C for 72 hours.
- Results was measured as positive or negative for growth at the respective concentrations.

4.10 Fermentation Experiment (A); SEC profiles

Fermentation by LAB is commonly used in industrial food processing and is implemented to affect taste and consistency but also to perceive a probiotic effect in the finished food product. Unfavourable sensory qualitites in food has been shown to be a result of the number of dipeptides and short-chained peptides present in the material. Fermentation by LAB has shown to metabolizing short peptides and free amino acids (Doeven, Kok & poolman., 2005). The capabilities of each LAB and the degree of fermentation may vary between different strains and in different substrates. The difference in fermentation may subsequently be a result of peptide preferences shown by the individual strains and so it is reasonable to expect that each strain will affect taste differently. An effort was made to obtain peptide-profiles, before and after LAB- fermentation in a small-scale experiment of four LAB on nine SMOPH media with 6mM glucose. Samples for SEC analysis were obtained before and after fermentation (samples before fermentation was not added glucose).

Procedure:

- 5 x 9 culture tubes were prepared with 3ml SMOPH-media (one series 0mM- and four series with 6mM glucose). Each series was visually checked for contaminations (tested over 48 hours at room-temperature).
- Subcultures of each strain was measured to 0.4 OD, and each strain was inoculated into the nine different growth media (total volume 250ml) with 7.5 µL to a final concentration of 0.001 OD/µl (same final concentration used in Bioscreen-C trials).
- Culture-tubes was incubated at 30^{o}C for 48 Hours with shaking applied every hour for the first 3 hours, and again 24 hours later).
- After complete incubation, for each sample, the media was extracted using a syringe and filtrated using 0,2µm sterile filter, leaving the bacterial pellet.
- 3 ml was extracted from each sample and stored in new Eppendorf-tubes at -20^{o}C (a control series from each SMOPH (0mM glucose) was taken which were not subjected to any fermentation).

4.11 Big-batch Fermentation experiment (B)

A big-batch experiment was performed to produce samples for sensory analysis (Napping). The experiment was performed for two strains on nine SMOPH media (6mM glucose concentration). All end-products was later sterile filtrated and lyophilized for later use (see method 4.12).

Procedure:

- Subculture of each strain was made using MRS-broth.
- The bacterial-pellet from each subculture was separated from the broth and suspended in PBS.
- The bacterial-solutions was then centrifuged and resuspended in new PBS (repeated twice).
- washed cells were resuspended in PBS and used as inoculum.
- The inoculum was measured to contain the same bacterial concentration as the Bioscreen-C (trial A-C), namely 0.001 OD/µl.
- The amount of inoculum was reduced whereas the concentration of the inoculum was increased. Each inoculum was measured to 0.48 OD and 0.750 ml was applied to ~250ml (two parallel series).
- The inoculated samples were incubated for 48h at 30^{o}C, and samples was shaken once every 60 min for 3 hours. Same was done 24 hours into the incubation process.
- After incubation each media was sterile filtrated, and samples was stored at -40^{o}C.

4.11.1 Lyophilization of Big-batch products

Drying of the fermented SMOPH media was done in the same manner as in method 4.1.2 Lyophilization (freeze-drying) of hydrolysis end-products. Samples with the same ID (parallel series) was pooled together to one sample.

4.10.1 pH-measurements of fermentation (A)

Samples from fermentation experiment (A) was pH-measured. This was mainly done to ensure that samples were not too protonated (high pH), which would otherwise interfere with SEC measurements and buffers. The pH was measured using pH-meter from VWR, pHenomenal™, pH 1000 H after finished fermentation over 48 hours.

4.12 Projective Mapping (Napping)

The fermented-products produced in method 4.11, together with the hydrolysate-product (method 4.1) was prepared for napping-analysis. Approximately five grams (+/- 10% water content) of dried sample was mixed with 250ml H_2O (2% dilution) at +/- 37^{o}C and each sample was given a randomized ID. Napping was performed by a semi-trained internal panel at Nofima, Ås. Note that fermented products was not completely dry with 5-8,2% water content in BC and MDCR samples. This was not adjusted for giving the fermented products a final dilution of 1.7-2%. Samples of the same raw-product ID (MDCR / BC /WP) was grouped and each raw material was analysed as a bulk. In total three napping trials was performed with each session consisting of nine samples out of the total 27 samples produced. The Napping analysis was performed on fermented samples from IS 93 and MF 1127 together with the respective non-fermented "blank samples". A max of two strains was analysed by a projective mapping due to the limitations in regard to the number of samples.

In the napping test, the task of the panelists is to list the attributes related to the sensory properties of each samples. The samples are then placed into a map, in a manner that place the samples that are most similar closest to each other. Measurement in the distance between the relative samples (with the respective notes of attributes) was plotted in Excel and analysed using Multiple Factor Analysis (MFA) and EyeOpenR statistical tools (performed by Mats Carlehøg at Nofima Ås).

4.13 SEC-analysis

For SEC analysis, four of the six candidate strains were chosen to partake in the fermentation process, whereof the two strains used in the Projective mapping analysis (method 4.12) were included (also IS 204 and MF 5214). The inclusion of four strains was to further investigate if the strains metabolized in same or similar manners.

In the characterization of the peptide profiles retrieved from LAB-fermentation (method 4.10) four fractions according to peptide size was produced. Fractionation of peptide sizes in the fermented and unfermented samples of protein hydrolysates was conducted after size exclusion chromatography (UHPLC-SEC) (Dionex UltiMate 3000 UHPLC$^+$ focused system (pump, autosampler and RS variable wavelength detector) from Thermo Scientific) using a SEC/GFC column from BIOSEP™-SEC-S (00H-2145-K0) (pH 3.00-7.00). Samples were eluted at a flow-rate of 0.9ml/min (ambient temperature) for 35 min, using the following mobile phase; (A) 30% ACN, 0.05% TFA solution and (B) 0.1M NaH_2PO_4 with 10µl sample-injection-volumes at 50 bars pressure. The chromatogram was obtained by measuring ultraviolet absorbance at 214nm and 254nm. A calibration-curve based on compounds and residues of known sizes was used to calibrate the column and used to fractionate the chromatogram according to peptide-size (free amino acids, peptides of 2-5-, 5-15-, and >15 residues) The crude grouping of peptides according to size was done according to Silvestre et al. 2012. The data was analysed using PSS WinGPC® UniChrom V8.00, Build 994 (analytical program from Polymer standards service (PSS). For calibration curve see table and figure A4.5.1 in appendix 4.

Procedure:

- Samples was injected to UHPLC-SEC instrument at 10µl volume at 50 bars and separated over 35 min. UV detection and measurements was performed at 214nm and 254nm.
- Data evaluation was performed using analytical-program: PSS (polymer standards service) and calibrated using measurements of molecules with known fragment-sizes.
- The chromatogram was trimmed down to contain data explaining the peptide fraction and free amino acids and is found to be between 5-15 mins (shown in figure 4.13.1-4.13.2 below). The data was further subdivided into four segments corresponding to the respective sized peptides; **F1**: 5-8.7 min elution time corresponds to >15 residues, **F2**: 8.7-10 min elution time corresponds to 5-15 residues, **F3**: 10-11.7 min elution time corresponds to 2-5 residues and **F4**: 11.7-15 min elution time corresponds to free amino acid.

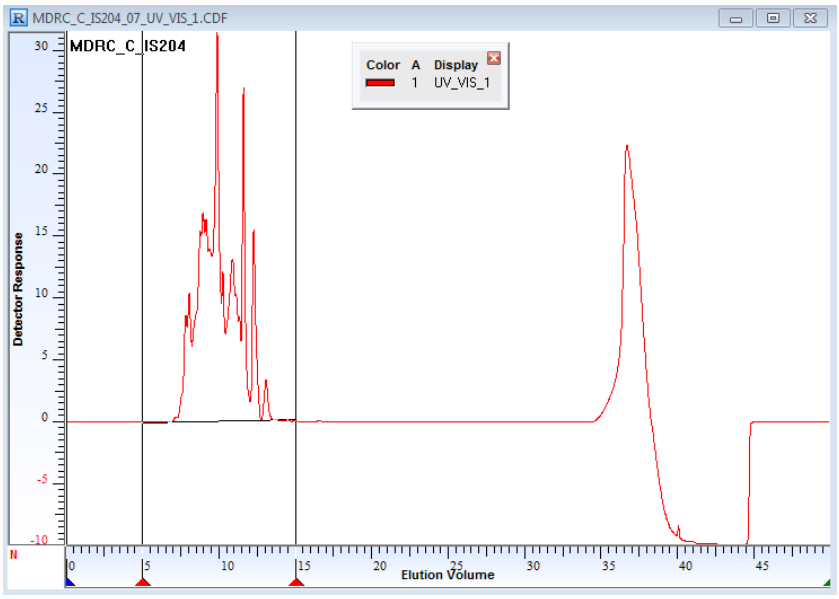


Figure 4.13.1 *Screen dump from the SEC analysis of MDCR_C_IS204 produced using Polymer Standards Service analysis tool (p=0.05). The first graph shows the sample run, with the 5-15minute stretch marked. The following graph are a zoom of the 5-15 minutes after calibration using the std.-curve shown in figure A4.5.1.*

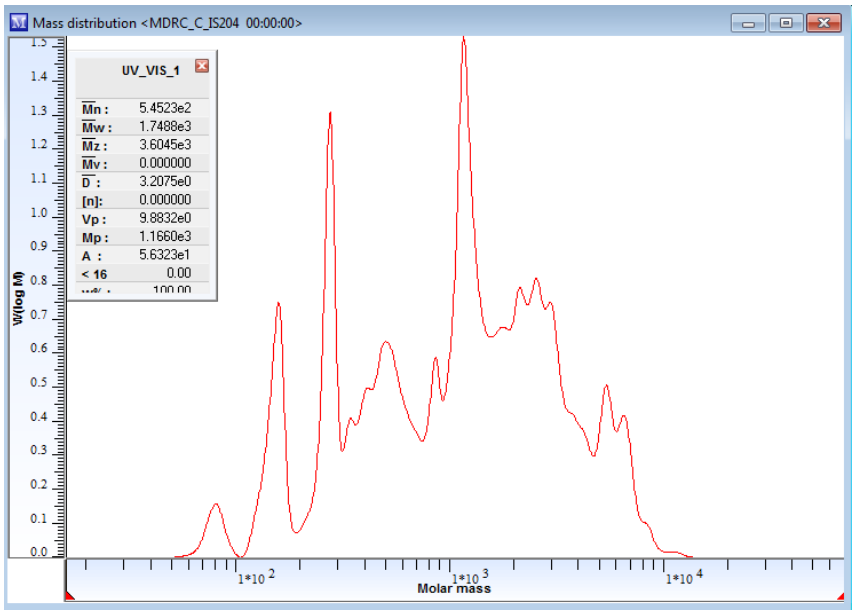


Figure 4.13.2 *Screen dump from the SEC analysis of MDCR_C_IS204 produced using Polymer Standards Service analysis tool (p=0.05). The graph shows the sample run, with the 5-15minute stretch marked and after calibration using the std.-curve shown in figure A4.5.1.*

5 Results

5.1 Sequencing by 16S rRNA

The Sequence-analysis of the 42 strains (retrieved from Bergen, Nofima) using 16S rRNA gave in almost all cases hit to LAB. Only strains that was viable after shipment was sequenced (30 isolates). The resulting Strain identification is found in the Table 3.2 (materials) and the resulting sequences are found Table A5.1 in the Appendix 5. Strain identification was not conclusive due to the size of the analysed fragment of 400-800 bp and that 16S rRNA sequence in some cases do not distinguish between closely related species. The strain identification is therefore an approximation towards group of related species within a genus.

5.2 Enzymatic Hydrolysis yield

The purpose of the enzymatic hydrolysis was to produce products which could support growth. The use of different enzymes and raw materials would provide the LAB with different compositions of peptides and peptide sizes. The access to different peptides would perhaps affect the growth and taste development by the LAB differently. The three by-products from animal origin (MDCR, BC and WP) was subjected to three different enzymes (A, C and F). The degree of Brix was measured for each hydrolyses by refractometer (results are listed in figure A6.4.1 to A6.4.3 in the appendix) and shows an increase in the degree of refraction (1-2 ^{o}B) over the course of hydrolysis (with exception of WP_C which stays at the same degree of brix throughout the hydrolyses. All replicates of the same enzyme and raw product composition progress in the same manner with exception to MDCR_C which deviates with 1.5^{o}B at most (40 min in to the hydrolysis).

Due to problems in the lyophilization, some material was lost from most but not all samples. The samples were stacked in series of four replicates, and at the initiation of lyophilization the samples were not sufficiently frozen. The liquids allowed air to escape too fast which resulted in a “foam” being produced. This continued beyond expectations and the process was stopped and restarted later, under right conditions. Furthermore, due to the uneven loss of sample material under lyophilization the mean deviation of the samples dry weight was not calculated. The amount of hydrolysate is the recovered amount of product after lyophilization. Based on the similarities in sample volume of the four replicates from each hydrolysis reaction, it was possible to estimate what samples lost the most material (explained under protein yield below).

The lipid fraction was only measured in MDCR samples, as it was less than 1% in BC and WP samples). The results show that Alcalase release more Lipids compared to Corolase and Flavourzyme, 96.22g compared to 69.22g and 61.78g, respectively (pooled results from both replicates (see table A6.1-A6.3).

The amount of sediment fraction (solids) is lower in BC compared to the two other by-products with roughly ~25% remaining product after hydrolysis (out of 1000g products of the pooled samples), only Flavourzyme gave ~36% sediment (256.8g, 256.8g and 365.38g respectively). The reduction was identical in terms of sediments between Alcalase and Corolase in BC series (table A6.2). The Main component of the sediment fraction was consisting of Bones and is perhaps explaining the similarities of Alcalase and Corolase.

Some of the same trends was seen in MDCR samples, giving less sediment by Alcalase compared to Corolase and Flavourzyme. The sediment fraction increased approximately with ~80-100g for each enzyme (A/C/F) (386.3g, 464.4g and 666.3g respectively) (table A6.1). The difference in sediment fraction by MDCR may be explained by the elevated content of lipids, collagen and elastin, but also by the fraction of Bones and tendons which is not degraded easily. Whereof the enzymes may release and interact with the components differently. For instance, the peptide fraction of Flavourzyme was relatively lower compared to Alcalase and Corolase, whereas Alcalase released more lipids compared to Corolase. Furthermore Flavourzyme produced a gel-like substance when centrifuged, indicating that collagen or/and elastin was released. This was not seen by Alcalase and Corolase which acted on the raw material in a more similar fasion. However, the differences in the interaction with the raw material preceived by the different enzymes helps explain why the sediment fraction is different. For instance the difference between Alcalase and Corolase is slightly less significant and is largely explained by the difference in the lipid fractions. Additionally Flavourzyme was less effective at releasing peptides, and is likely the main reason for the increased sediment fraction. It is important to remember that some loss of peptide fractions may futher explain the decrepency seen in the results perhaps more so in relation to Alcalase and Corolase.

The sediment fraction from whey protein is based on the pooled sampled of replicate series which in total consisted of 250g dry weight mixed into a total of 1000g (diluted with dH_2O). Due to the more complex nature of the sample preparation it is difficult to estimate the sediment fraction. However, by subtracting the yield in terms of lyophilized sample from the dry weight of the added samples it is possible to get an estimate of the dry sediment fraction. Alcalase and corolase gave less sediments compared to flavourzyme with roughly 111.4 (44,5%), 114.8 (45.9%) and 160.4 (64.1%) grams material respectively.

Based on the similarity between parallel series of products with same raw material and enzyme it is possible to estimate which samples experienced the highest sample loss through lyophilization (dry weight) (table A6.1-A6.3). It is reason to believe that a dramatic loss in BC_A series occurred with an estimated loss of ~30g (23.6%) (dry weight) (believed to be close to BC_C in terms of yields). Loss of weight in other samples was estimated to be less than 5g on average. All samples were slightly more hydrated after hydrolysis. This would affect MDCR and WP more than BC due to higher raw material content of the sediment phase.

The dry weight of the aqueous-phase would normally explain the yield of the hydrolysis process in terms of weight. However, due to losses this is just the relative yield in terms of recovered material. Based on the total amount of raw-material added and the total isolated yield after lyophilization, the hydrolysis managed to recover between 12-16% in MDCR, ~11-13% in BC and 8-14% in WP of the total product (weight). In all cases the lowest yield was produced with flavourzyme whereas the highest yield from Alcalase and Corolase was fairly similar in all cases (with exception to BC_A with high sample-loss) with a mean-deviation between alcalase and corolase series of 0.40% and 1.04% in MDCR and WP series respectively.

After lyophilization of the hydrolysates (see methods 4.1 and 4.2) each product was tested for protein content using Kjeldahl-analysis (performed by Nofima, Bergen). It was established from this method that the hydrolysis products contained 67% - 88% protein, whereas the highest protein content in the products were in Cod (86 – 88%, followed by chicken- (73 – 83%) and whey products (67-75%) (see table 5.2.1 Products of Corolase (C) gave the highest protein content in all three cases shown in the results from the Kjeldahl-analysis listed below.

The lyophilized hydrolysates were sent for Kjeldahl-analysis to estimate the protein content in order to produce growth media with 20g/L. The results are listed in table 5.2.1 and the adjusted amounts are listen in table 4.4.1.

Table 5.2.1*: shows the adjusted weight of each protein-hydrolysate (according to Kjeldahl-analysis, Nofima Bergen) needed to make 20g/l final concentration of each SMAPT media. The table shows the results from Kjeldahl-analysis performed by Nofima Bergen and approved by laboratory-leader Arne Brodin. The analysis was performed on request from Diana Lindberg Nofima Ås. The results are listed in percentage after conversion with the Total Kjeldal Nitrogen (TKN) conversion factor 6.25.*

Sample ID	Raw-protein (%) Kjeldahl (N*6,25)
MDCR_A	82
MDCR_C	83.5
MDCR_F	72.7
BC_A	87.6
BC_C	87.7
BC_F	85.7
WP_A	73.2*
WP_C	75.0*
WP_F	67.4*

* values were initially converted with TKN 6.25 when analysed, and later experimental exercises was consistently based on the listed measurements throughout the study. A more accurate value would have been achieved with the TKN 6.38 for WP products. giving 74.7-, 76.56- and 68,8% respectively. The difference is roughly ~ +1.4-1.6% in WP samples.

Protein yield

The protein dividend or the total amount of protein recovered from each enzyme and raw-material was calculated using the Dumas results shown in table A3.3. Note that a sample-loss was observed though the lyophilization step, and so the total weight of each hydrolysate is somewhat higher with an average loss of ~5g in most samples (uncertainties towards which samples). Furthermore, BC_A was estimated to have lost ~30 g (23.6%) and would in reality reflect the values observed with BC_C (table A3.3)

The total water content of MDCR; BC and WP raw-material were calculated to 59.50-, 73.30- and 3.09% respectively. The total amount of raw material was measured to ~500g per hydrolysis with materials of MDRC and BC, whereas WP was applied as 125g dry weight and mixed with 375 to produce 500g wet product. For each product and enzyme, two parallel hydrolyses-reactions was performed. This would include an approximate total amount of ~1000g (+/- 5g) for MDCR and BC and 250g (+/- 1g) for WP that was hydrolysed for each enzyme in order to produce the hydrolysates. To get the total protein yield from the hydrolyses', the protein content was estimated form the total RM (g) using the converted Dumas values by TKN shown table A3.3 for each respective sample. The same was done with the lyophilized samples and subtracted from the total protein of RM and converted to percentage. The resulting protein yield is shown in grams and in percentage of the total protein content of each raw material (table 5.1). Alcalase and

Corolase seemed to produce the same yield with +/- 2% recovered proteins, whereas Flavourzyme produced less yield in all cases. The highest yield was seen in BC series ranging from ~53-45%, followed by WP ~46-27 % and MDCR with ~36-24%.

Table 5.1: *Shows the total amount of proteins in the raw-material (duplicate series pooled), the converted total Kjeldahl protein (g) in RM, the converted total Kjeldahl protein in the produced hydrolysates (based on Kjeldahl values from table A3.3) and the final protein yield as % of the total protein recovered from the Raw material. Data was retrieved by Dumas method and converted with TKN 6.25 for MDCR, BC and 6.38 for WP Data from Dumas Tot. N (%) may give higher values than a typical Kjeldahl measure and so depending on substrate the results may be 5-10% lower in reality (5-6% in WP and 9-10% in MDCR and BC). The results are not total Kjeldahl protein but rather a conversion from Dumas method to total Kjeldahl nitrogen.*

Sample ID	Tot. RM (g)	Tot. Kjeldahl protein in RM (g)	Tot. Kjeldahl protein in Hydrolysates (g)	Protein yield (%) from hydrolysis
MDCR_A	998.92	404.56	139.93	34.59 %
MDCR_C	997.93	404.16	145.93	36.11 %
MDCR_F	995.75	403.28	98.94	24.50 %
BC_A	1000.86	237.20	96.26	40.58 %**
BC_C	1000.50	237.12	126.05	53.16 %
BC_F	1000.93	237.22	105.84	44.62 %
WP_A	250.54	242.80	110.05	46.27 %
WP_C	250.95	243.20	108.99	45.75 %
WP_F	250.66	242.91	65.50	27.52 %

* RM- Raw material (MDCR; BC; WP).

**Sample-loss at lyophilization. Estimated to be close to identical to BC_C values (explained elsewhere).

5.3 Bioscreen-C Trials

Prior to Bioscreen-C trials, all SMAPT media was prepared in 100ml and 50ml batches. The medium preparation steps revealed several potential obstacles and problems in regard to the resulting media. For instance, several key aspects were affected by the different techniques (autoclavation, filtration and centrifugation) used, whereas Turbidity, discoloration and loss of protein or nitrogen concentration was mainly of the concerns. To ensure that no significant change occurred in the finished media, the concerns were investigated in more detail. The results from the media preparation showed that centrifugation and sterile filtration was the best choice of method, with little to no loss of proteins and no discoloration with a clear media. The results the nitrogen content shows that each media contain >20g/L protein with most likely an approximate concentration of 22-24g/L in each SMAPT media. For more details on the media preparation, see the results in appendix 2 (A2.1-A2.3)

Bioscreen-C trial A was performed on all 46 LAB strains and was a wide screening for growth on nine different SMAPT media and on two basis media; MAPT and MRS. The initial screening process in trial A was used as the basis for picking out the 6-9 best performing strains under the given conditions.

5.3.1 Trial A

For each media and strain, the measurements were performed in triplicates. The growth rates (μ_{max}), Max growth (OD_{max}) and the dormant periods (Lag-phase) of each strain was calculated based on the growth curves (listed in appendix 8) and are listed in the respective tables A1.1, A1.8 and A1.9. An OD_{max} range of values was set for each media whereas all strains within the range are marked with “X” in table 5.3. strains with no “X” marked in MAPT or MRS indicates a higher growth in one or more of the SMAPT-mediums.

Table 5.3: *Shows the best performing strains from each media, where X indicate good performing strains in a given media. Based on the over-all growth in a given media, a top OD_{max} range was set. Strains within the top OD_{max} range was considered to perform above average ("X"). The OD_{max} range are based on the max growth achieved in the given media, whereof the OD_{max} range varies depending on the media. A list of the OD_{max} range is found below the table. The results from table 5.3 are based on the OD_{max} values from Bioscreen-C trial A found in table A1.1.*

strain ID	MRS	MAPT	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F
IS 61											
IS 64	x			x						x	x
IS 79	x	x	x	x	x	x	x	x		x	x
IS 93			x	x	x	x	x	x			x
IS 118.B	x	x	x	x	x	x	x	x		x	x
IS 118.4	x	x								x	x
IS 145	x	x	x	x	x	x				x	x
IS 185	x	x		x							x
IS 196.1										x	
IS 196.2				x	x				x	x	x
IS 196.3										x	x
IS 196.4									x	x	
IS 200											
IS 204	x		x	x	x		x				x
IS 269											
IS 352		x	x	x	x					x	x
IS 357	x								x		
IS 361	x	x	x	x	x				x		x
IS 371	x								x		
IS 380	x										
IS 384	x										x
MF 9											
MF 110											
MF 150	x										
MF 1127	x		x	x	x	x	x	x	x	x	x
MF 1964											
MF 1965	x										
MF 1974	x										
MF 1978											
MF 1979											
MF 1980		x		x							
MF 2033	x										
MF 2035	x									x	
MF 2357											
MF 2576									x		
MF 2900				x	x	x		x			x
MF 2983											
MF 02996											
MF 3579											
MF 5214			x	x	x			x	x		
MF 6580	x				x						x
MF 6581											

- the following values corresponds to the OD_{max} range of the best performing strains for each media, whereas all strains marked with "X" are within this OD range.

- MDCR_A: 1500-1750
- MDCR_C: 1500-1800
- MDCR_F: 1450-1600
- BC_A: 1400-1700
- BC_C: 1400-1650
- BC_F: 1350-1550
- MAPT: 1450-1860
- WP_A: 750-1050
- WP_C: 1050-1450
- WP_F: 1350-1650
- MRS: 1600-1990

Several strain performed good in the individual growth media, whereas the table 5.3 depicts all strains which performed above average. In the following text, the best performing strain with the achieved Max values are listed for all tested media.

The best performing strain in MRS was with IS 371 (1.99 OD), whereas the highest growth measured in MAPT was with IS 79 (1.86 OD),

The highest growth measured in MDCR_A (SMAPT 25mM glucose) was with IS 361 and IS 145 (1.76 and 1.71 OD respectively), whereas the highest growth measured in MDCR_C (SMAPT 25mM glucose) was with IS 118.b, -145, -79, -361 (1.82, 1.77, 1.77 and 1.77 OD respectively). The highest growth measured in MDCR_F (SMAPT 25mM glucose) was with IS 79 and MF 6580 (1.61 OD)

The highest growth measured in BC_A (SMAPT 25mM glucose) was with IS 79 and IS 118.b (1.5 OD), whereas the highest growth measured in BC_C (SMAPT 25mM glucose) was with IS 79, IS 118.b, IS 64 and IS 204 (1.57 OD). The highest growth measured in BC_F (SMAPT 25mM glucose) was with IS 79, -93, -118.b and MF 1127 (1.4 OD).

The highest growth measured in WP_A (SMAPT 25mM glucose) was with MF 1127 and MF 2576 (1.01 and 0.91 OD), whereas the highest growth measured in WP_C (SMAPT 25mM glucose) was with IS 79 and IS 118.b (1.44 and 1.42 OD). The highest growth measured in WP_F (SMAPT 25mM glucose) was with IS 118.b and IS 79 (1.62 and 1.57 OD)

Growth rate (μ_{Max})

The highest growth rates (μ_{Max}) was observed with MF 1127, ranging from 0.52-0.59 in MDCR and BC series (compared to 0.44 in MAPT), closely followed by MF 3579, MF 5214 and IS 93 (see table A1.1). the highest growth-rates observed in the basis media MAPT was with MF 3579, IS 196.2, -196.4, -200, -269, -352 and MF 1127 ranging from 0.44-0.51. Only IS 185 acquired high growth rate in MRS with 0.52.

Lag-phase

All strain showed relatively short lag-phase, with 3-10 hours being the average. Some strains showed lag-phase beyond 24 hours, however few cases (MF 150 and MF 110). For lag-phase see table A1.8

Negative control

Two negative-control samples for each media was included in each run. The negative control was not subtracted from the replicate series (graphs), however was subtracted for Max OD values listed in tables A1.9-A1.15 and table 5.3 (due to problems with the converted file acquired from workbook.exe and the Bioscreen-C PC (excel, version from 1997)). However, in all trials, the average negative-control value was as following for each of the series; MDCR_A 0.091, MDCR_C 0.081, MDCR_F 0.088, BC_A 0.081, BC_C 0.079, BC_F 0.082, WP_A 0.133, WP_C 0.155, WP_F 0.18. The control series did deviate with, +/- 0.004 of the average negative control (mention above). Through all Bioscreen-C runs, the first measurement (at 0min) was generally higher (~20%) than the following measurement (at 20min).

Candidate strains

A few strains were picked as candidates based on their growth relative to the growth in basis media (MAPT) (table 5.3). Both OD_{max} and μ_{Max} values was used to pick candidates. Furthermore, several strains were identified to same genus and so strain ID was used as a restricting criterion to maintain diversity of the candidates. The chosen strains were; IS 79, IS 93, IS 118.b, IS 204, IS 361, MF 1127, MF 5214. Furthermore, MF 150 and MF 110 was added to check if temperature was the limiting factor for these strains. MF 3579 did perform relatively good and could have been one of the candidate strains however was not included due to lower OD_{max}. Good performance in SMAPT was in general relative to the achieved growth in MAPT for each strain, and so the choice of strain was not purely base on high growth, but rather relatively high growth compared to the basis media.

5.3.2 Trial B and C

In Trial B and C, both temperature-conditions and glucose-concentration effect on growth was tested for the chosen candidate strains from trial A. For each media and strain, the measurements were performed in triplicates.

Comparison of 30°C and 37°C Conditions

Growth rates (μ_{Max}) results from 30°C trials was compared to the results from 37°C trials. The Growth rate at 37°C increased most notably in MF 5214 (all BC, WP and MAPT series), IS 204 (all media with exception to WP_C and MAPT) and IS 93 (MDCR_F, BC_A and BC_C). The increase in growth rates was on average +/- ~0.1-0.25μ_{Max} compared to the measurements at 30°C. A substantial improved growth was not observed in the remaining strain. In a subset of the strains, the increased temperature resulted in reduced growth, most notably in MAPT media. For the entirety of the results see table A1.3 & A1.2 in the appendix.

The OD_{Max} results from trails at 30°C and 37°C (SMAPT 25mM glucose) in some cases gave the highest optical density at lower temperature (see table A1.10 & A1.11), however on average the same OD was achieved (+/- 0.2 OD). The highest OD value in both cases was measured to 1.8-1.87 OD (in MAPT and MDCR_C at 30°C and 37°C). No strains perceived to grow to higher concentrations (in all growth mediums) at 37°C in terms of optical density.

Lag-phase

On average, the lag phase was reached within the first 24 hours (3-12 hours being the average) in all strains with exception to MF 150 and MF 110 which never reached stationary phase. The Lag-phase for 37°C-trails is not listed as the parameters was not substantially changed compared to the Lag phase from the 30°C trial (A1.8).

Glucose concentrations

MF 150 and MF 110 performed poorly in all following cases and is not included in the results. IS 93 (MDCR_C, BC_A, MDCR_C_12.5mM) and IS 79 (BC_A_12.5mM) were dead in trial B or C and throughout measurements and is not included in the comparison (dead or weak inoculation culture). The μ_{Max} and OD_{Max} values for all SMAPT trials are listed in table A1.1 – A1.13 with exception to A1.6-A1.7 in the appendix.

25mM and 12.5mM glucose (30 ^{o}C with SMAPT)

Good growth was observed in SMAPT at both 25mM and 12,5mM. The resulting OD_{Max} was not changed significantly between cultivations at 25mM and 12.5mM glucose in SMAPT and MAPT with the highest OD_{max} values in MAPT with OD 1.87 and 1.90 respectively. In SMAPT the OD_{max} value was slightly higher as a result of cultivation in the 25mM media, with the highest values ranging between 1.6-1.82. Whereas, OD_{max} in SMAPT with 12.5mM glucose ranged between 1.4-1.72 (A1.10 – A1.12 in the appendix).
Lower OD_{max} as a result of reduced glucose concentrations was mainly observed in MAPT (-0.01-0.21 OD) and in BC_F series (-0.2-0.9 OD), with some exceptions by MF 5214, IS 204 and IS 361.
The μ_{Max}-values were slightly higher in 25mM SMAPT compared to 12.5mM SMAPT with ~0.1-0.2 μ_{Max} on average. Few exceptions were observed; MDCR_A showed higher μ_{Max}-values as a result of cultivation in 12.5mM glucose SMAPT with values 0.1-0.2 μ_{Max} on average. Furthermore, a few other strains showed higher μ_{Max} in 12.5mM SMAPT (in some of the SMAPT media), mainly from IS 204, IS 118.b and IS 361. (table A1.2 and A1.4 in the appendix).

0mM glucose (30 ^{o}C with SMAPT)

Poor growth was observed in most SMAPT media with 0mM glucose, with the highest OD_{max}-values ranging between ~0.6-1.18. The growth-rates of MF 5214 and MF 1127 outperformed other strains with the highest μ_{Max}-values ranging from 0.36-0.56. IS 93 performed moderately good in some cases (MDCR_A/C/F and WP_F). The OD_{Max}-values in media with 25mM glucose ranged from 0.4-1.9OD whereas media with 12.5mM reached values between 0.2-1.30OD. values below 0.2 was not considered (see table A1.13).

In general, MAPT outperformed all SMAPT media with exception to MDCR_F_MF_5214 (with the highest optical density of the SMAPT media of 1.18 OD). Of all SMAPT media, the best growth was observed in MDCR_F, followed by MDCR_C, WP_F, MDCR_A and BC_F (with the highest values between ~0.4-0.85 OD). Poor growth (OD of 0.17 and 0.41) was observed in WP_C, WP_A, BC_A and BC_C series (with exception MF 5214_BC_A/C and MF1127_BC_C with an OD of 0.35-0.53 (moderate growth)). MDCR_A/C/F series supported the highest growth ranging between 0.4-1.18 OD, followed by BC_F ranging between 0.4-0.7 OD.

5.3.3 Trial D

SMAPT media had to be adjusted to ensure that no media components are of any health risk if consumed. SMOPH was made, containing only a protein- and carbohydrate source. 0- and 12.5mM glucose SMOPH was prepared for all candidate strains (excluding MF 150 and MF 110). Note that one error was made in the preparation of 0mM glucose SMOPH, and the bacteria-inoculum was measured at 440nm and not 600nm, giving lower inoculation concentration. Remeasurements indicated more than 2X reduction in OD of each sample inoculum from 0.05OD down to 0.018-0.025 OD (see table A1.15).

Growth in SMOPH with 0mM and 12.5mM glucose are listed in table A1.14-1.15. The results show better growth in series containing glucose with average ODs of ~0.65. MDCR_A, MDCR_F, WP_A, WP_F, MDCR_C and WP_C gives more drastic growth with the presence of glucose compared to the other growth media (highest series showing increases of 0.7-1.3 OD). The most successful growth in multiple mediums was seen in cultivation of IS 93, followed by MF 1127. MF 1127 performed above average in all mediums (with exception to BC_C, BC_F and WP_C). MF 5124 performed poorly in SMOPH with quite similar growth at 0- and 12.5mM glucose but with a small increase of ~0.1-0.2 OD in the presence of glucose. The best max-values (highest OD_{max}) were found in the same media for all bacteria's, namely, MDCR_A, MDCR_F and WP_A. Poor growth was observed in the same media for all bacteria's (namely BC_A and BC_C). In SMOPH with 12.5mM glucose the best growth was observed in WP_A, WP_F, MDCR_A and MDCR_F (0.6-1.35 OD).

Growth rate (μ_{Max})

A log-phase was in the majority of cases not observed when cultivating in SMOPH, in absence of glucose, which generally resulted in low μ_{Max} values. Contrary to this, a log-phase was observed in many cases for 12.5mM SMOPH, which generally resulted in higher μ_{Max} values. μ_{Max}-values ranged from 0.05-0.45 in SMOPH containing glucose, whereas the absence gave values ranging between 0.01-0.24. (see table A1.6 – A1.7.

5.4 Antibiotic-resistance test

The six candidates from Bioscreen-C Trial D was checked for ampicillin and erythromycin antibiotic-resistance.

In the case of ampicillin, it was observed that there was resistance up to concentrations of 25µg/ml in all strains. MF 1127 was somewhat more susceptible to ampicillin at higher concentration with barely any growth at 25µg/ml. At a concentration of 50µg/ml no growth was observed in any of the strains. All strains were highly susceptible to erythromycin with no growth observed at 2.5µg/ml (lower concentrations was not tested).

5.5 SEC analysis

SEC-analysis was performed on non-fermented and fermented samples of MDCR, BC and WP (using SMOPH media). The results are listed in table 5.6.1 where the non-fermented samples are referred to as "Blank samples" and fermented samples are marked with the respective strain ID (MF 1127, MF 5214, IS 93 and IS 204). The results are grouped together according to enzyme with the respective F1-F4 fractions (explained below). Values for the standard-curve is listed in the table and figure A4.5.1 in the appendix 4, with a R^2-values of 0.92 and was produced by plotting the meanRT-values against LogMW-values.

The pH was measured prior to SEC analysis, to verify that the pH was not too low for the column (method 4.10.1). The pH of the media ranged from pH 6-7.1 (MDRC/BC between 6.8-7.1 and WP between 6.0-6.2). After 48 hours of fermentation with MF 5214, MF 1127, IS 204 and IS 93, the pH of all series of growth media ranged between pH 4.2-5.3 (results not listed).

The SEC results listed in table 5.6.1 shows the relative peptide sizes divided into four partitions which indicates the percentage of the given peptide size relative to the total peptides in each sample explained in method 4.13 and briefly in table 5.6.1.

	Peptide fraction:	F1	F2	F3	F4
Sample ID	Hydrolysates		Alcalase		
Blank Sample	MDRC_A	23,30	30,95	33,37	12,37
	BC_A	14,10	40,95	36,91	8,02
	WP_A	26,99	29,56	36,16	7,28
IS 93	MDRC_A	→0,44	→0,08	→0,75	→0,39
	BC_A	→0,26	→0,67	→1,37	→0,45
	WP_A	→0,16	→0,17	→0,43	→0,09
IS 204	MDRC_A	→2,53	→0,38	→1,38	→0,75
	BC_A	→2,46	→0,99	→2,63	→0,83
	WP_A	→4,01	→1,52	→5,01	→0,51
MF 1127	MDRC_A	→1,24	→0,60	→0,01	→0,65
	BC_A	→0,10	→0,45	→1,19	→0,64
	WP_A	→0,39	→0,50	→0,97	→0,07
MF 5214	MDRC_A	→0,22	→0,56	→0,93	→1,27
	BC_A	→0,05	→0,38	→0,12	→0,43
	WP_A	→3,59	→1,35	→4,49	→0,44
			Corolase		
Blank Sample	MDRC_C	23,06	37,18	29,32	10,43
	BC_C	20,01	40,27	32,19	7,52
	WP_C	18,73	32,51	40,31	8,42
IS 93	MDRC_C	→3,84	→1,38	→1,50	→0,95
	BC_C	↓12,24	↓11,49	↓12,43	→0,43
	WP_C	→3,26	→0,43	→0,22	→3,48
IS 204	MDRC_C	→6,90	→1,87	→3,94	→1,07
	BC_C	→3,67	→0,35	→3,07	→0,93
	WP_C	→6,83	→2,04	→5,63	→3,25
MF 1127	MDRC_C	→4,19	→1,55	→1,64	→0,99
	BC_C	→0,52	→0,24	→0,27	→0,54
	WP_C	→3,10	→0,16	→0,61	→2,33
MF 5214	MDRC_C	→3,71	→1,37	→3,12	→0,78
	BC_C	→0,54	→0,11	→0,95	→0,53
	WP_C	→6,79	→1,92	→5,39	→3,33
			Flavorzyme		
Blank Sample	MDRC_F	19,61	23,51	35,59	21,27
	BC_F	12,28	19,08	53,12	15,50
	WP_F	20,63	27,05	38,14	14,17
IS 93	MDRC_F	→2,89	→1,74	→1,96	→2,66
	BC_F	↑11,46	↑11,79	↓20,50	→2,74
	WP_F	↓9,75	↓7,42	↑14,39	→2,78
IS 204	MDRC_F	→3,88	→1,58	→0,97	→4,45
	BC_F	→4,82	↑8,84	↓14,32	→0,64
	WP_F	↓11,27	↓7,97	↑16,73	→2,49
MF 1127	MDRC_F	→3,10	→1,47	→1,82	→2,75
	BC_F	→7,21	↑21,43	↓21,20	↓7,44
	WP_F	↓8,89	→6,33	↑13,00	→2,22
MF 5214	MDRC_F	→2,11	→1,36	→2,21	→1,26
	BC_F	↑7,46	↑8,52	↓14,06	→1,90
	WP_F	↓11,08	↓7,84	↑16,74	→2,18

Table 5.6.1: *Shows the relative content of free amino acids, di- tri- and oligopeptides present in each media after lactic acid fermentation. Blank samples are SMOPH-media which has not undergone any fermentation whereas the compared series of fermented-SMOPH samples are subtracted from the Blank values. Arrows indicate the relative compositional change in percentage (%) of the fermented samples. Green arrows indicate dramatic increase in a peptide fraction, whereas red arrow indicates dramatic reductions. Yellow arrows indicate Low-moderate alteration. black numbers indicate a reduction in the peptide-fraction of the fermented sample compared to the Blank series. Red values indicate an increase in in the peptide-fraction of the fermented sample compared to the Blank series. The probability threshold was set in the PSS program to P< 0.05. The peptide-size fractions are listed as F1-F4 (**F1**: 5-8.7 min elution corresponds to >15 residues, **F2**: 8.7-10 min elution corresponds to 2-5 residues, **F3**: 10-11.7min elution corresponds to 5-15 residues and **F4**: 11.7-15 min elution corresponds to free amino acids). The percentage is the relative composition of different sized fragments given as relative % nmol. An example of peptide profile with the 5-15min cut off and use of calibration curve is shown in Figure 4.13.1-4.13.2 in methods*

5.6 Napping (Projective mapping of sensory attributes)

Napping is a projective mapping method used to explain a products attributes in relation to the product's flavour perception. Napping may include several aspects such as taste, smell, looks and texture of a given food and gives an indication of how the specific food-product will be perceived by other consumers. However, in this work the screening was purely based on the perceived flavour. It is by no means a decisive method but rather a statistical approach to answer an opinionated perception of the food product.

The samples were tested internally at Nofima by a semi-trained sensory panel with no guidelines towards the use of discriminatory attributes. The following figures 5.6.1-5.6.6 shows the result from the napping analysis. Each co-product (MDCR, BC, WP) is included in one graph, explaining the distance in taste between samples (similarities), whereas the following graph represents the discriminatory attributes perceived by the given products. The graphs from the same raw material are connected and should be evaluated as a whole. The X- and Y-axis, or dimensions, each helps to explain a percentage of the similarity/dissimilarity among samples and is not identical for each dataset.

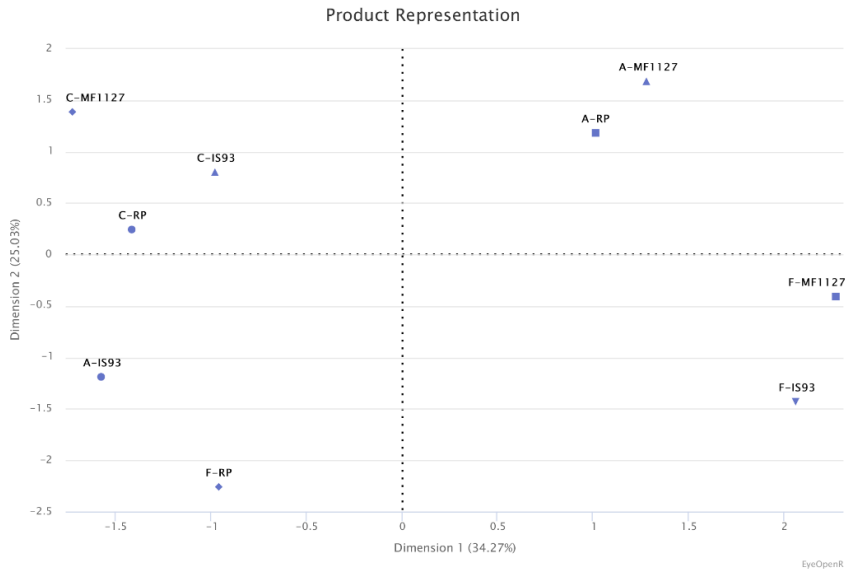


***Figure 5.6.1**: Shows the relative similarities and differences of MDCR products of raw product (RP) hydrolysed by the three different enzymes (A, C, F) and compared to the fermented products of MF 1127 (A-MF1127, C-MF1127 & F-MF1127) and IS 93 (A-IS93, C-IS93 & F-IS93). The corresponding graph 5.6.2 shows the perceived attributes. The placement within plots will correspond the attributes localized in the same area. The graph was produced by collected data from Napping analysis using EyeOpenR statistical analysis tool.*

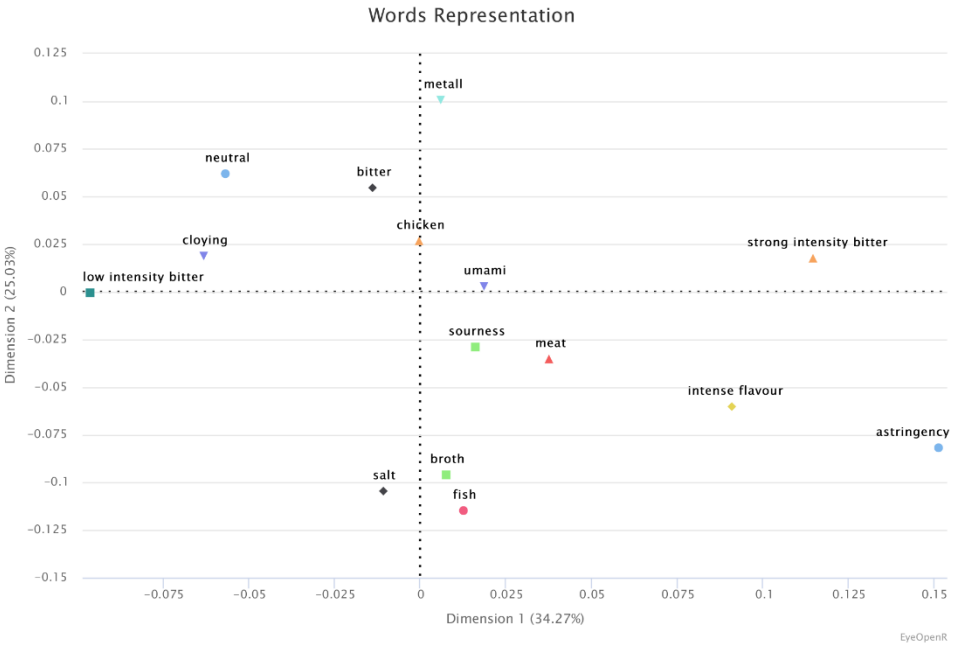


Figure 5.6.2: This figure shows the perceived attributes from napping analysis for MDCR samples. The attributes shown in the graph correspond to the samples of the plots in 5.6.1 localized in the same graph-area. The graph was produced using collected data from Napping analysis by EyeOpenR statistical analysis tool.

The MDCR processed with Alcalase, Corolase and Flavourzyme resulted in products with noticeable different taste attributes.

The hydrolysate product from Flavourzyme was associated with a somewhat salty, perhaps even slight broth-like taste. The fermentation process with MF 1127 and IS 93 seemingly altered the taste towards a strong/intense bitter or even astringent taste.

The hydrolysate product from Corolase were perceived as somewhat sickening or disgusting mild and bitter taste. Fermentation with IS 93 seemed to alter the taste towards a more neutral flavour (not particularly resembling a distinct taste). MF 1127 progressed somewhat in the same manner although was perceived as slightly more bitter.

The hydrolysate product from Alcalase was plotted far away from any discriminatory attributes although the taste may perhaps be somewhat metallic or a strong bitterness. Fermentations seemed to further increase the intensity in flavour and was characterized as stringent. Both MF 1127 and IS 93 seemed to be quite similarly perceived in taste.

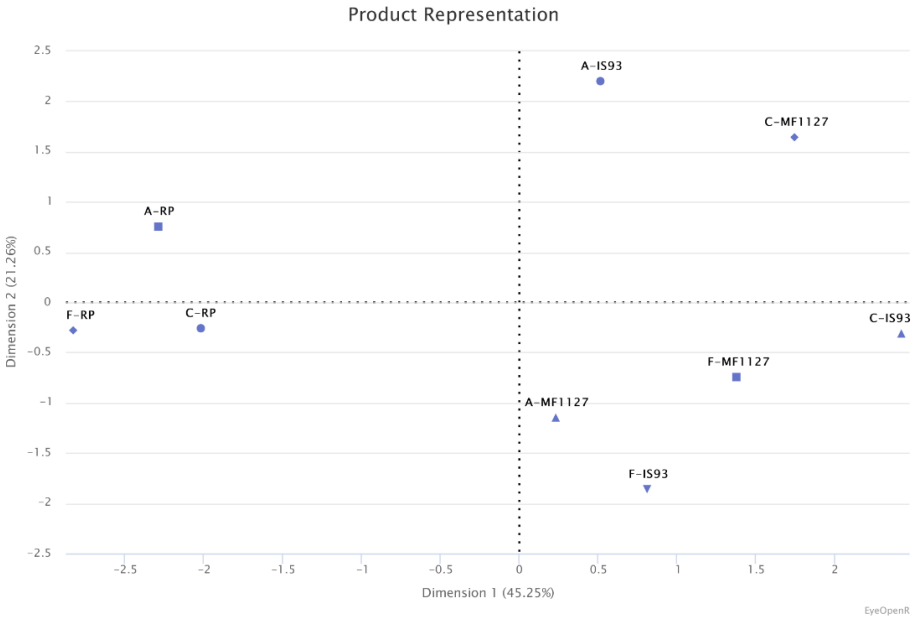


Figure 5.6.3: Shows the relative similarities and differences of BC products of raw product (RP) hydrolysed by the three different enzymes (A, C, F) and compared to the fermented products of MF 1127 (A-MF1127, C-MF1127 & F-MF1127) and IS 93 (A-IS93, C-IS93 & F-IS93). The corresponding graph 5.6.4 shows the perceived attributes. The placement within plots will correspond the attributes localized in the same area. The graph was produced by collected data from Napping analysis using EyeOpenR statistical analysis tool.

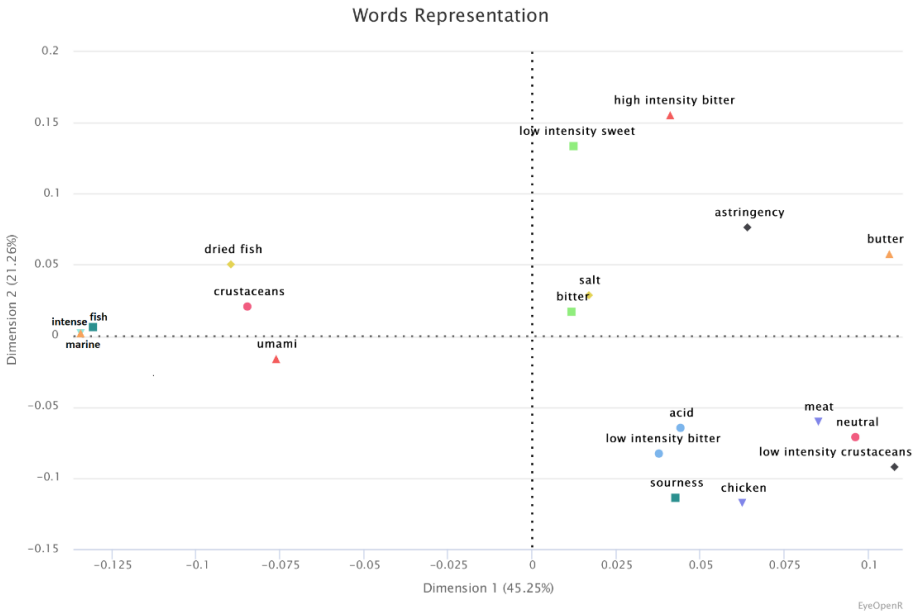


Figure 5.6.4: This figure shows the perceived attributes from napping analysis for BC samples. The attributes shown in the graph correspond to the samples of the plots in 5.6.3 localized in the same graph-area. The graph was produced using collected data from Napping analysis by EyeOpenR statistical analysis tool.

The BC processed with Alcalase, Corolase and Flavourzyme resulted in a raw-product with seemingly similar characteristics and attributes, namely a dried-fish, fish or crustacean-like flavour with Corolase tasting more of umami.

The raw product form Flavourzyme was associated with an intense marine/fish-like taste, whereas raw product from Alcalase was associated with a dried fish or crustacean-like taste. The raw product of Corolase was more closely associated as umami, perhaps crossing towards a crustacean-like taste.

The fermented products seemed to alter the taste in general towards a more tart, bitter or acidic flavour.

The raw-product from Alcalase fermented by IS 93 were perceived as intensely bitter, but yet somewhat sweet, whereas MF 1127 were perceived as more acidic, sour or bitter in taste.

The raw-product from Corolase fermented by IS 93 and MF 1127 were perceived as somewhat similar with attributes for MF 1127 as being somewhat butter-like or stringent in taste, whereas IS 93 was perhaps more butter-like or neutral in flavour.

The raw-product from Flavourzyme fermented by IS 93 and MF 1127 were perceived as quite similar in taste and was more closely associated with a meat-like flavour although yet acidic, whereas IS 93 were recognized as more sour, bitter and chicken-like in taste.

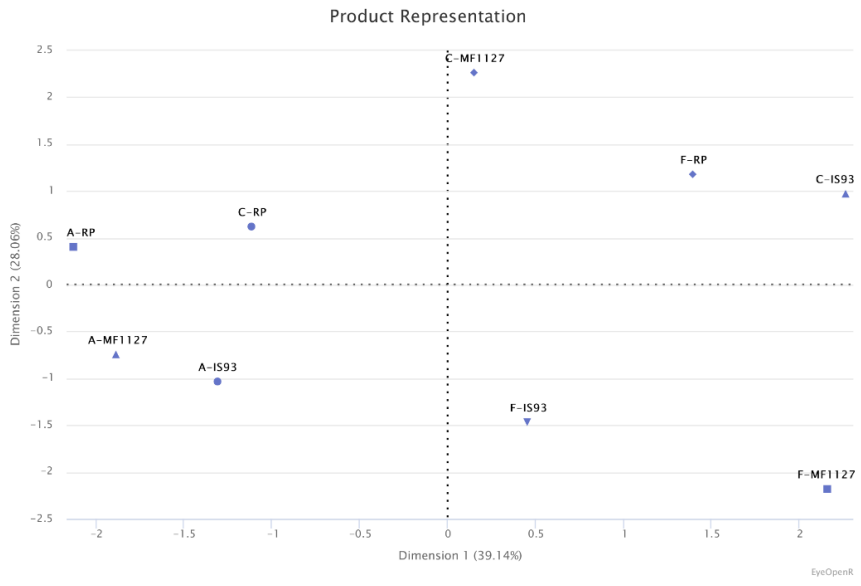


Figure 5.6.5: Shows the relative similarities and differences of WP products of raw product (RP) hydrolysed by the three different enzymes (A, C, F) and compared to the fermented products of MF 1127 (A-MF1127, C-MF1127 & F-MF1127) and IS 93 (A-IS93, C-IS93 & F-IS93). The corresponding graph 5.6.6 shows the perceived attributes. The placement within plots will correspond the attributes localized in the same area. The graph was produced by collected data from Napping analysis using EyeOpenR statistical analysis tool.

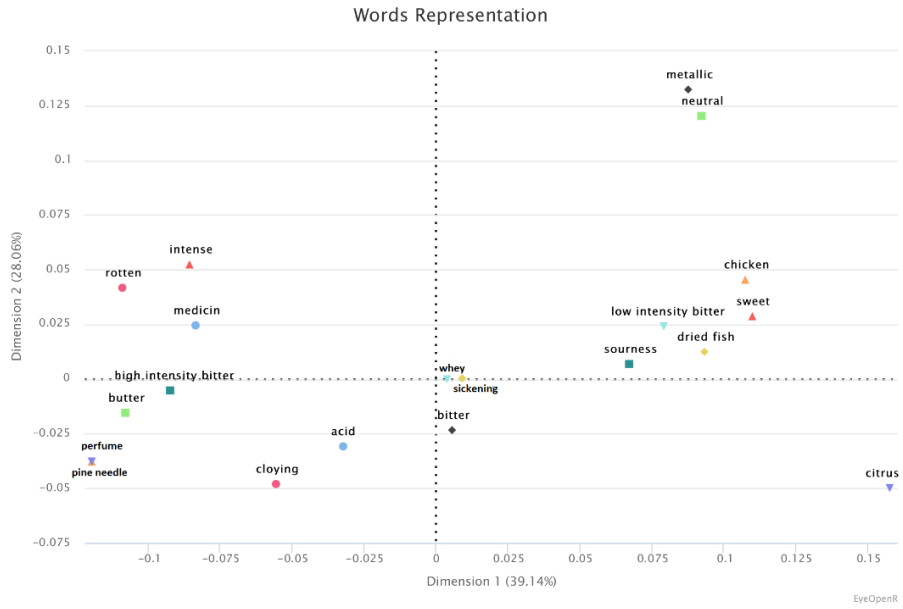


Figure 5.6.6: *This figure shows the perceived attributes from napping analysis for WP samples. The attributes shown in the graph correspond to the samples of the plots in 5.6.5 localized in the same graph-area. The graph was produced using collected data from Napping analysis by EyeOpenR statistical analysis tool.*

The WP processed with Alcalase, Corolase and Flavourzyme resulted in a hydrolysate product with seemingly different characteristics with Alcalase and Corolase being more similar to each other as compared to Flavourzyme. Alcalase and Corolase was both characterized as being somewhat intense with a taste of being rotten or even medicine-like. Flavourzyme produced a product that was best characterized as sweet or slightly bitter chicken-like in taste.

The hydrolysate product form Alcalase fermented by MF 1127 was associated with a perfume/pine needle or even bitter butter-like flavour, whereas IS 93 conceived perhaps some of the same characteristics, however was perceived as somewhat more sickening or disgusting in flavour.

The hydrolysate product form Corolase fermented by MF 1127 and IS 93 was not characterized as a particular flavour, although MF 1127 was more closely characterized as metallic or neutral in taste, whereas IS 93 were perhaps sweeter or chicken-like in flavour.

The hydrolysate product form Flavourzyme fermented by MF 1127 were perceived as being citrus-like in flavour, whereas IS 93 resembled a more bitter and sickening whey-like flavour.

6 Discussion

Food-waste is a global scandal and an enormous waste of resources. Today the industry is dictated by the economic aspects of the food production, it has simply been cheaper to discard of waste-products rather than to process and to further refine into additional products. This is perhaps most obvious when investigating the product-ratio of food to food-waste in the western world which ranges between 2:1 and 3:1 (Helgesen., 2013; Hall et al. 2009). It is imperative that strict guidelines are made in order to reduce food-waste all together, and to create a system where wasting high-quality resources is less economical compared to additional product-refining. Enzymatic protein hydrolysis is a good example of a gentle method for providing further utilization of a raw-material and to reduce the waste-fractions of valuable nutritional components to a minimum. The applicative use of enzymatic hydrolysis is increasing as a reflection of the process being an inexpensive method for an industrialized setting. However, it is still limited due to a few shortcomings of the method. Although enzymes and protein hydrolysis are effective and cheap methods for extracting proteins/peptides and lipids, the resulting peptide-fraction are often perceived as less palatable for us humans. The flavour-profile is an important criterion of hydrolysates utilized for human consumption. Improvements in the hydrolysis method could help improving the perceived taste of the end-products, or by developing additional processing-steps to improve the flavour perception.

In addition to their main function as a preservation method through fermentation, lactic acid bacteria (LAB) are today utilized to produce refined flavours in a wide variety of food-products. They do so by metabolising peptides and carbohydrates and produce additional compounds besides lactic acid in the food-product which may alter the flavour profile. The use of LAB may therefore be applicative to hydrolysates for improving the perceived taste and to further increase the product-value. Improving the end-products of hydrolysates and to maintain high-quality and desirable flavours would shift the economic incentive-scheme of the food-industry. This would mean a further refinement of raw waste-materials for downstream applications in food products for human consumption.

The use of LAB-strains as food modifiers in protein-hydrolysates for human consumption is a seemingly new application. As this field of science is still in its infancy, several aspects are yet to be discovered, and in order to do so we had to start from the very beginning, with protein hydrolysis, media preparations and growth experiments with trials and errors.

6.1 Author's remarks

This study includes both microbiological and biotechnological aspects each of which could perhaps be its own topic for investigation. To study the effect of lactic acid fermentation and its alterations to flavour in protein hydrolysates, while at the same time investigating the aspects of the respective enzymes and raw-materials used in the substrate-production is undoubtedly a difficult topic as a whole. The sheer size of the collected data and the experimental setup with several affecting dimensions makes it difficult to make decisive conclusions on the topic. For instance, it is not easy to explain how a strain performs in relation to flavour across three raw materials processed by three different enzymes, nor is it easy to determine good growth when including a large set of strains on the premise of multiple media-factors. To ensure that all strain-requirements are reached and to make sure false positives are of minimal occurrence is a rather difficult task in these regards. Furthermore, a seeming paradox of this study is to produce data on the perception of flavour. Each dataset produced by one bacterial strain contained (in this case) nine samples for napping-analysis, while the max number of samples was 30, and so to extrapolate the perception to taste by the LAB-genera based on our data is seemingly impossible. This would also mean that data on the flavour formation is merely an indication of the potential, and a lot of work is yet to be done. On the other hand, our data strongly indicates that by-products of chicken, cod and whey proteins processed by a hydrolysis reaction using enzymes (A/C/F) results in suitable materials for LAB growth, and so this study provides the foundation for further studies to elucidate the flavour formation by LAB.

In the following text, I will argue that this study has produced reliable data applicable to answering key aspects related to the use of enzymatic protein hydrolysis of co-products and furthermore, try to elucidate the effect of LAB as a food modifier of hydrolysate-products in order to affect the flavour formation.

6.2 Hydrolysis

The production of hydrolysates with MDCR, BC and WP using Alcalase, Corolase and Flavourzyme did mostly go as predicted with quite similar products in both duplicates of each enzyme in respects to brix measurements. However, exceptions did occur. This was especially noticeable in MDCR_C product where four enzymatic hydrolysis-reactions was performed in order to produce two duplicate that was seemingly similar (based on Brix measurements). Furthermore, the progression of each hydrolysis process in our study was interpreted in a bachelor's thesis written by Aurélien Godard (2017); the use of FT-IR analysis of hydrolysates of animal origin (not available Online). A plausible interpretation is that the hydrolysis progress is seemingly predictable until 60 minutes with the relative composition of peptides shifting towards shorter fragments and free amino acids. However, in certain cases, the 80-minute fractions are more similar to the fraction at 50 minutes, in relation to the relative composition of peptide fragments. This observation indicates that the enzymatic hydrolysis is quite effective until 60 minutes but shows a reduction in the efficiency afterwards, which may indicate that the amount of available substrate for the enzymes to hydrolyse is reduced. Out of all raw-materials, hydrolysates from MDCR showed more variation at 60 and 80 minutes compared to the other raw-materials. Based on some observations made after completed hydrolysis this may be due to the use of a propeller in the hydrolysis reactor core, which spun the chicken material into a lump around the propeller-blades. This may have led to materials being unavailable for the enzymes, and individual occurrences' in each hydrolysis-reaction may have led to different amounts being clogged together. This was not a problem shared in other raw-materials that dissolved more evenly and may help explain why the protein recovery in chicken was less efficient in comparison.

From the three by-products, the highest hydrolysis yield in terms of protein recovery was achieved with cod-materials and Corolase with ~53% (theoretically BC_A is around 50% as well). Both Alcalase and Corolase achieved closely the same yield in the same materials (less than 2% difference), whereas Corolase resulted in higher yield in chicken-material and Alcalase more in whey protein (BC not included due to sample-losses). The differences seen between chicken and other materials is likely due to the elevated levels of collagen found in land animals, needed to maintain structural integrity. Flavourzyme produced in general less yield with about 25% protein recovery in chicken and whey. However, from cod-materials the recovery was high with almost 45%. Whey and Cod gave the highest protein recovery in most cases being between 44-53% (excluding WP_F ~27% and BC_A, with a theoretical recovery of ~53%), whereas the chicken proteins were somewhat lower with a recovery of 24-35%.

6.2.1 Media preparation

The finished SMAPT-media was prepared by centrifugation and sterile-filtration in contrast to the initial plan of autoclavation. This was due to the drastic change in colour seen in all growth mediums. Later Dumas and Kjeldahl-analysis validated that minimal loss of nitrogen or proteins occurred through-out the centrifugation process. However, the use of both Dumas and Kjeldahl-method elucidated a gross discrepancy in the measurement and results between the two methods. For instance, in materials of chicken and cod, the values deviated with 9-10%, whereas for whey protein ~5-6% after conversion by TKN (6.25 and 6.38).

Initially each produced hydrolysate was measured using the Kjeldahl-method (table 5.2.1). Based on the results from the Kjeldahl analysis, each media was measured to contain ~20g/l proteins. Each hydrolysate contained an impurity (not proteins) which was in most cases centrifuged to a minimum. The reduction in added weight might have increased the protein concentration somewhat, but not significantly. However, potential nitrogen-loss was investigated by use of the Dumas method. After accounting for any additional nitrogen from other sources, it seemed as if the real protein concentration in the SMAPT media was approximately 5-10% higher, which was further explained by the difference in methods mentioned above.

The mean deviation between Dumas and Kjeldahl method of 3-12% was based on the conclusion drawn by Oftedal et al. 2014. Whereof other studies have previously reported similar results; Simonne et al. 1997 and Thompson et al. 2002. In Our case this seems to be quite accurate to the actual 5-10% observed in this study. Due to the discrepancy between the two methods (table 5.2.1 and A3.3), this indicates that the corrected content of each finished SMAPT media was roughly at 21-23g/l protein. It is believed that Kjeldahl-analysis is more accurate towards measuring protein content of a sample whereas the Dumas method reportedly overestimates the crude protein content and therefore most results based on the Dumas method were 5-10% lower in reality. It is also believed that factors such as homogeneity of the measured samples and the preparation of media may have caused slight deviations (observed between parallel measurements). Furthermore, a small reduction in %nitrogen was observed in MDCR_F. This was likely due to the formation of a gel in the filtration and centrifugation step, roughly accounting for 10% of the total volume of the media. The gel likely formed a complex which trapped the reported missing ~1% nitrogen but also a relatively large amount of water. It makes sense to expect that this led to an increase in the protein concentration of the finished MDCR_F media.

Lastly the results from the protein-assay was deemed as inaccurate in determining the protein content. This was likely due to the complexity of the SMAPT-media with several reagents that could possibly skew the results.

6.3 LAB Growth in Bioscreen-C

In order to further investigate the perceived taste of each hydrolysate product, each media had to be able to sustain growth by LAB. Arguably good growth was observed by several strains achieving up to and above ~1.7 OD_{max} across multiple growth media (especially MDCR and BC series). Only whey protein produced a media with moderate capabilities to sustain LAB growth dependant on the used enzyme. In media with whey protein, both Corolase and Flavourzyme outperformed Alcalase with OD_{max} values up to 20-35% higher across multiple strains. Based on the growth observed in the nine hydrolysate medias; the chicken materials produced the best growth results, closely followed by cod and lastly by whey proteins. Although the highest growth was observed in MAPT with close to 2.0 OD_{max}, few strains achieved noticeably higher OD in this media compared with one or more of the SMAPT media. The Bioscreen-C results gives a strong indication that chicken, cod and whey are suitable protein-sources for growth-media for microbial applications. In general, among all tested strains, the average max growth was seemingly similar within the same waste-materials across the three enzymes (A/C/F) with exception to the whey proteins. The choice of enzyme clearly affects the achieved growth and OD_{max}-values in whey protein products. The growth was best supported in Flavourzyme compared to Corolase and Alcalase with 36.6 and 16.1% increased growth respectively (based on the observed OD_{max}-values of the averaging strains, excluding the best performing strains). Out of all the media types produced with hydrolysates it was MDCR_C that seemed best suited to sustain bacterial growth. The growth was close to identical to the once seen in MAPT, although perhaps slightly better in MDCR_C due to the more uniform growth-curves it produced (few deviations or spikes in the graphs, with smooth log-phases all the way to stationary-phase).

Interestingly a few different trends occurred throughout the growth experiments. In general, the growth curves of the bacteria's progressed in four different ways; (1) short lag-phase with a fast exponential phase followed by an abrupt stationary-phase; (2) similar to first mentioned although lower OD_{max} and slightly longer lag-phase; (3) about doubled lag-phase with a good log-phase and highest OD_{max}-values, followed by a smooth transition to stationary-phase; (4) long lag-phase, slow log-phase with some occurrence of not reaching stationary-phase within 48hours. These trends were most obvious in MDCR_C and MAPT although

visible across all growth mediums, perhaps less noticeably in whey protein and MRS. The highest growth was observed in the first growth trial with MRS, MAPT and MDCR_C/A. MRS contains about four times the amount of carbohydrates compared to MAPT and SMAPT-media making the media less comparable. With that said, MRS was able to provide some degree of growth from all 46 strains whereas the same could not be said for MAPT/SMAPT with four strains performing particularly poorly compared to others (MF 2033, MF 2983, MF 1965 and MF 6580). Although growth was higher in MRS (and MAPT in some cases) this difference was minimal and both chicken and fish (cod) raw-materials produced comparable results to the two basis media. Both MRS and MAPT are known to sustain good growth in LAB, whereas the similarity in max growth may indicate that both protein-sources of cod and chicken are good sources for complex nitrogen for microbial applications (same conclusion was drawn by Aspmo et al. 2005, in regard to Cod proteins from viscera).

With no exception, the access to carbohydrates was shown to greatly affect growth. Due to the abrupt ending of the logarithmic growth seen in MAPT and SMAPT, it is reason to believe that growth could have continued somewhat further with higher glucose concentrations. With this said, the goal was not growth optimization but rather indicating good candidate strains for growth in SMAPT. Based on the growth rate and OD_{max}-values form all bacteria's (Bioscreen-C Trial A), several high performing strains could be observed. Whereof, MF 5214, MF 1127, IS 361, IS 204, IS 93, IS 79 and IS 118.b was chosen for further studies. MF 150 and MF 110 were initially added to validate that temperature was not the limiting condition for the growth observed by the two strains. This was subsequently proved to be true. Due to this, MF 150 and MF 110 were excluded from further studies. In retrospect it was noted that MF 3579 could have been one of the candidate strains (being perhaps the best strain from Bioscreen-C trial A).

Further investigation of carbohydrate dependency of the LAB candidates gave somewhat different reactions to alterations in glucose and other growth-promoting components. The results from growth in SMOPH (12.5mM glucose) indicated that perhaps IS 93 and MF 1127 performed best across media with less nutrition (with incidents of poor growth in certain media by MF 1127, BC_A/C and WP_C). however, with SMOPH_0mM it seemed to be only MF 1127 and MF 5214 that was able to grow noticeably on MDCR and BC media, whereas all strains grew in WP media. This may indicate that the proteolytic abilities of the LAB are better suited for whey proteins in a solution due to the natural behaviour of casein with branches of amino acids being highly flexible and allows for peptide-cleavage (Holt & Sawyer., 1988), whereas this occurs less frequently in BC and MDCR media. Alternatively, trace amounts of lactose and glucose may be found in the whey products and may give subsequently support for growth.

The differences in results from alteration of glucose concentrations may indicate that MF 1127 and IS 93 perhaps were not the best suited strains based on growth, but rather belonged to a larger group of potentially well-suited strains. Furthermore, several candidate strains did prefer a few media types opposite to others. These strains are perhaps good at changing taste and flavours in specific media but falls short in terms of growth capabilities sought out in our candidate strains used for Napping-analysis. The effect on flavour from each LAB is perhaps more obvious when looking at the perceived flavours from each non-fermented hydrolysate in relation to improvements in flavour of the fermented products from MF 1127 and IS 93 (results 6.4).

Lastly, on the topic of Bioscreen-C data it is important to mention that all data was manually produced using excel with established methods for estimating Lag-phase, µmax and OD_{max}. However, data-programs do exist which does this work in a bulk-collection, namely GrowthCurver CRAN-R for R-studio. Regrettably, this was discovered late in the course of the study. A working program would allow a perfect fit of each growth-curve out of 400 possible fits with the highest possible R-squared value based on the three replicates for each series (not obtained in this study). R^2-value could also be obtained by a F-test or "moving average" using "analysis-toolpak" in excel but was deemed unnecessary for the purpose of this study due to the size of the data-set. Although commonly used, averaging replicate-series to produce bacterial growth-curves is less accurate than a comprehensive adjustment of a graph based on the replicates. This would mean that the data from the Bioscreen-C are not definite but rather an approach to explain each strains growth-phase. This would also hold true for all parameters obtained though Bioscreen-C trials; µmax, ODmax and lag-phases, which is rather an approximation to explain the difference in growth and not definite values.

6.4 Amino acid metabolism

SEC-analysis was used to try to explain the relative change in fractions of differently sized peptides from four LAB strains and how they metabolize the raw materials in respects the different enzymes. The SEC results are not comparable to growth results directly as OD_{max} and μ_{max} values were not obtained for the SEC samples, and so the change in peptide fractions may not be reflected by a growth observed earlier in the study by a given LAB strain. However, the SEC results gave a possible approach to try to explain the flavour perception retrieved from the projective mapping of each food product and fermentation process. This would mean that the projective mapping and the peptide profiles are not directly comparable but rather helpful if a pattern emerged. Four of the seven remaining candidate strains was used to investigate the peptide metabolism. The choice of candidate strains for which the peptide metabolism was investigated, was reduced to four strains due to limitations in regard to the remaining time and the work needed to complete all samples.

Interestingly, the results from the Bioscreen-C indicates that products of the same raw-material are perhaps close to equally good to sustain growth. However, when looking at table 5.6.1 its seems to be quite different amounts of peptides that are utilized by each bacterium. This may indicate that growth is not necessarily directly depicting the bacteria's peptide utilization, but rather a function of the dependency in carbohydrates and the energy cost of peptide metabolism. In general, based on the peptide metabolism, all strains perceived to change or shift the peptide fractions significantly in the products from Flavourzyme, while at the same time affecting mostly the same peptide fractions (across all raw-materials).
Somewhat of the same observation was in products processed by Corolase with exception to IS 93 whereof a drastic change in the peptide fraction was observed with ~35% reduction of the total peptides available and perhaps the highest degree of fermentation observed (based on peptide fractions). In general, a bigger change was observed in the relative peptide sizes of products processed with Flavourzyme compared to the other enzymes. Furthermore, oligopeptides seemed to be the metabolized fragment-size in products processed by Corolase and perceived fermentation processes which affected the peptides fractions considerably. Purely based on the relative change in peptide fractions, Alcalase produced a product which was not well liked by the LAB strains, shown in the low metabolism of peptides.

The identification of the analysed strains is found in table 3.1 and 3.2 and all strains analysed by SEC had different ID, namely; *L. plantarum* (IS 93), *L. paracasei* (IS 204), *L. sakei* (MF 1127) and *C. divergens* (MF 5214). The peptide-profiles of each strain gave several indications of patterns between the four chosen strains although individualism was a factor. For instance, L. plantarum and *L. sakei* showed similar preferences towards metabolism of the same fraction of peptide-sizes, same as with *L. paracasei* and *C. divergens* respectively, although none performed identical. This would perhaps mean that fermented products of *L. sakei* and *L. plantarum* would have more similarities compared to the products from *C. divergens* and *L. paracasei* and vice versa. Looking at the resulting metabolism in all products from Alcalase and Corolase (table 5.6.1) it becomes somewhat apparent that each strain would produce quite specific products in relation to taste. This would entail that growth is perhaps not a good criterion for screening of LAB in protein hydrolysates in relation to flavour developments. Screening for flavour in LAB would demand more copious exploration of the fermentation process, peptide-profiles and the effect of LAB on flavour from multiple strains. A wide understanding of multiple lactobacilli could perhaps elucidate and forecast flavour alteration by the given LAB strains in a group of food products. The comparison of the peptide metabolism and perceived taste in this data-set, does however not indicate that strains with similarities in metabolism would produce similar perceptions to taste. However, it does illustrate strains individual differences with a dependency of the given substrate which dictates the perceived flavours. whereof, it is difficult to explain how flavour formation proceeds based on this data alone. Perhaps most interesting was the difference in the utilization of peptide sizes that resulted from hydrolysis of each enzyme. For instance, there was no close similarities in the peptide uptake by each bacterium in materials processed by different enzymes, although similarities between strains was not uncommon. This indicates that the use of enzyme affects how the LAB strains will metabolize the substrate. This may also indicate that the potential for flavour improvements is perhaps found in materials and enzymes that promotes the utilization of shorter peptides or vice versa.

The results with regard to flavours is perhaps somewhat random in nature and may be explained by the size of the data-set provided (table 5.6.1.). Aside from the mentioned data-size, a clear weakness of this method lies in the measurement itself. The spectral region of peptides and proteins are found by wavelengths of <190 and from 190-380 nm, giving detection to saturated/mono-saturated peptides and poly-unsaturated and aromatic compounds respectively (kolpik R., 2009). With this said, further studies have indicated that wavelength of 214nm and 254nm are best suited to detect free amino acids, di-, tri-

and oligo-peptides (Silvestre et al. 2012). By detecting peptides using 214 and 254 nm it is expected to observe the majority of compounds at a given size, although not all can be detected. Furthermore, it is reason to believe that the fractions F1 to F4 in table 5.6.1 are the relative fraction-sizes. Meaning that not all peptides of a given size may absorb at the expected wavelengths and so some peptides may be wrongfully placed into another fraction. Furthermore, the separation by size from the utilized column is more suited for peptides of 2-15 amino acid residues, and so the free amino acids and peptides above 15 residues are underrepresented. This means that mainly the aromatic amino acids are detected in the free amino acid range. Furthermore, the fraction of bigger oligopeptides is likely to be truncated or compressed. This would entail that the importance of F1 and F4 is likely to only have an impact if the relative peptide fraction is dramatically changed. However, the fraction F2-F3 is assumed to be quite accurate although not perfect. Furthermore, for the comparison of peptide metabolism and perceived flavours it would have been beneficial with OD measurements of each fermentation trial A and B (SEC-analysis and Napping-analysis). Although growth was observed in each case, one cannot know if they arose to the same values. To improve the use of SEC in microbiological applications would include continues growth data and perhaps improving the segment fractions of different peptide sizes to be more accurate. A possible use of SEC to evaluate changes in a peptide fraction may be produced by a new experimental setup, with an active LAB inoculation which is sampled, sterile-filtrated and analysed by SEC continuously throughout fermentation. This may show the nature of the peptide uptake over time and give further insight to LAB's flavour formation in food products.

6.5 Perceived taste of fermented products

The results from Napping trials and the perceived flavours was produced by an internal semi-trained judge-panel. It was expected that all fermented samples would be perceived as mildly sour or acidic compared to the non-fermented sample (confirmed by measured pH produced by the elevated amounts of lactic acid and reduced glucose concentrations). However, it was hoped that one could link this to the subsequent changes in the peptide profile that would perhaps lead to palatable flavours, or that a fermentation process would help to mask the bitterness of a given sample. The screening of flavour formation was limited to two LAB strains (out of the seven remaining candidate strains) due to limitations of the sensory panel and the restricting number of samples viable for a sensory screening. Furthermore, due to scarcity of MDCR_F media and some WP media it would not be possible to produce samples from all media substrates.

Interestingly it was not observed any clear trends from any LAB strains in relation to the use of enzyme nor raw-material (substrate). This perhaps means that a wide range of LAB may produce small differences in the food product which is perceived differently in taste. This may indicate that improved taste due to LAB fermentation is somewhat strain-specific with dependency to the given media. Furthermore, the complexity of flavour perception is likely to be affected by several other factors not included in this study. For instance, the utilization of peptide sizes in this study does not explain what specific peptide chain which is utilized or too what extent, nor how these changes affects taste in the end-product. In order to perhaps explain the relations between peptide composition and flavour perception, an in-depth analysis of single peptides is required. A detailed understanding of the 3D structure of single peptides may help explain their perception to taste when compared to the respective sensory receptors and their dimerization's (Hervé., 2012; Temussi., 2011). Furthermore, another aspect not accounted for in this study is the interactions between peptides and minerals, salts and other peptides which may further affect taste (Temussi., 2011).

Based on our results from the napping analysis the choice of enzyme is perhaps the most important aspect when producing hydrolysates. The taste of each product processed with the three enzymes seemed to be in general better in Flavourzyme and Corolase compared to Alcalase with occasionally pleasant perceived flavours (perhaps most noticeable with Flavourzyme in cod and whey proteins). The relevance of product choice in regard to enzyme usage was also somewhat important, with generally better perceived flavours in Cod materials (all enzymes), whereas less pleasant flavours was described in some chicken- and whey protein products. This was perhaps especially noticeable in whey protein products. Whey proteins were in general perceived as having undesirable sensory properties after hydrolysis (with exception to

Flavourzyme) and so any fermentation with resulting improvements in the flavour-profile would indicate a potential.

According to the perception of flavour, one product from each strain showed potentially good improvements compared to the non-fermented sample. The perhaps most promising results was observed with IS 93 in Whey protein processed with corolase where an astringently bitter medicine or rotten flavour in the non-fermented sample, was changed to tasting mildly sweet chicken with perhaps somewhat bitterness after fermentation. The initially perceived taste of Whey proteins was in general characterized with unwanted sensory attributes (with perhaps slightly better result in regard to Flavourzyme). Whey proteins processed by flavourzyme had a mild sweet chicken or bitter/sour dry-fish-like flavour and was characterized as quite similar to the fermented product of IS 93 with WP_C. This observation may give indications of the potential of lactic acid fermentation to improve flavour-profiles, while at the same time elucidate the importance of enzyme choice. For the most part the non-fermented product of chicken and cod were perceived as more palatable compared to the fermented products, meaning that the hydrolysis in itself produced on average a better product for human consumption (chicken and cod). However, some products were different. For instance, in the case of IS 93 in cod processed with corolase where the raw material was initially perceived as Umami or crustacean-like with an “after-taste” of dry fish. The fermentation process removed most of the “after-taste” and was perceived as mildly meaty or crustacean-like in flavour.

Although not all flavour-profiles improved to a degree where the fermented product was perceived as pleasant or palatable, some did improve relatively to the non-fermented product. For instance; Fermentation of whey proteins processed by alcalase produced objectively better flavours opposed to hydrolyses itself. Interestingly, citrus and pine-needle was described in some product of MF 1127 (flavourzyme and alcalase respectively). The improvements seen by IS 93 and MF 1127 in some hydrolysates may illustrate a potential for further optimization of the use of LAB-strains to improve flavouring.

It is worth mentioning that for that most part LAB-fermentation resulted in less desirable flavours. The flavour improvements were in comparison a rare occurrence, whereas more bitter or astringent flavours was generally associated with LAB-fermentation. This may be due to the Lactic acid formation being too high (max fermentation) which ultimately increased the sour or tart flavours. whereas a controlled fermentation or addition of carbohydrates to the end-product may help to improve the flavour-profiles of hydrolysates. Furthermore, it is possible that heat-treatment is a vital processing-step for improving the

flavour perception of each product. Prior to Dumas-analysis of all products it was noticed that heat-treatment at 60^{o}C dramatically improved smell and flavour of some hydrolysates. This was however discovered with a lack of experimental setup, making this a mere observation for further investigation. Furthermore, due to observations made in this study, Heat-treatment, smoking or acidity regulation is perhaps methods which could further help to improve the poor flavour profiles of some hydrolysates obtained.

Interestingly enough, a relatively low metabolism of peptides was observed in some products that gave relatively superior flavours (Assuming that equal fermentation occurred prior to SEC analysis and Napping analysis). In particular, IS 93 in whey protein processed with corolase seemed to undergo minimal alterations of the relative peptide fraction while still experiencing drastic changes to the perception of taste. On the other hand, a huge change in the peptide fractions may lead to a low impact on flavour. An observation seen with IS 93 in cod processed with Corolase. The initial raw-product was characterized with an “after-taste” of dry fish which was reduced after roughly 35% of the peptides was metabolized while still maintaining other attributes. This discrepancy between degree of fermentation and the change in sensation of taste does not harmonize with prior expectations made before experimental trials. In theory it was expected to see an increasing change in taste which would be relative to the degree of fermentation. In its simplicity this would mean that longer fermentation would give a big relative change of peptide-fraction leading to an increased change of taste. However, this was not the case in this study and may indeed indicate that partly fermenting the product may lead to a wide range of flavours and that fully fermented products are not necessarily related to palatability. Because of this observation it is perhaps not only the choice of strain which is important when selecting LAB strains for protein hydrolysate modification. It may be of equal importance to control the cultivation period. However, it is important to remember that the data on flavour formation in this study are rather limited and so observations made in this study may not be supported if a more comprehensive dataset was produced.

Another shortcoming of the experimental setup was, the size of the Napping-analysis with 27 samples. This may have been too much information to process and categorize in one session. On the other hand, to explain the flavour development by LAB will require a more extensive investigation and so testing two strains against the raw-material is not representative for the bacterial-group. Furthermore, sterile-filtration may have skewed the results of the sensed flavours and is likely to be different from the product with the bacteria present. All samples were treated in the same manner, so any change would occur in all samples. However, for an industrial setting, it might be preferable to keep the LAB strains in the finished product

and so in this regard, the fermented hydrolysates may not illustrate the same flavour-profile which desired for an industrial setting. The sterile-filtration was, however, necessary to do in this study due to concerns of antibiotic resistance in the tested strains. Furthermore, it is important to note that the antibiotic-resistance test was limited but was a mere indication if antibiotic resistance would be an issue. The use of a standardised methods for investigating antibiotic resistance with a screening towards multiple antibiotic agents would perhaps give a negative result in regard to the overall antibiotic resistance perceived by each strain (Korhonen et al. 2008) (was not available for the study at that current time). Especially in the case of ampicillin resistance where resistance was observed above 25µg/ml, it is reason to believe that the experimental setup was flawed. Based on earlier reports on Lactobacilli and antibiotic resistance indicated that most Lactobacilli was susceptible for ampicillin at 8µg/ml (Korhonen et al. 2008). Furthermore, in a discussion with Axelsson L. on the results from ampicillin it was state that the available ampicillin was probably outdated and where the use of MRS is thought to further affect the antibiotics in a negative manner which inhibits the effect of antibiotics. This may help explain the big difference in antibiotic resistance which was seen between erythromycin and ampicillin. Furthermore, some of the LAB strains utilized in this study was among prior investigated strains at Nofima with results that directly disputes the results in this study. in the revelation of this information it is reasonable to believe that the ampicillin used to investigate the antibiotic resistance was out of date and so the results on ampicillin resistance is likely not true where susceptibility would likely be found in the range of ~8µg/ml.

7 Conclusion

Throughout the progression of this thesis the goal has been to explore the possibilities of LAB fermentation and its effect on flavour development in different processed raw materials from the food industry. Our findings indicate that processing hydrolysates by LAB is a possible method for improving flavour-profiles, although too achieve “palatable flavours”, further work is demanded to find the right LAB relative to the choice of raw-material. The choice of enzyme was found to be of high importance when producing hydrolysate for LAB fermentation. In certain cases, the hydrolysis produced arguably good products. Furthermore, our data indicate that the choice of enzymes dictates the peptide uptake of the bacteria by giving access to differently desirable peptide sizes and perhaps sequence. The uptake of shorter peptides was thought to affect taste more than bigger peptides and so this observation is likely to be of importance when investigating the change of flavour.

Interestingly our data seems to disprove some prior expectations. For instance, increased utilization of peptides was expected to give higher effect on flavour formation, whereas this work shows that this might not be the case. Although the example in mind was IS 93 WP_C where max fermentation took place, the peptide-profile comparison may indicate that almost no peptide uptake took place. The same was observed in BC_C with a high relative peptide change but little change to flavour. This observation with contradicting results may be indicative of a more complex nature of flavour development. This problem is perhaps solved by controlling the fermentation process or adding carbohydrates. Further observations may simply indicate that heat-treatments or acidity regulation may improve the flavour even further. It is regrettably still unknown what effect the LAB strain would have had on flavour if kept in the product. This is likely highly important information for an industrial setting and is yet to be explored.

With regard to the topic, the results presented on flavour development are rather limited and we have only scratched the surface with the first pioneering steps into the field of LAB fermentation as a food modifier of hydrolysates. Arguably, the few results on the improvement of flavour perception achieved by two LAB strains speaks of a much bigger picture of the potential of LAB for processing hydrolysed raw-material for commercial purposes.

The results on flavour changed by LAB fermentation may not be impressive as a whole but did, however, show occasional improvements. This is reflected by the observation that each strain shows to be quite substrate specific in relation to flavour improvements. Two strains are a very limited selection indeed of the large number of potentially useful LAB in this regard. In addition, the use of SEC as an investigative tool

towards bacterial peptide metabolism seems to produce reliable and distinguishable data, apart from a few shortcomings in regard to monitoring the fermentation degree, SEC may be an interesting new field for microbial applications. Although promising data have been revealed throughout this study, several key aspects are yet to be answered, and so the effect of flavour formation is far from concluded. Further research is warranted to address the topic of LAB as a food modifier of protein hydrolysates and may help to reveal the nature of flavour development in food processing of co- and waste materials by LAB.

8 Further Research

To our knowledge and as far as what could be found in the literature, the use of LAB fermentation on different protein hydrolysates is a new field of study, hence, multiple aspects are still unanswered. First, due to our small size of the napping analysis and projective mapping of flavour perception, it is reasonable to believe that multiple strains may be suitable for differently processed raw material. Temperatures of 30^{o}C and 37^{o}C gave small changes in the max OD and growth rate. The small change in peptide metabolism may be affecting taste even if the change may be small. In this case, raw-material or enzymes which in general produce poor perception to taste are especially of interest. In this case this would entail further studies on whey proteins but also the use of Alcalase and Corolase. In general, the perception of taste of the fermented hydrolysates was recognized as mostly bitter. This is likely further affected by the high concentration of lactic acid and subsequently low concentration of glucose giving a sour or tart flavour. Sweetening the end-product may produce better overall results from the napping analysis. Other approaches may simply be acidity regulation or using a controlled fermentation in order to maintain a higher sugar content. Furthermore, the observed discrepancy where the degree of fermentation is not necessarily in coherent with the degree of flavour development. Our data indicate that certain LAB may achieve better results in different hydrolysates by a controlled fermentation method. Additionally, the use of multiple strains in one inoculation process is commonly used in the food industry, and so it raises the question; How does the use of multiple strain inoculation affect the flavour development? Perhaps the most obvious criteria for further investigate is the effect of LAB strains on taste if they are kept intact in the food product. Another interesting and perhaps an important observation was the drastic improvement of smell which was produced from drying the samples (measuring water content). Preliminary observations indicated that treating the samples at 60^{o}C improved the flavour perception of the end-product.

9 References

Adler-Nissen J.A., (1986)., *Enzymatic hydrolysis of food proteins*., Elsevier Applied science publishers. London.

Alberts B., Johnson A., Lewis J.H., Morgan D., Raff M., Roberts K. & Walter P., (2014)., *Molecular Biology of The Cell*., 6th ed. Garland Science. Taylor & Francis Group. Chapter 12, 13, 14, 15 & 16.

Ardö Y., (2006)., *Flavour formation by amino acid catabolism.,* Science Direct, Biotechnology advances, 24(2). pp. 238-242.

Arsky, G.H et al., (2016)., *Kosthåndboken – veileder i ernæringsarbeid i helse- og omsorgstjenesten*., Helsedirektoratet. IS-1972. Andvord Grafisk AS. (Norwegian).

Aspmo S.I., Horn S.J. & Eijsink V.G.H., (2005*)., Use of Hydrolysates from Atlantic cod (Gadus morhua L.) viscera as a complex nitrogen source for lactic acid bacteria.*, federation of European Microbiological Societies letters 1;248(1):65-8.

Axelsson L., Ahrné S. (2000) *Lactic Acid Bacteria*. In: Priest F.G., Goodfellow M. (eds) *Applied Microbial Systematics*. Springer, Dordrecht.

Axelsson L., Rud I., Naterstad K., Blom H., Renckens B., Boekhorst J., Kleerebezem M., Hijum S. V. & Seizen J. R., (2012)., *Genome sequence of the Naturally Plasmid-Free Lactobacillus plantarum Strain NC8 (CCUG 61730)*., American Society for microbiology. Bacteriology. 194 (9). pp. 2391-2392.

Axelsson, L., 2004. Lactic acid bacteria: classification and physiology. In: Salminen S., von Wright A., Ouwehand A. (Eds.)., Lactic Acid Bacteria: Microbiological and Functional Aspects. 3rd Ed. Revised and Expanded. Marcel Dekker Inc. CRC Press, New York, pp. 1-66

Azarkan M., El Moussaoui A., Wuytswinkel D., Dehon G. & Looze Y., (2003)., *Fractionation and Purification of the enzymes stored at the latex of Carica Papaya.,* Journal of Chromatography B. 790(1-2): p. 229-238.

Baker P.J. & Numata K., (2013)., *Polymerization of peptide polymers for Biomaterial applications.,* Polymer Science. Chapter 9. pp 229-241.

Barth H.G., Boyes B.E. & Jackson C., (1996)*., Size exclusion chromatography*., Analytical chemistry. 68(12): p. 445-466.

Berntsson R. P-A., Doeven M. K., Fusetti F., Duurkens R. H., Sengupta D., Marrink S-J., Thunnissen A-M. W.H., Poolman B. & Slotboom, D-J., (2009)., *The structural basis for peptide selection by the transport receptor OppA.,* The EMBO journal. 9:28. 1332-1340.

BIOSEP™-SEC-S specification chart, 2017., Phenomenex. Available at: https://www.brechbuehler.ch/fileadmin/redacteur/pdf/columns-sampleprep/lc-columns/zhbss.pdf.

Bremmer J.M. & Mulvaney C.S., (1982)., *Nitrogen-Total, methods of soil analysis, Chemical and Microbiological Properties*. Part 2., Agronomy Monograph 9 (2nd Ed).

Bucci L.R. & Unlu L., (2000)., *Protein and amino acid supplements in exercise and sport*. In: Wolinsky I., Driskell J.A (eds)., *Energy-yielding macronutrients and energy metabolism in sports nutrition*. CRC Press, Boca Raton. pp 191–212.

Calo-Mata P., Arlindo S., Boehme K., Miguel T.d., Pascoal A., & Barros-Velazquez J. (2008)., *Current applications and future trends of lactic acid bacteria and their bacteriocins for the biopreservation of aquatic food products*. Food and Bioprocess Technology, 1, 43 –63. doi:10.1007/s11947-007-0021-2.

Campbell-Platt G., (1987)., *Fermented Foods of the World. A Dictionary and Guide*., Butterworth, London, Boston, Durban u. a.

Chae J.H., In J.M., Kim H.M., (1998)., *Process development for the enzymatic hydrolysis of food protein: effects of pre-treatment and post-treatments on degree of hydrolysis and other product characteristics*., Biotechnol Bioprocess Eng. 3:35–39.

Chaijamrus S., (2002)., *Enzymatic Hydrolysis of renewable vegetable Proteins to Amino Acids.,* Hannover University, Hannover.

Chopin A., (1993)., *Organization and regulation of genes for amino acid biosynthesis in lactic acid bacteria*. FEMS Microbiology. Rev. 12:21-38.

Clark D.P. & Pazdernik N.J., (2012)., *Molecular biology*. 2nd ed., Academic Cell.

Crawford Scientific., (2013)., *Silica for HPLC stationary phases*., Available at http://www.Crawfordscientific.com/chromatography-technical-Tips-Silica-for-HPLC-Stationary-Phases.html.

Diffen., (2018)., *"Catalyst vs Enzyme"*. Diffen LLC, n.d. Web. 04.05.2018, available at: http://www.diffen.com/difference/catalyst_vs_enzyme

Doeven M. K., Kok J. and Poolman B., (2005)., *Specificity and selectivity determinants of peptide transport in Lactococcus lactis and other microorganisms.,* Molecular Microbiology 57(3), 640-649.

EFSA., (2013)., *updated list of Qualified Presumption of Safety (QPS) Status recommended biological agents for safety risk assessments*., European Food Safety Authority. EFSA Journal. EFSA Scientific Panels and Units. 4th revision.

Evans J. B., and Niven C.F., (1951)., *Nutrition of the heterofermentative lactobacilli that cause greening of cured meat products*., Journal of Bacteriology. 62, 599-603.

Gasson M. J., (1983)., *Plasmid complements of Streptococcus lactis NCDO 712 and other lactic streptococci after protoplast-induced curing*. Journal of Bacteriology. 154. 1-9.

Gold R. S., Meagher M. M., Hutkins R. & Conway T., (1992). *Ethanol tolerance and carbohydrate metabolism in lactobacilli*. Journal of Industrial Microbiology & Biotechnology. 10. 45-54.

Hall K.D., Guo J., Dore M., Chow C.C., (2009)., *The Progressive Increase of Food Waste in America and Its Environmental Impact*. Public Library of Science. 4(11): e7940.

Harris D.C., (2010)., *Quantitative chemical analysis*. Ed 8. Freeman W.H. Co Ltd.

Hauge J.G., (2009)., *Proteolytiske enzymer*., Store Norske Leksikon (Norwegian).

Hervé T., (2012)., *The Science of the Oven*., Columbia University Press, New York 2012, pp 26-32.

Holt C. & Sawyer L., (1988)., *Primary and predicted secondary structures of the caseins in relation to their biological function*. Protein Engineer. 2:251-259.

Holzapfel W. H., Wood B. J.B (eds)., (1995)., *The Genera of Lactic Acid Bacteria*., Blackie Academic and Professional. London. Chapter 1, 2, 3 & 4. pp 1-55.

Horn S. J., Apsmo S. I. & Eijsink V. G., (2005)., *Growth of Lactobacillus plantarum in media containing hydrolysates of fish viscera.,* Journal of Applied Microbiology. 99(5):1082-9.

Kawai M., Sekine-Hayakawa Y., Okiyama A., Ninomiya Y., (2012)., *Gustatory sensation of (L)- and (D)-amino acids in humans.* US National Library of Medicine. National institute of Health. 43(6):2349-58.

Klein G., Pack A., Bonaparte C. & Reuter G., (1998)., *Taxonomy and physiology of probiotic lactic acid bacteria*., International Journal of Food Microbiology, 41(2) pp. 103-125.

kolpik R., (2009)., Ultraviolet and visible spectrometry; theoretical overview. UV/VIS spectrometry., Advanced strategies in food analysis.

Korhonen, J. M., Danielsen, M., Mayo, B., Egervärn, M., Axelsson, L., Huys, G., von Wright, A., (2008)., *Antimicrobial susceptibility and proposed microbiological cut-off values of lactobacilli by phenotypic determination*. International Journal of Probiotics and Prebiotics 3. 257-268.

Kunji E. R. S., Mierau l., Poolman B., Konings W. N., Venema G., and Kok J., (1996)., *Fate of peptides in peptidase mutants of Lactococcus Lactis.* Molecular Microbiology. 21:123-131.

Kunji E. R.S., Mierau I., Hagting A., Poolman B. & Konings W. N., (1996)., *The proteolytic systems of lactic acid bacteria*., Kluwer Academic Publishers. Netherlands, Antonie van Leeuwenhoek 70: 187-221.

Kuratsu M., Hamano Y. & Dairi T., (2010)., *Analysis of the Lactobacillus Metabolic Pathway*., Applied Environmental Microbiology. 76. 217299-7301.

Lane D.J. (1991)., 16S/23S rRNA sequencing., In: Stackebrandt E., Goodfellow M., (eds). *Nucleic acid techniques in bacterial systematics*. Chichester, United Kingdom. John Wiley and Sons. pp. 115–175.

Lesk M. A., (2016)., *Introduction to protein science; architecture, function, and genomes*., 3rd edition. Oxford University press. United Kindom. chapter 1, 2, 5 pp. 21, 76, 209-211.

Liggieri C., Obregon W., Trjeo S. & Priolo N., 2009., *biochemical analysis of a papain-like protease isolated from the latex of asclepias curassavica L.,* Acta Biochimica Biophysica Sinica (Shangai) 41(2): p. 154-162.

Ludwig W., Schleifer K.H. & Whitman W.B., (2009)., *Revised road map to the phylum Firmicutes*. In: De Vos, P., Garrity, G.M., Jones, D. et al., (2009)., *Bergey's Manual of Systematic Bacteriology*. 2nd ed. Dordrecht, Heidelberg, London, New-York: Springer. pp. 1–13.

Mao B., Pear M., McCammon J., Quiocho F., (1982)., *Hinge-bending in L-arabinose-binding protein. The venus's flytrap model*., Journal of biological chemistry. 257: 1131-1133.

McLeod A., Brede D. A., Rud I. & Axelsson L., (2013)., *Genome Sequencing of Lactobacillus sakei subsp. Sakei LS25, a Commerical starter culture strain for fermented Sausage.,* Genome Announcement. 1 (4) e00475-13.

Mills O.E. & Thomas T.D., (1981)., *Nitrogen sources for growth of lactic streptococci in milk*. New Zealand Journal of dairy science and technology. 16:43-55.

Nadkarni M. A., Martin F.E., Jacques N.A. & Hunter N., (2002)., *Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set*., Microbiology 148 (1): 257-266.

Nelson G., Chandrashekar J., Hoon M. A., Feng L., Zhao G., Ryba N. J. P. & Zuker C.S., (2002)., *An amino-acid taste receptor*., Nature vol. 416, pp. 199–202.

Oftedal O. T., Eisert R. and Barrel G. K., (2014)., *Comparison of analytical and predictive methods for water, protein, fat, sugar, and gross energy in marine mammal milk*., Science Direct. Journal of Dairy Science. 9 (8). Pp 4713-4732.

Oliver J.M. & Duncan H.G., (2002)., US Patent 6387241; *Method of sterilization using ozone*

Olsen D., Chang R., Willimas K.E., Polarek J.W., (2010)., *The development of novel recombinant human gelatins as replacements for animal-derived gelatin in pharmaceutical applications*. In: Pasupuleti V.K. (eds) *Protein hydrolysates in biotechnology*. Springer. The Netherlands.

Olson N. F., (1990)., *The impact of lactic acid bacteria on cheese flavor*., FEMS Microbiology. 87:131-148.

OpenWetWare., (2016)., *McClean N.M: Colony PCR (yeast).* Web 05.05.2018, Available at: https://openwetware.org/mediawiki/index.php?title=McClean:_Colony_PCR_(Yeast)&oldid=939180

Pagès. J., (2003)., *Collection and analysis of perceived product inter-distance using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley*. Food Quality and Preference s, 16, 642-649.

Pasupuleti V.K. & Braun S. (eds.)., (2010)., *State of the Art Manufacturing of protein hydrolysates*. chapter 2 In: Pasupuleti V.K. (eds) *Protein hydrolysates in biotechnology*. Springer. The Netherlands. pp 11-30.

Perkampus H.H., (2012)., *Uv-vis spectroscopy and its applications*., Springer Science. Berlin. Chapter 1. pp. 1-2.

Pokusaeva K., Fitzgerald G.F. & Sinderen D. V., (2011)., *Carbohydrate metabolism in Bifidobacteria*., Genes & Nutrition. 6(3): 285–306.

Pontonio E., Lorusso A., Gobbetti M., Rizzello C. G., (2017)., *Use of fermented milling by-products as functional ingredients to develop a low-glycaemic index bread.,* Elsevier. Journal of Cereal Science, Vol. 77, pp. 235-242.

Ringø, E., Wesmajervi, M. S., Bendiksen, H. R., Berg, A., Olsen, R. E., Johnsen, T., Mikkelsen, H., Seppola, M., Strom, E., Holzapfel, W., (2001)., *Identification and characterization of carnobacteria isolated from fish intestine*. Systematic Applied Microbiology. 24. 183-191.

Rønningen P.O., (2017)., *Væskekromatografi, i Instrumentering, mobilfaser og stajonærfaser.*, HiOA. Oslo. (Norwegian)

Safari R., Motamedzadegan A., Ovissipour M., Regenstein J. M., Gildberg A., & Rasco B., (2009)., *Use of hydrolysate from yellowfin tuna (thunnus albacares) heads as acomplex nitrogen source for lactic acid bacteria.,* Food and Bioprocess Technology. 5: 1. pp 73-79.

Salvetti E., Torriani S. & Felis, G.E., (2012)., *The genus lactobacillus: a taxonomic update*., Probiotics and antimicrobial Proteins. 4:217-226.

Schleifer K. H. & Ludwig W., (1995)., *Phylogeny of the genus Lactobacillus and related genera.,* Systematic and Applied Microbiology., 14. pp. 461-467.

Schmidt D.G., (1982)., *Association of caseins and casein micelle structure*. In: Fox P.F. (Ed) Developments in Dairy Chemistry. 1. pp 61-68. Elsevier. London.

Silvestre M.P.C., Silva M.R., Silva V.D.M., de Souza M.W.S., Junior C. d. O. L. & Afonso W. d. O ., (2012)., *Analysis of whey protein hydrolysates: peptide profile and ACE inhibitory activity*., Brazilian Journal of Pharmaceutical Sciences. Sao Paulo. 48(4).

Silzyte R., Grimsmo L. & Storrø I., (2010)., *prossesering av bioråstoff fra sild til olje of protein hyrdrolysat*., SINTEF-Rapport ISSN 1504-9795. (Norwegian)

Simonne A.H., Simonne E.H., Eitenmiller R.R., Mills H.A., Cresman III CP., (1997)., *Could the Dumas method replace the Kjeldahl digestion for nitrogen and crude protein determination in foods?*., Journal of Science of Food and agriculture. 73(1):39-45.

Stiles M.E. & Holzapfel W.H., (1997*)., Lactic acid bacteria of foods and their current taxonomy*., international journal of food Microbiology. 36(1). pp. 1-29.

Stuart B., George W.O., Mcintyre P.S, (1996)., *Modern infrared spectroscopy*., University of Greenwich., John Willey & sons. p. 180.

Sun Z., Harris H.M.B., McCann A., Guo C., Argimón S., Zhang W., Yang X., Jeffery I.B., Conney J. C., Kagawa T.F., Liu W., Song Y., Salvetti E., Wrobel A., Rasinkangas P., Parkhill J., Rea M.C., O'sullican O., Ritari J., Douillard F.P., Ross R.P., Yang R., Briner A.E., Felis G.E., Vos W.M.d., Barrangou R., Klaenhammer T.R., Caufield P.W., Cui Y., Zhang H. & O'Toole P.W., (2015)., *Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera*., Nature Communications. 6:8322.

Temussi P.A., (2011)., *The good taste of peptides*., Journal of Peptide Science. 18(2):73-82.

Teusink B. & Molenaar D., (2017)., *Systems biology of lactic acid bacteria: For food and thought.,* Elsevier. Current Opinion in System Biology. 6. pp 7-13.

Theron M.M. & Lues J.F.R., (2007)., *Organic Acids and Meat Preservation: A Review*., Food Reviews International. 23:2, 141-158.

Thompson M., Owen L., Wilkinson K., Wood R., Damant A., (2002)., *A comparison of the Kjeldahl and Dumas method for the determination of protein in foods, using data from a proficiency testing scheme*. Analyst 127(12):1666-8.

Tymoczko J.L., Berg J.M. & Stryer L., (2015)., *Biochemistry, A short course.* 3 ed. Parker K.A., Freeman W.H and company. US.

Tynkkynen S., Buist G., Kunji E., Kok J., Poolman B., Venema G., Haandrikman A., (1993)., *Genetic and biochemical characterization of the oligopeptide transport system of Lactococcus lactis*., Journal of Bacteriology. 175: 7523-7532.

U.I.O., (2011)., *Hydrolyse*., [cited 2018 14.04]; Available at; http://www.mn.uio.no/ibv/tjenester/kunnskap/plantefys/leksikon/h/hydrolyse.html

Uchida S., Houjo M., Tochikubo M., (2008)., *Efficient sterilization of bacteria by pulse electric field in micro-gap*. Journal of Electrostatics. 66:427–431.

Vojcic L., Pitzler C., Körfer G., Jakob F., Martinez R., Maurer K.H., Schwaneberg U., (2015)., *Advances in protease engineering for Laundery detergents*., New biotechnology. 25;32(6):629-34.

Whitaker J.R., Voragen A.G.J & Wong D.W.S., (2003)., *Handbook of Food Enzymology.,* Marcel Dekker Inc. US. pp. 10-30.

10.APPENDIX

APPENDIX 1 – GROWTH VALUES FROM THE BIOSCREEN-C TRIAL A/B/C/D

Table A1.1 *The μ_{Max}-values from Bioscreen-C trial A with cultivation of all 42 strains, at 30°C, 25mM glucose over 48 hours (MRS not altered). μ_{Max}-values was based on values from a time frame of 1-4 hours (ca. 2X of OD) taken from the Log-phase. In cases with inconsistent growth or poor log-phase, Microsoft Excel forecast sheet was applied to aid in the prediction of μ_{Max}-values). Diauxic growth rates were not considered for μ_{Max}-values.*

LAB specie	strain ID:	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT	M.R.S
Carnobacterium inhibens subsp. Gilichinskyi / Ca. Divergens	IS 61	0,16	0,18	0,13	0,28	0,29	0,30	0,20	0,18	0,19	0,19	0,10
L.casei/paracasei	IS 64	0,23	0,27	0,15	0,19	0,20	0,22	0,09	0,13	0,11	0,32	0,16
L.casei/paracasei	IS 79	0,28	0,43	0,25	0,33	0,28	0,29	0,10	0,25	0,23	0,30	0,27
L. Plantarum/paraplantarum / L. Pentosus	IS 93	0,47	0,48	0,50	0,43	0,45	0,43	0,00	0,14	0,22	0,28	0,13
L.casei/paracasei	IS 118.B	0,27	0,33	0,24	0,27	0,25	0,22	0,08	0,19	0,22	0,33	0,23
L. Sakei	IS 118.4	0,06	0,15	0,09	0,09	0,11	0,11	0,00	0,07	0,16	0,14	0,22
L.casei/paracasei	IS 145	0,26	0,34	0,24	0,22	0,19	0,17	0,10	0,17	0,16	0,25	0,29
L.casei/paracasei	IS 185	0,24	0,30	0,26	0,24	0,28	0,25	0,10	0,16	0,14	0,31	0,52
Lactobacillus sakei subsp. Sakei	IS 196.1	0,18	0,16	0,21	0,28	0,27	0,30	0,15	0,20	0,19	0,23	0,09
Lactobacillus sakei subsp. Sakei	IS 196.2	0,15	0,14	0,17	0,27	0,28	0,31	0,32	0,27	0,23	0,46	0,20
Lactobacillus sakei subsp. Sakei	IS 196.3	0,14	0,13	0,14	0,24	0,27	0,26	0,16	0,21	0,23	0,34	0,16
Lactobacillus sakei subsp. Sakei	IS 196.4	0,17	0,16	0,20	0,27	0,27	0,31	0,21	0,20	0,21	0,47	0,19
Ca. Maltaromaticum	IS 200	0,32	0,35	0,35	0,37	0,41	0,42	0,21	0,22	0,18	0,48	0,13
lactobacillus paracasei JCM 8130/Casei ATCC	IS 204	0,24	0,31	0,19	0,09	0,25	0,26	0,11	0,16	0,13	0,25	0,33
Carnobacterium inhibens subsp. Gilichinskyi	IS 269	0,17	0,17	0,18	0,24	0,29	0,31	0,15	0,14	0,11	0,48	0,14
L. paracasei / L.casei	IS 352	0,25	0,29	0,23	0,20	0,16	0,19	0,07	0,13	0,15	0,47	0,23
L. Plantarum/paraplantarum / L. Pentosus	IS 357	0,08	0,15	0,10	0,15	0,16	0,13	0,15	0,20	0,21	0,19	0,36
L.paraplantarum/ planaturm	IS 361	0,30	0,38	0,27	0,20	0,15	0,14	0,17	0,20	0,17	0,30	0,25
L. Plantarum/paraplantarum / L. Pentosus	IS 371	0,11	0,11	0,11	0,19	0,19	0,16	0,17	0,21	0,18	0,37	0,37
L. Plantarum/paraplantarum / L. Pentosus	IS 380	0,13	0,14	0,12	0,16	0,18	0,15	0,17	0,19	0,21	0,34	0,35
L. Plantarum/paraplantarum / L. Pentosus	IS 384	0,13	0,17	0,09	0,24	0,24	0,22	0,16	0,23	0,26	0,34	0,35
Lactobacillus plantarum NC8	MF 9	0,37	0,35	0,36	0,34	0,37	0,37	0,22	0,24	0,28	0,40	0,18
Lactobacillus reuteri DSM20016	MF 110	0,06	0,12	0,05	0,06	0,11	0,21	0,05	0,04	0,15	0,02	0,08
Lactobacillus amylovorus NRRL B4542	MF 150	0,11	0,14	0,09	0,11	0,15	0,11	0,16	0,09	0,09	0,14	0,35
Lactobacillus sakei LS25	MF 1127	0,59	0,58	0,57	0,53	0,52	0,57	0,29	0,31	0,42	0,44	0,33
Lactobacillus brevis DSM20054	MF 1964	0,16	0,15	0,17	0,02	0,12	0,13	0,00	0,00	0,04	-0,01	0,08
Lactobacillus alimentarius DSM20249	MF 1965	0,04	0,04	0,09	0,00	0,00	0,01	0,00	0,00	0,00	0,02	0,21
Leuconstoc paramesenteroides DSM 20193	MF 1974	0,05	0,08	0,05	0,06	0,08	0,07	0,05	0,04	0,05	0,31	0,09
Lactobacillus («Pediococcus») dextrinicus DSM 20335	MF 1978	0,09	0,20	0,11	0,13	0,01	0,16	0,23	0,08	0,07	0,19	0,38
Pediococcus pentosaceus DSM 20336	MF 1979	0,32	0,38	0,34	0,20	0,33	0,30	0,00	0,04	0,17	0,41	0,29
Lactobacillus helveticus ATCC15009	MF 1980	0,23	0,24	0,23	0,22	0,22	0,20	0,00	0,07	0,16	0,27	0,33
Lactobacillus delbrueckii ATCC12315	MF 2033	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,42	0,25
Lactobacillus casei ATCC393	MF 2035	0,18	0,18	0,20	0,12	0,17	0,16	0,16	0,09	0,12	0,17	0,19
Lactobacillus salivarius DSM 20555	MF 2357	0,16	0,23	0,12	0,15	0,22	0,19	0,11	0,14	0,14	0,29	0,16
Lactobacillus coryniformis NCFB 2741 (=DSM 20001)	MF 2576	0,15	0,12	0,10	0,18	0,16	0,14	0,14	0,12	0,08	0,42	0,26
Lactobacillus paracacei NCDO151	MF 2900	0,24	0,28	0,30	0,25	0,25	0,30	0,14	0,13	0,15	0,27	0,12
Lactobacillus sanfransiscensis	MF 2983	0,05	0,14	0,07	0,01	0,11	0,10	0,00	0,02	0,06	0,09	0,19
Leuconostoc mesenteroides ATCC 8293 (=DSM 20343)	MF 02996	0,05	0,10	0,00	0,07	0,08	-0,02	0,07	0,08	0,03	0,21	0,36
Lactococcus lactis MG1363	MF 3579	0,44	0,51	0,51	0,51	0,54	0,52	0,30	0,34	0,38	0,51	0,33
Carnobacterium divergens Lab01	MF 5214	0,37	0,44	0,35	0,48	0,47	0,53	0,26	0,31	0,33	0,33	0,18
Pediococcus clausenii DSM 14800 (Type strain)	MF 6580	0,20	0,14	0,20	0,27	0,29	0,33	0,10	0,08	0,17	0,00	0,22
Weissella confusa DSM 20194	MF 6581	0,20	0,23	0,16	0,24	0,25	0,20	0,16	0,16	0,18	0,35	0,37

Table A1.2 *Resulting μ_{Max}-values from Bioscreen-C cultivation at 30°C over 48 hours with 25mM glucose (SMAPT and MAPT). Values are extracted from table A1.1 with the nine-chosen candidate strains.*

Strain ID	MDCR_	MDCR_	MDCR_	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT
MF 5214	0,37	0,44	0,35	0,48	0,47	0,53	0,26	0,31	0,33	0,33
MF 1127	0,59	0,58	0,57	0,53	0,52	0,57	0,29	0,31	0,42	0,44
IS 204	0,24	0,31	0,19	0,09	0,25	0,26	0,11	0,16	0,13	0,25
IS 93	0,47	0,48	0,50	0,43	0,45	0,43	0,00	0,14	0,22	0,28
IS 79	0,28	0,43	0,25	0,33	0,28	0,29	0,10	0,25	0,23	0,30
IS 118.b	0,27	0,33	0,24	0,27	0,25	0,22	0,08	0,19	0,22	0,33
IS 361	0,30	0,38	0,27	0,20	0,15	0,14	0,17	0,20	0,17	0,30
MF 150	0,11	0,14	0,09	0,11	0,15	0,11	0,16	0,09	0,09	0,14
MF 110	0,06	0,12	0,05	0,06	0,11	0,21	0,05	0,04	0,15	0,02

Table A1.3 *The resulting μ_{Max}-values from Bioscreen-C cultivation at 37°C over 48 hours with 25mM glucose (SMAPT and MAPT). μ_{Max}-values was based on values taken from a time frame of 1-4 hours of the Log-phase. In cases with inconsistent growth or poor log-phase, Microsoft Excel forecast sheet was applied to aid in the prediction of μ_{Max}-values). Diauxic growth rates were not considered for μ_{Max}-values.*

Strain ID	MDCR_	MDCR_	MDCR_	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT
MF 5214	0,44	0,43	0,41	0,23	0,29	0,26	0,19	0,26	0,22	0,28
MF 1127	0,37	0,51	0,39	0,34	0,36	0,39	0,27	0,24	0,43	0,58
IS 204	0,37	0,30	0,19	0,21	0,32	0,40	0,16	0,18	0,36	0,51
IS 93	0,50	0,00	0,38	0,37	0,27	0,42	0,17	0,34	0,26	0,50
IS 79	0,58	0,32	0,36	0,34	0,37	0,21	0,24	0,23	0,30	0,41
IS 118.b	0,33	0,36	0,35	0,38	0,42	0,43	0,22	0,23	0,23	0,58
IS 361	0,35	0,43	0,35	0,51	0,18	0,30	0,15	0,18	0,22	0,53
MF 150	0,13	0,09	0,12	0,14	0,28	0,44	0,20	0,14	0,14	0,28
MF 110	0,12	0,07	0,07	0,08	0,07	0,16	0,05	0,13	0,13	0,19

Table A1.4 *The resulting μ_{Max}-values from Bioscreen-C cultivation at 30°C over 48 hours with 12.5mM glucose (SMAPT and MAPT). μ_{Max}-values was based on values taken from a time frame of 1-4 hours of the Log-phase. In cases with inconsistent growth or poor log-phase, Microsoft Excel forecast sheet was applied to aid in the prediction of μ_{Max}-values). Diauxic growth rates were not considered for μ_{Max}-values.*

Strain ID	MDCR_	MDCR_	MDCR_	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT
MF 5214	0,59	0,58	0,59	0,48	0,46	0,39	0,25	0,11	0,22	0,39
MF 1127	0,54	0,60	0,57	0,32	0,35	0,37	0,23	0,24	0,24	0,42
IS 204	0,34	0,44	0,26	0,25	0,39	0,17	0,12	0,09	0,09	0,30
IS 93	0,50	0,52	0,48	0,34	0,33	0,34	0,15	0,12	0,10	0,40
IS 79	0,37	0,01	0,26	0,00	0,27	0,19	0,26	0,16	0,06	0,24
IS 118.b	0,38	0,38	0,25	0,28	0,41	0,59	0,05	0,14	0,10	0,36
IS 361	0,48	0,36	0,25	0,32	0,34	0,44	0,25	0,09	0,16	0,25
MF 150	0,01	0,12	0,08	0,00	0,00	0,09	0,01	0,03	0,06	0,01
MF 110	0,10	0,09	0,17	0,21	0,08	0,16	0,07	0,04	0,07	0,12

Table A1.5 *The resulting μ_{Max}-values from Bioscreen-C cultivation at 30°C over 48 hours with 0 mM glucose (SMAPT and MAPT). μ_{Max}-values was based on values taken from a time frame of 1-4 hours of the Log-phase. In cases with inconsistent growth or poor log-phase, Microsoft Excel forecast sheet was applied to aid in the prediction of μ_{Max}-values). Diauxic growth rates were not considered for μ_{Max}-values.*

Strain ID	MDCR_	MDCR_	MDCR_	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT
MF 5214	0,36	0,37	0,56	0,20	0,24	0,38	0,14	0,03	0,17	0,29
MF 1127	0,20	0,18	0,53	0,25	0,21	0,43	0,03	0,08	0,29	0,30
IS 204	0,11	0,05	0,04	0,04	0,03	0,07	0,13	0,00	0,11	0,13
IS 93	0,07	0,22	0,49	0,09	0,10	0,30	0,05	0,08	0,22	0,01
IS 79	0,10	0,07	0,14	0,00	0,03	0,10	0,00	0,04	0,10	0,16
IS 118.b	0,02	0,04	0,24	0,09	0,01	0,24	0,03	0,15	0,11	0,12
IS 361	0,07	0,09	0,25	0,13	0,09	0,13	0,08	0,09	0,10	0,02
MF 150	0,11	0,03	0,09	0,10	0,10	0,12	0,00	0,04	0,10	0,01
MF 110	0,09	0,06	0,10	0,03	0,04	0,13	0,04	0,03	0,10	0,07

Table A1.6 *The resulting μ_{Max}-values from Bioscreen-C cultivation at 30°C over 48 hours with 12.5 mM glucose (SMAPT and MAPT). μ_{Max}-values was based on values taken from a time frame of 1-4 hours of the Log-phase. In cases with inconsistent growth or poor log-phase, Microsoft Excel forecast sheet was applied to aid in the prediction of μ_{Max}-values). Diauxic growth rates were not considered for μ_{Max}-values.*

Strain ID	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F
MF 5214	0,24	0,10	0,15	0,17	0,09	0,10	0,20	0,14	0,12
MF 1127	0,40	0,23	0,35	0,31	0,15	0,16	0,45	0,18	0,18
IS 204	0,32	0,19	0,04	0,22	0,13	0,05	0,28	0,20	0,07
IS 93	0,34	0,21	0,05	0,26	0,17	0,10	0,30	0,18	0,05
IS118.b	0,31	0,20	0,06	0,23	0,16	0,05	0,29	0,19	0,07
IS 361	0,41	0,25	0,12	0,25	0,10	0,10	0,45	0,29	0,14

Table A1.7 *The resulting μ_{Max}-values from Bioscreen-C cultivation at 30°C over 48 hours with 0mM glucose (SMOPH). μ_{Max}-values was based on values taken from a time frame of 1-4 hours of the Log-phase. In cases with inconsistent growth or poor log-phase, Microsoft Excel forecast sheet was applied to aid in the prediction of μ_{Max}-values). Diauxic growth rates were not considered for μ_{Max}-values.*

Strain ID	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F
MF 5214	0,19	0,19	0,23	0,13	0,16	0,24	0,03	0,00	0,12
MF 1127	0,05	0,01	0,08	0,06	0,03	0,06	0,00	0,01	0,05
IS 204	0,03	0,02	0,03	0,02	0,00	0,04	0,05	0,02	0,02
IS 93	0,02	0,01	0,04	0,03	0,00	0,02	0,03	0,02	0,03
IS118.b	0,02	0,02	0,09	0,03	0,01	0,04	0,06	0,02	0,04
IS 361	0,00	0,01	0,07	0,01	0,01	0,03	0,00	0,01	0,05

Table A1.8 *The resulting Lag-phase values from Bioscreen-C cultivation of all 42 strains, at 30°C, 25mM glucose over 48 hours (MRS not altered). The time of the lag phase was calculated by use of a ruler and drawing a straight line through both the log phase and lag phase, where the point at which these lines crossed marked the end time of lag phase.*

LAB specie	strain ID:	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT	M.R.S
Carnobacterium inhibens subsp. Gilichinskyi / Ca. Divergens	IS 61	4,5	5	4,5	5	5	5,5	5	5	4	8	8,2
L.casei/paracasei	IS 64	7	10	8	8,5	9	8,5	11	8	9	10	10
L.casei/paracasei	IS 79	9	12	9	11,5	8,5	9	15,5	10	9	11,5	8
L. Plantarum/paraplantarum / L. Pentosus	IS 93	4	4	4,5	4	3,5	4	13	6,5	3,5	6	5,5
L.casei/paracasei	IS 118.B	9	10	8	12	7,5	7	11,5	9	7	9,5	9,9
L. Sakei	IS 118.4	6	9	9	6	9	9	N/A	15	9,5	17	17,5
L.casei/paracasei	IS 145	7	10,5	8,5	8	8	8,5	13	6	6	11,5	9,5
L.casei/paracasei	IS 185	7	10	9	8	8,5	6,5	11,5	8	8	11,5	5,5
Lactobacillus sakei subsp. Sakei	IS 196.1	4	4,5	4	4,5	4,8	5	19	6,5	5	5,5	5
Lactobacillus sakei subsp. Sakei	IS 196.2	4	5	4	4,5	3	4	27	7	5	6,5	5
Lactobacillus sakei subsp. Sakei	IS 196.3	3	4,5	4	4,5	3	4	15	7	5	6,5	5
Lactobacillus sakei subsp. Sakei	IS 196.4	3	4	4	4,5	3	4,2	21	7	5	5,5	5
Ca. Maltaromaticum	IS 200	4,5	5	4	4,5	4	4,5	4	4	4	6	6,5
lactobacillus paracasei JCM 8130/Casei ATCC	IS 204	8	11,5	8	12	9	9	12	8	9	14,5	12,1
Carnobacterium inhibens subsp. Gilichinskyi	IS 269	4	4	4	4	3,5	4	3,8	3	4	5	6,5
L. paracasei / L.casei	IS 352	7	9,5	7	7	7,5	6,5	12	6	6,5	5	8,2
L. Plantarum/paraplantarum / L. Pentosus	IS 357	3,5	5	5,5	5	4,5	4,5	4	5	5	6	6
L.paraplantarum/ planaturm	IS 361	7	8,5	10	8	8	7	8	8	9	9,5	9,9
L. Plantarum/paraplantarum / L. Pentosus	IS 371	4	5,5	5,5	5	4,5	5,5	5	5	5	6	6
L. Plantarum/paraplantarum / L. Pentosus	IS 380	3,5	5	5,5	5	4,5	4,5	5,5	5	5	6,2	6
L. Plantarum/paraplantarum / L. Pentosus	IS 384	5	5	5,5	4,5	4,5	4,5	4	4	5	6	6
Lactobacillus plantarum NC8	MF 3	5	5	5	5	4	4	5	4	5	6	5,5
Lactobacillus reuteri DSM20016	MF 110	18,5	23,5	27	18	19	32	24	13	34	10	8
Lactobacillus amylovorus NRRL B4542	MF 150	17,5	21,5	25,5	17	20,5	18	23	18	20	25	12
Lactobacillus sakei LS25	MF 1127	3	3	3,5	4	3	3	8	6	3	5	3,5
Lactobacillus brevis DSM20054	MF 1364	7,5	7	6	13	6,5	5	N/A	N/A	11	15	7
Lactobacillus alimentarius DSM20249	MF 1365	10	10	12,2	N/A	N/A	N/A	N/A	N/A	N/A	10	5
Leuconstoc paramesenteroides DSM 20193	MF 1374	12	11,5	12	13	10	12	11	13	12	13	5,5
Lactobacillus («Pediococcus») dextrinicus DSM 20335	MF 1378	4,5	6	5,5	5,5	4,5	5	13	4	4,5	10	6,2
Pediococcus pentosaceus DSM 20336	MF 1379	5,5	6	5,5	5	5	4,5	N/A	9	6,5	6,5	5,5
Lactobacillus helveticus ATCC15009	MF 1380	5	5,5	6	6	6	4	N/A	7	5,5	9	7
Lactobacillus delbrueckii ATCC12315	MF 2033	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	14,2	4,2
Lactobacillus casei ATCC393	MF 2035	12,5	15	13	15	11	15	22	13	12	16	17,2
Lactobacillus salivarius DSM 20555	MF 2357	10	10	9	10	10	10,5	12	9	7,5	14	6
Lactobacillus coryniformis NCFB 2741 (=DSM 20001)	MF 2576	6,5	8	6	5,5	7	6,5	5	5	7	11,5	8
Lactobacillus paracacei NCDO151	MF 2900	7	7	8,5	8	8	9	6	6,5	7,5	12	8
Lactobacillus sanfransiscensis	MF 2983	7,5	7	8	N/A	6,5	6,5	N/A	N/A	7,5	5	5,5
Leuconostoc mesenteroides ATCC 8293 (=DSM 20343)	MF 02996	2,8	3	3	4	4	3	4	4	3,5	5	4,5
Lactococcus lactis MG1363	MF 3579	3,5	2,8	2,5	2,5	2,8	2,5	2,5	2	2,5	4,5	4
Carnobacterium divergens Lab01	MF 5214	4	3	4	4	4	3,5	4	4	4	6	5
Pediococcus clausenii DSM 14800 (Type strain)	MF 6580	11	10	11	8	6	9	23	9	9,5	42	12,4
Weissella confusa DSM 20194	MF 6581	3	2,5	2,5	2,5	2,5	2,8	2	2,2	2,5	3	3

Table A1.9 *The resulting OD_{Max}-values from Bioscreen-C cultivation of all 42 strains, at 30°C, 25mM glucose over 48 hours (MRS not altered). The max OD was taken from when the slope of the graph went over to stationary phase. Diauxic growth rates were not considered for OD_{Max} -values.*

LAB specie	strain ID:	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT	M.R.S
Carnobacterium inhibens subsp. Gilichinskyi / Ca. Divergens	IS 61	1,32	1,27	1,36	1,04	1,02	1,17	0,59	0,85	1,11	1,16	0,67
L.casei/paracasei	IS 64	1,11	1,67	1,36	1,03	1,62	1,22	0,57	1,07	1,27	1,56	1,77
L.casei/paracasei	IS 79	1,56	1,77	1,61	1,60	1,62	1,55	0,42	1,45	1,57	1,86	1,80
L. Plantarum/paraplantarum / L. Pentosus	IS 93	1,59	1,57	1,54	1,37	1,37	1,42	0,63	1,02	1,27	1,26	1,37
L.casei/paracasei	IS 118.B	1,69	1,82	1,56	1,67	1,62	1,57	0,49	1,42	1,62	1,69	1,87
L. Sakei	IS 118.4	0,40	1,15	1,06	0,26	1,22	0,32	N/A	1,25	1,22	1,41	1,77
L.casei/paracasei	IS 145	1,71	1,77	1,54	1,47	1,47	0,85	0,39	1,10	1,32	1,71	1,77
L.casei/paracasei	IS 185	1,26	1,70	1,34	1,22	1,37	1,35	0,54	0,32	1,22	1,66	1,67
Lactobacillus sakei subsp. Sakei	IS 196.1	1,44	1,47	1,41	1,22	1,12	1,15	0,74	1,17	1,07	1,29	1,37
Lactobacillus sakei subsp. Sakei	IS 196.2	1,43	1,47	1,46	1,27	1,30	1,35	0,82	1,12	1,32	1,21	1,32
Lactobacillus sakei subsp. Sakei	IS 196.3	1,44	1,47	1,44	1,30	1,30	1,30	0,64	1,25	1,23	1,19	1,39
Lactobacillus sakei subsp. Sakei	IS 196.4	1,46	1,42	1,41	1,22	1,20	1,10	0,78	1,25	1,02	1,16	1,37
Ca. Maltaromaticum	IS 200	1,26	1,17	1,16	1,12	0,97	1,15	0,72	0,90	0,90	1,11	0,57
lactobacillus paracasei JCM 8130/Casei ATCC	IS 204	1,01	1,72	1,51	0,82	1,57	1,27	0,71	1,00	1,22	0,61	1,53
Carnobacterium inhibens subsp. Gilichinskyi	IS 269	1,26	1,17	1,19	1,03	0,97	1,05	0,69	0,82	0,82	1,11	0,52
L. paracasei / L.casei	IS 352	1,66	1,77	1,56	1,16	1,10	1,15	0,27	1,22	1,22	1,49	1,49
L. Plantarum/paraplantarum / L. Pentosus	IS 357	1,21	1,22	1,06	1,10	1,12	1,07	0,89	1,00	1,12	1,21	1,87
L.paraplantarum/ planaturm	IS 361	1,76	1,77	1,51	1,10	1,25	0,87	0,77	0,78	1,30	1,69	1,87
L. Plantarum/paraplantarum / L. Pentosus	IS 371	1,26	1,27	0,96	1,17	1,20	1,20	0,79	0,97	1,10	1,26	1,39
L. Plantarum/paraplantarum / L. Pentosus	IS 380	1,14	1,27	1,06	1,02	0,97	0,95	0,59	0,85	1,02	1,21	1,82
L. Plantarum/paraplantarum / L. Pentosus	IS 384	1,02	1,17	0,96	1,00	0,97	1,17	0,32	1,00	1,22	1,21	1,87
Lactobacillus plantarum NC8	MF 3	1,24	1,12	1,21	1,17	1,17	1,20	0,57	1,02	1,17	1,31	1,29
Lactobacillus reuteri DSM20016	MF 110	1,06	0,74	0,19	1,02	1,02	1,02	0,74	0,70	0,92	0,41	0,78
Lactobacillus amylovorus NRRL B4542	MF 150	1,11	1,12	0,46	1,27	1,27	1,17	0,54	0,90	1,07	1,21	1,87
Lactobacillus sakei LS25	MF 1127	1,56	1,57	1,54	1,42	1,44	1,47	1,02	1,30	1,37	1,36	1,59
Lactobacillus brevis DSM20054	MF 1364	1,29	0,80	1,01	0,16	0,73	0,72	N/A	N/A	0,42	0,44	1,20
Lactobacillus alimentarius DSM20249	MF 1365	0,11	0,08	0,46	N/A	N/A	N/A	N/A	N/A	N/A	0,45	1,87
Leuconstoc paramesenteroides DSM 20193	MF 1374	0,47	0,54	0,51	0,32	0,37	0,37	0,27	0,32	0,35	1,31	1,68
Lactobacillus («Pediococcus») dextrinicus DSM 20335	MF 1378	1,24	1,17	1,29	1,12	1,17	1,27	0,64	1,00	1,02	1,09	1,37
Pediococcus pentosaceus DSM 20336	MF 1379	1,16	1,32	1,21	0,84	1,22	1,00	N/A	0,60	1,07	1,11	1,42
Lactobacillus helveticus ATCC15009	MF 1380	1,24	1,52	1,26	1,02	1,20	1,35	N/A	0,70	1,17	1,69	1,52
Lactobacillus delbrueckii ATCC12315	MF 2033	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1,24	1,67
Lactobacillus casei ATCC393	MF 2035	1,31	1,32	1,44	0,78	1,12	1,12	0,59	1,07	1,17	1,21	1,72
Lactobacillus salivarius DSM 20555	MF 2357	0,84	1,00	0,96	0,71	0,82	0,62	0,62	0,70	0,87	0,81	1,27
Lactobacillus coryniformis NCFB 2741 (=DSM 20001)	MF 2576	1,11	0,97	1,06	0,92	0,87	0,75	0,92	0,87	0,59	0,81	1,29
Lactobacillus paracacei NCDO151	MF 2900	1,59	1,47	1,51	1,37	1,30	1,52	0,67	0,86	1,32	1,11	1,24
Lactobacillus sanfransiscensis	MF 2983	0,09	0,22	0,39	N/A	0,10	0,17	N/A	N/A	0,32	0,15	0,75
Leuconostoc mesenteroides ATCC 8293 (=DSM 20343)	MF 02996	0,79	0,95	0,86	0,70	0,75	0,67	0,54	0,56	0,55	0,71	1,02
Lactococcus lactis MG1363	MF 3579	1,14	1,12	1,11	1,17	1,17	1,30	0,64	0,82	1,15	1,31	0,89
Carnobacterium divergens Lab01	MF 5214	1,54	1,47	1,49	1,25	1,17	1,32	0,82	0,87	1,07	1,29	0,87
Pediococcus clausenii DSM 14800 (Type strain)	MF 6580	1,26	0,87	1,61	1,25	1,22	1,37	0,17	1,00	1,45	N/A	1,64
Weissella confusa DSM 20194	MF 6581	1,01	1,02	0,96	0,92	0,92	0,85	0,54	0,75	0,87	0,76	1,24

Table A1.10 *The resulting OD_{Max}-values from Bioscreen-C cultivation at 37°C over 48 hours with 25mM glucose (SMAPT and MAPT). The Max OD was taken from when the slope of the graph went over to stationary phase. Diauxic growth rates were not considered for OD_{Max}-values*

Strain ID	MDCR_	MDCR_	MDCR_	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT
MF 5214	1,43	1,17	1,49	0,95	0,65	0,97	0,72	1,03	1,05	1,00
MF 1127	1,57	1,47	1,52	1,45	1,27	1,23	1,12	1,14	1,48	1,60
IS 204	1,52	1,52	1,59	1,52	1,53	1,30	1,15	1,29	1,61	1,76
IS 93	1,32	0 (DEAD	1,39	1,12	0,95	1,10	0,82	1,12	1,35	1,47
IS 79	1,12	1,52	1,45	1,50	1,75	1,40	1,02	1,17	1,57	1,68
IS 118.b	1,52	1,80	1,45	1,34	1,53	1,45	1,22	1,40	1,48	1,75
IS 361	1,52	1,32	1,45	1,50	1,53	1,10	0,52	1,32	1,40	1,84
MF 150	0,30	0,28	0,23	0,70	0,30	1,10	0,28	0,55	0,93	1,40
MF 110	0,70	0,30	0,55	0,80	0,40	0,78	0,30	0,44	1,15	0,56

Table A1.11 *The resulting OD_{Max}-values from Bioscreen-C trials at 30°C over 48 hours with 25mM glucose (SMAPT and MAPT). The Values are taken out from table A1.9 of the 9 chosen candidate strains. The Max OD taken from when the slope of the graph went over to stationary phase. Diauxic growth rates were not considered for OD_{Max}-values*

Strain ID	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT
MF 5214	1,54	1,47	1,50	1,24	1,17	1,32	0,82	0,87	1,05	1,29
MF 1127	1,57	1,57	1,55	1,42	1,44	1,47	1,02	1,30	1,35	1,37
IS 204	1,02	1,72	1,52	0,82	1,57	1,27	0,71	1,00	1,20	0,62
IS 93	1,59	1,57	1,55	1,37	1,37	1,42	0,63	1,02	1,25	1,27
IS 79	1,57	1,77	1,62	1,59	1,62	1,55	0,42	1,45	1,55	1,87
IS 118.b	1,69	1,82	1,57	1,67	1,62	1,57	0,49	1,42	1,60	1,69
IS 361	1,77	1,77	1,52	1,09	1,25	0,87	0,77	0,78	1,27	1,69
MF 150	1,07	0,74	0,20	1,02	1,02	1,02	0,74	0,70	0,90	0,42
MF 110	1,12	1,12	0,47	1,27	1,27	1,17	0,54	0,90	1,04	1,22

Table A1.12 *The resulting OD_{Max}-values from Bioscreen-C cultivation at 30°C over 48 hours with 12.5mM glucose (SMAPT and MAPT). The Values are taken out from table A1.9 of the 9 chosen candidate strains. The Max OD is taken from when the slope of the graph went over to stationary phase. Diauxic growth rates were not considered for OD_{Max}-values*

Strain ID	MDCR_	MDCR_	MDCR_	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT
MF 5214	1,42	1,32	1,32	1,04	0,35	0,97	1,10	0,82	0,92	1,40
MF 1127	1,42	1,02	1,42	1,09	0,30	1,03	0,70	0,92	1,12	1,33
IS 204	1,48	1,65	1,42	1,52	1,53	0,30	0,92	0,67	0,88	1,88
IS 93	1,44	1,02	1,42	0,87	0,70	0,80	0,62	0,52	0,77	1,30
IS 79	1,48	0,00	1,42	0,00	1,55	1,30	1,32	0,67	0,88	1,88
IS 118.b	1,72	1,62	1,42	1,21	1,55	1,25	0,68	0,67	1,04	1,70
IS 361	1,48	1,59	1,42	1,32	1,55	1,30	1,32	0,82	1,07	1,90
MF 150	0,00	1,00	0,30	0,00	0,00	0,30	0,00	0,00	0,44	0,00
MF 110	1,52	0,70	0,70	0,72	0,60	0,55	0,40	0,54	0,48	0,42

Table A1.13 *The resulting OD_{Max}-values from Bioscreen-C trials cultivation at 30°C over 48 hours with 0mM glucose (SMAPT and MAPT). The max OD was taken from when the slope of the graph went over to stationary phase. Diauxic growth rates were not considered for OD_{Max}-values.*

Strain ID	MDCR_	MDCR_	MDCR_	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F	MAPT
MF 5214	0,82	0,57	1,18	0,53	0,35	0,70	0,25	0,32	0,54	1,05
MF 1127	0,20	0,62	0,82	0,17	0,41	0,45	0,12	0,18	0,62	0,70
IS 204	0,62	0,42	0,72	0,17	0,05	0,35	0,38	0,42	0,72	1,15
IS 93	0,42	0,42	0,62	0,19	0,21	0,40	0,24	0,20	0,52	0,42
IS 79	0,38	0,32	0,69	0,02	0,10	0,22	0,08	0,22	0,52	1,10
IS 118.b	0,24	0,62	0,82	0,05	0,10	0,13	0,27	0,32	0,72	1,30
IS 361	0,54	0,52	0,78	0,05	0,05	0,13	0,24	0,32	0,56	1,15
MF 150	0,22	0,19	0,30	0,20	0,18	0,48	0,25	0,20	0,52	0,10
MF 110	0,28	0,22	0,40	0,30	0,20	0,40	0,40	0,20	0,54	0,28

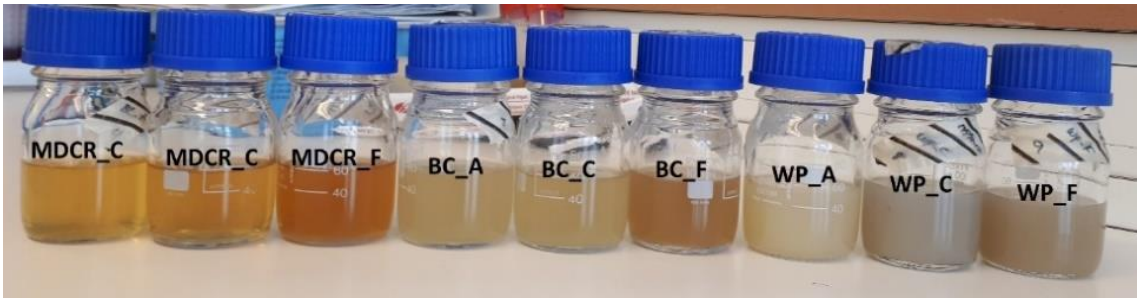
Table A1.14 *The resulting OD_{Max}-values from Bioscreen-C trials cultivation at 30°C over 48 hours with 12.5 mM glucose (SMOPH). The Max OD was based on values from time-frames of 1-4 hours (ca. 2X increase of OD) of the Log-phase. In cases with inconsistent growth or poor log-phase, Microsoft Excel forecast sheet was applied to aid in the prediction of μ_{Max}-values). Diauxic growth rates were not considered for OD_{Max}-values.*

Strain ID	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F
MF 5214	0,62	0,283	0,618	0,613	0,136	0,425	0,603	0,106	0,593
MF 1127	1,02	0,723	1,218	0,433	0,326	0,775	0,953	0,166	1,213
IS 204	1,15	0,85	1,1	0,85	0,5	0,55	1,28	0,8	1,08
IS 93	1,32	0,773	1,318	1,213	0,806	0,525	1,033	0,796	1,133
IS118.b	1,15	0,85	0,7	0,9	0,6	0,6	1,28	0,85	1,2
IS 361	1,1	0,77	0,6	0,9	0,5	0,45	1,28	0,78	1,15

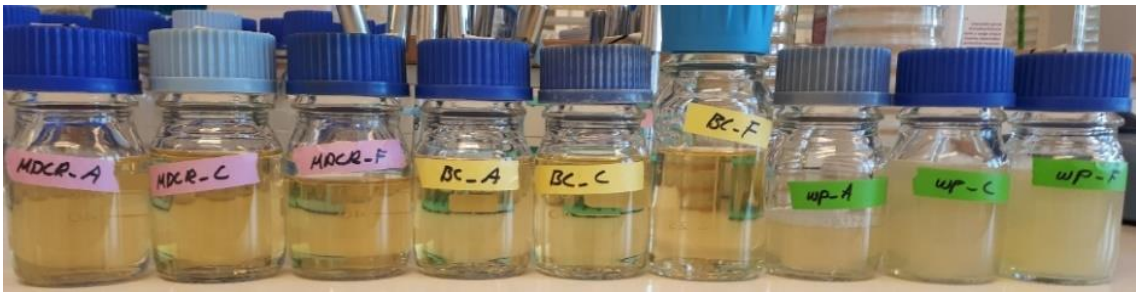
Table A1.15 *The resulting OD Max values from Bioscreen-C cultivation at 30°C over 48 hours with 0 mM glucose (SMOPH). The Max OD was based on values from time-frames of 1-4 hours (ca. 2X increase of OD) of the Log-phase (in cases with inconsistent growth or poor log-phase, Microsoft Excel forecast sheet was applied to aid in the prediction of μ_{Max}-values). Diauxic growth rates were not considered for μ_{Max}-values.*

Strain ID	MDCR_A	MDCR_C	MDCR_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F
MF 5214	0,5	0,223	0,718	0,113	0,126	0,345	0,033	0,006	0,193
MF 1127	0,1	0,023	0,238	0,073	0,046	0,155	0,103	0	0,313
IS 204	0,1	0,1	0,25	0,1	0,08	0,15	0,35	0,1	0,55
IS 93	0,02	0,023	0,018	0,013	0,026	0,025	0	0	0,113
IS118.b	0,1	0,1	0,25	0,1	0,08	0,15	0,38	0,1	0,45
IS 361	0,1	0,1	0,25	0,1	0,08	0,15	0,25	0,1	0,4

APPENDIX 2 – MEDIA PREPARATIONS



Picture A2.1*: The resulting nine SMAPT-media after autoclavation at 115°C for 15 min*



Picture A2.1: *The resulting nine SMAPT-media after centrifugation at 10.000 rpm followed by sterile-filtration (WP_A has reduced volume due to the breakage of a sample-tube while subduing centrifugation).*

A2.1 Media preparation and protein content analysis

A2.2 Preparation of SMAPT and SMOPH

Several methods were attempted to produce SMAPT and SMOPH media of the nine hydrolysates with sufficiently low turbidity (required to measure growth in Bioscreen-C). Autoclavation at 115^{o}C and 121^{o}C gave a drastic change in coloration and in turn the turbidity of the given media. Sterile-filtration with 0.2µm pore-size and vacuum was attempted as a second approach. This method gave low yield before the filter clogged. Centrifugation at 10.000 rpm for 10 min before sterile-filtration gave a sufficient outcome (see picture A2.1-A2.2 in the appendix).

A significant volume reduction was observed with MDCR_F. Centrifugation resulted in a gel-like pellet which roughly accounted for 10% of the volume (consistent in all centrifugation processes with MDCR_F). Furthermore, a small volume reduction was seen regardless of gel formation in all growth media except BC series) with 0.48-1.73g from centrifugation or 0.5-1.5ml on average.

Preparation of the growth media for the big-batch fermentation trial used for Napping analysis (method 4.11) required a more extensive centrifugation protocol due to the increase sample volume of 1 litre, with samples being centrifuged at 14.000 rpm for 15 min at +4^{o}C. Supernatant was then centrifuged a second time under the same conditions before sterile-filtration. On average 2-3x filters was needed per 1L medium, with whey protein being the most difficult of sample types (3-5 filters per 1L medium).

A2.3 Comparison of resulting protein content in SMAPT, analyzed by Dumas or Bio-Rad methods

Several methods were investigated to confirm the protein content of each SMAPT-media after centrifugation and filtration (see method 4.4.2 for media preparation). The Bio-Rad method was first applied (explained in method 4.4) on the prepared media and compared to non-filtrated samples. The results from the Bio-Rad method are shown in figure A3.4 in the appendix and shows a reduction in the protein content to an average of ~15g/l (down from 20g/l). The inconsistency in the results from the protein-Assay warranted a second analysis method.

The Dumas method measures the total nitrogen content, and it was used on SMAPT media and results were compared to the non-filtrated sample. The results ranged from 6.18-6.77 total nitrogen (%) in all

samples before and after centrifugation/filtration (values in Table A3.1 are shown multiplied with the Kjeldahl-conversion factor (TKN) of 6.25 (MDCR/BC) and 6.38 (WP) for all Tot. N (%) series).

After conversion with the TKN (Total Kjeldahl Nitrogen (TKN, commonly used to estimate the protein content in meat samples), the resulting protein in the samples ranged from 39-42% protein.

The Dumas analysis was performed in duplicate series giving a std.-deviation between series of 0.01-0.04 total N (%) and converted by TKN to 0.03-0.24 % deviation.

The Dumas method showed that each medium contained roughly the same protein content in the finished media compared to that of the non-filtrated samples with a reduction or increase in the protein concentration ranging from (- 0.82% to + 0.09%. (std. deviation 0.03-0.24%). All SMAPT media showed less than 1% reduction in the protein concentration in the finished media (see table A3.1). Samples from BC (Cod) was not subjected in the Dumas analysis as BC samples did not produce any pellet after centrifugation, nor did it show any problems under filtration, hence giving no reason to suspect protein losses.

Each of the measured SMAPT media contained 39.5 – 42.12% proteins (see table A3.1) which includes all sources of nitrogen measured as protein. By subtracting the access nitrogen of ~1.44g from other sources from the total nitrogen and by accounting for the weight loss by filtration from each media of ~0.5 – 1.7g (per litre medium). A crude estimation of protein (g) per litre indicates that each sample contained 26.02 – 27.88 g/l protein (based on results from table A3.1). A marginal error of 3-12% was applied to estimate the actual protein content. This was done due to the elevated values often obtained by Dumas method when converted by TKN 6.25 and 6.38. This would constitute ~0.8 -3.34g deviation and each media was estimated to contain ~22- 24 g/L proteins (Oftedal et al. 2014). The marginal error between Dumas method and the conversion by TKN factor (6.25 for MDCR and BC, 6.38 for whey) was shown to be 9-10% in MDCR and BC and 5-6% in WP series (comparison of table 5.2 and A3.3). A crude estimation would entail that each media contains a protein content within the marginal error shown in table A3.2, whereas MDRC and BC perhaps contain 22-23g/L and WP are slightly higher with about 23-24g/L protein.

APPENDIX 3 – DATA FROM THE PROTEIN-HYDROLYSATES USING LOWRY PROTEIN ASSAY, DUMAS-METHOD AND KJELDAHL-ANALYSIS

Table A3.1 *Results after nitrogen analysis with the Dumas method. The protein concentration in dried samples of each media is listen below in percentage, after conversion with Kjeldahl conversion factor TKN 6.25 for MDCR and BC and 6.38 for WP (Each media contains reagents listed in method 4.4.2). the std. deviation between parallels were between 0.01-0.04 Tot N (%) or by TKN 0.03-0.24 % deviation in protein content. The change in protein content is shown in percentage with negative values indicating loss in protein concentration compared to unfiltered samples.*

	Before filtration		Average	After filtration		Average	
Sample ID	Tot. N (%) parallel 1	Tot. N (%) parallel 2	Tot. N (%)	Tot. N (%) parallel 1	Tot. N (%) parallel 2	Tot. N (%)	change in Protein-content (%)
MDCR_A	41.33	41.82	41.58	41.18	41.93	41.55	-0.02
MDCR_C	41.93	42.13	42.03	41.93	42.31	42.12	0.09
MDCR_F*	40.13	40.53	40.33	39.48	39.53	39.50	-0.82
WP_A	41.16	41.28	41.22	41.06	41.42	41.24	0.02
WP_C	40.83	40.90	40.86	40.42	41.12	40.77	-0.10
WP_F	39.59	39.71	39.65	39.43	39.78	39.61	-0.04

*samples of MDCR_F creates a gel-like-pellet which binds ~10% of the total dH_2O volume, as was measured by drying.

Table A3.2 Total reagents content and estimations of the protein content (g/L) based on the total nitrogen of hydrolysates (%) given in table A3.1. Mean deviation between Dumas and Kjeldahl method of 3-12% is based on the conclusion drawn by Oftedal et al. 2014.

#	MDCR_A	MDCR_C	MDCR_F	WP_A	WP_C	WP_F
Hydrolysate (protein %)	82	83.5	72.7	73.2	75	67.4
Reagents (g)	41.70	41.70	41.70	41.70	41.70	41.70
Amount hydrolysate	24.39	23.95	27.51	27.32	26.66	29.67
tot. Ingredients	66.08	65.65	69.21	69.02	68.36	71.37
loss (g) after centrifugation	1.74	0.83	1.66	0.7	0.48	0.63
New tot. weight	64.35	64.82	67.55	68.32	67.88	70.74
NITROGEN (%)	41.55	42.12	39.5	40.4	39.94	38.8
NITROGEN (g)	27.46	27.65	27.34	27.88	27.30	27.69
access N (other sources)	1.43	1.43	1.43	1.43	1.43	1.43
N (g) minus access N (other Sources)	26.02	26.22	25.90	26.45	25.87	26.25
12% error	3.12	3.15	3.11	3.17	3.10	3.15
3% error	0.78	0.79	0.78	0.79	0.77	0.78
Tot. Protein of added hydrolysate (g) (12% error)	21.46	21.63	21.36	21.84	21.33	21.67
Tot. Protein of added hydrolysate (g) (3% error)	23.81	23.99	23.69	24.22	23.66	24.03

Table A3.3 *The resulting total N % as measured by the Dumas method and use of the Kjeldahl protein conversion factor (TKN) 6.25 for MDCR, BC and 6.38 for WP. RM indicates samples from the raw-material and is the % of protein in each material. The remaining samples are hydrolysates and their respective % of protein, recovered with Alcalase, Corolase and Flavourzyme.* Triplicates were taken of the Raw material and the std.-deviation is listed in "()" after the tot.N %.

Sample ID	Dumas method (Tot. N%)	Kjeldahl protein (%) (N*6.25 & 6.38)
MDCR_A	14.60	91.25
MDCR_C	14.70	91.88
MDCR_F	13.10	81.88
BC_A	15.60	97.50
BC_C	15.60	97.50
BC_F	15.20	95.00
WP_A	12.70	81.03
WP_C	12.90	82.30
WP_F	11.70	74.64
MDRC_RM	6.98 (0.43)	43.64
BC_RM	10.55 (0.11)	65.94
WP_RM	13.01 (0.05)	83.01

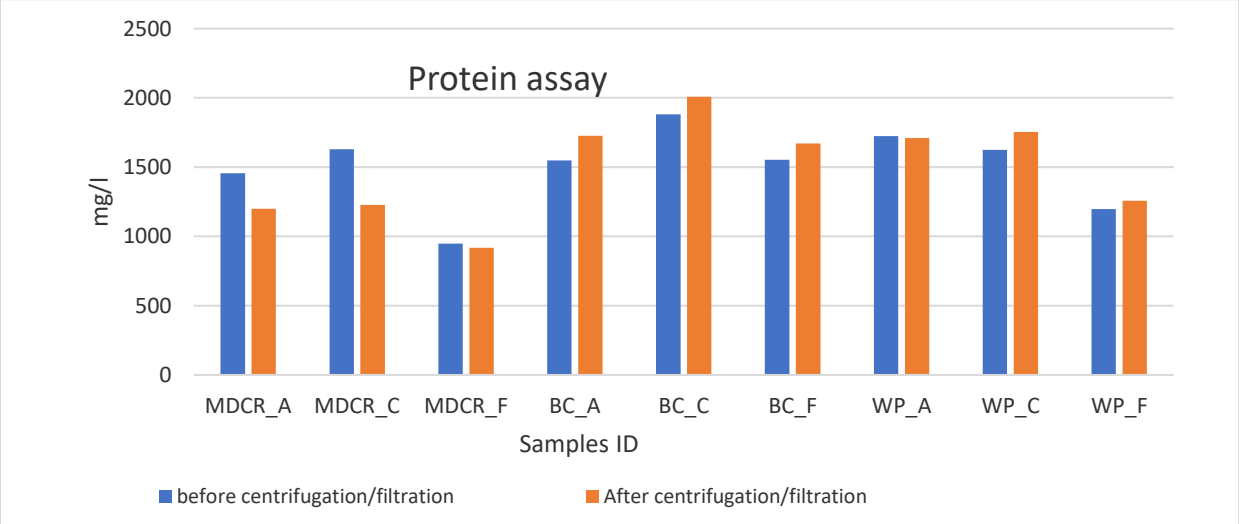


Figure A3.4*: The resulting protein content after measurements with the Lowry protein Assay method (Bio-Rad). BSA (bovine serum albine) was used as standard in the concentrations 125-,250-, 500-,750-, 1000-, 1500-, 2000 µg/ml. Each media was diluted to 10^{-1}-10^{-3} in duplicate series. The linear regression (r^2) from the two std.-curves (for samples "before" and "after" centrifugation/filtration) was calculated to 0.994 and 0.997 respectively.*

APPENDIX 4 – DATA FOR SEC-ANALYSIS

Table A4.5.1 *Resulting values/retention times from SEC measurements on calibration samples to produce a calibration curve. This is based on the retention time (RT) of the individual molecules through SEC separation. The values and known sizes of each compound was used for a crude estimate where to set the line between the different sized peptides for Table 5.6.1, according to Silvestre et al. 2012. The std.-curve and the data were obtained and produced by Mari Linnea Ruud.*

Name Compound	Code	M, Wt,	RT_1	RT_2	RT_3	MeanRT	LogMW	STDV RT
Albumin from chicken egg-white	AlbChi	44287	6.08	6.08	6.08	6.08	4.65	0.00
Carbonic anhydrase	CarbAn	29000	6.10	6.10	6.11	6.10	4.46	0.00
Lysosyme	Lysosyme	14300	6.62	6.63	6.63	6.63	4.16	0.01
Aprotinin from bovine lung	Aprotinin	6511	7.15	7.15	7.17	7.15	3.81	0.01
Insulin Chain B Oxidized from bovine pancreas	InsChB	3496	8.76	8.76	8.77	8.76	3.54	0.01
Renin Substrate Tetradecapeptide porcine	Renin	1759	8.45	8.46	8.47	8.46	3.25	0.01
Angiotensin II human	AngiII	1046	8.99	9.01	9.01	9.00	3.02	0.01
Bradykinin Fragment 1-7	Brad17	757	9.49	9.49	9.50	9.49	2.88	0.01
[D-Ala2]-Leucine enkephalin	LeuEnk	570	11.51	11.53	11.53	11.52	2.76	0.01
Val-Tyr-Val	ValTyrVal	379	11.12	11.12	11.14	11.13	2.58	0.01
Trp	Trp	204	11.95	11.96	11.97	11.96	2.31	0.01

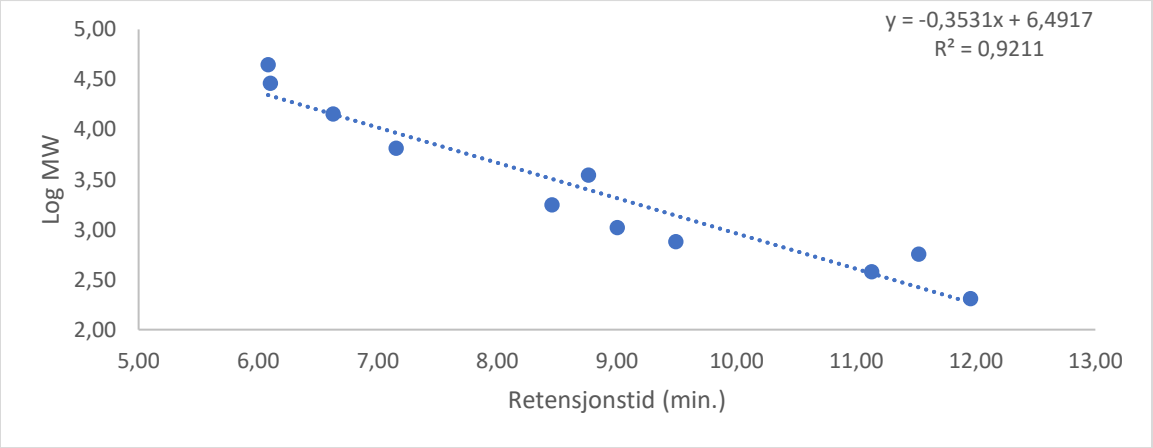


Figure A4.5.1: *Resulting calibration curve used in the SEC analysis tool (PSS) and corresponds to table A4.5.1.*

APPENDIX 5 – RESULTS FROM 16S RRNA SEQUENCING

Table A5.1: *Results from 16S rRNA sequencing (using V3-V5 stretch of the gene) of strains from Nofima Bergen. Sequences was read in MVF (Minor Variant Finder v.1 applied biosystems) and sequence-stretches was trimmed to only contain sequence with high quality. Trimmed sequences were identified using nBLAST (NCBI) with penalties and parameters; Excluding models (XM/XP) & uncultured/environmental sample sequences with limits to sequences from type material and mega-blast with scoring parameters (match/mismatch) 4/-5, Existence:12 and extension 8. strain identification was not conclusive (due to the size of the analysed fragment 200-800bp) but rather in approximation towards family/genus. ID is shown in percentage and as total hits in terms of base pairs. Several hits were found for all sequence.*

Isolate No.	ID (Blast query)	% ID (basepair)	Sequence
IS 185	*L. paracasei* *L. casei*	(99%)761/765bp (98%) 751/765bp	AGTCGAACGAGTTCTCGTTGATGATYGGTGCTTGCRCCGAGATTCAACATGGAACGAGTGGCGGAC GGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATAC CGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGA CCCGCGGCGTATTAGCTAGTTGGTGAGGTAAYGGCTCACCAAGGCGATGATACGTAGCCGAACTGA GAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGA ATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGT AAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGYCGGCGTGACGGTATCCAACCA GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAG GAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGT GAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCT GAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTG
IS 118.b	*L. paracasei* *L. casei*	(99%) 749/753bp (98%) 739/753bp	AGTCGAACGAGTTCTCGTTGRTGATYGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGCGGAC GGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATAC CGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGA CCCGCGGCGTATTAGCTAGTTGGTGAGGTAAYGGCTCACCAAGGCGATGATACGTAGCCGAACTGA GAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGA ATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGT AAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGYCGGCGTGACGGTATCCAACCA GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAG GAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGT GAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCT GAGGCTCGAAAGCATGGGTAGCGAACAGGA
IS 204	*L. paracasei*	(99%) 395/396bp	GAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGYC GGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTG AAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAG TGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCT GTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTA GTC
IS 196.1	*L. sakei subsp. Sakei*	(99%) 760/761bp	GATGGACCCGCGGTGCATTAGTTAGTYGGTGAGGTAAAGGCTCACCAAGACCGTGATGCATAGCCG ACCTGAGAGGGTAATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAG TAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCG GATCGTAAAACTCTGTTGTTGGAGAAGAATGTATCTGATAGTAACTGATCAGGTAGTGACGGTATCC AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCC GGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCTTCGGCTC
IS 196.2	*L. sakei subsp. Sakei*	(99%) 553/553bp	ATGGACCCGCGGTGCATTAGTTAGTTGGTGAGGTAAAGGCTCACCAAGACCGTGATGCATAGCCGA CCTGAGAGGGTAATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGT AGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCG GATCGTAAAACTCTGTTGTTGGAGAAGAATGTATCTGATAGTAACTGATCAGGTAGTGACGGTATCC AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCC GGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCTTCGGCTCAA CCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTA GCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTG ACGCTGAGGCTCGAAAGCATGGGTAGCA

IS 196.3	*L. sakei subsp. Sakei*	(99%) 390/390bp	GAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTGGAGAAGAATGTATCTGATAGTAACTGATCAG GTAGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG GTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTG AAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAG TGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCT GTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACAGGATTAGATACCCTG
IS 196.4	*L. sakei subsp. Sakei*	(99%) 760/761bp	TGCAAGTCGAACGCACTCTCGTTTAGATTGAAGGAGCTTGCTCCTGATTGATAAACATTTGAGTGAG TGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTAAAGTGGGGGATAACATTTGGAAACAGR TGCTAATACCGCATAAAACCTAACACCGCATGGTGTAGGGTTGAAAGATGGTTTCGGCTATCACTTT AGGATGGACCCGCGGTGCATTAGTTAGTTGGTGAGGTAAAGGCTCACCAAGACCGTGATGCATAG CCGACCTGAGAGGGTAATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG CAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTT TTCGGATCGTAAAACTCTGTTGTTGGAGAAGAATGTATCTGATAGTAACTGATCAGGTAGTGACGGT ATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT GTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCTTCGGC TCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATG TGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTA ACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACAGG
IS 200	*Carnobacterium maltaromaticum* *Carnobacterium inhibens subsp gilichinskyi*	(100%) 380/380bp (99%) 378/380bp	GTAAAACTCTGTTGTTAAAGAAGAACAAGGATGAGAGTAACTGCTCATCCCCTGACGGTATTTAACC AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGA TTTATTGGGCGTAAAGCGAGCGCAGGCGGTTCTTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCG GGGAGGGTCATTGGAAACTGGAGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGC GGTGAAATGCGTAGATATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGAC GCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA
IS 64	*L. Paracasei* *L. casei*	(99%) 600/601bp (99%) 599/601bp	CATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGT TGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATT GGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCA AGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGA AGAATGGTCGGCAGAGTAACTGTTGYCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTAC GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAG CGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTG GGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATG GAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGG TAGCGAACAGG
IS 145	*L. paracasei* *L.casei*	(99%) 673/677bp (99%) 666/672bp	CATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTG GGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGA AAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGC TCACCAAGGCGATGATACGTAGCCGAACTGARAGGTTGATCGGCCACATTGGGACTGARACACGGC CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAAC GCCGCGTGAGTGAARAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGA GTAACTGTTGYCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTA AGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCA GAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGG CGAAGGCGGCTGTCTGGTCTGTAACTGACGCTG
IS 376	*L. paracasei* *L. casei*	(99%) 748/751bp (98%) 738/751bp	GTCGAACGAGTTCTCGTTGATGATYGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGCGGACG GGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACC GCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGAC CCGCGGCGTATTAGCTAGTTGGTGAGGTAAYGGCTCACCAAGGCGATGATACGTAGCCGAACTGAG AGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAA TCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTA AAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGYCGGCGTGACGGTATCCAACCAG AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTT ATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGG AAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTG AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGA GGCTCGAAAGCATGGGTAGCGAACAGG
IS 366	*L. plantarum* *L. pentosus* *L. paraplantarum*	(100%) 403/403bp (100%) 403/403bp (100%) 403/403bp	CCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGT AACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT AATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAG TCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAA GAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGA AGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGAT ACCCTGGTA

IS 118,4	*L. sakei* *L. fuchuensis* *L. fuchuensis* *L. sakei*	(99%) 393/394bp (100%) 394/394bp (100%) 394/394bp (100%) 394/394bp	TGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTGGAGAAGAATGTATCTGATAGTAACTGATCAG GTAGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG GTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTG AAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAG TGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCT GTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACAGGATTAGATACCCTGGTA
IS 79	*L. paracasei* *L. casei*	(99%) 546/548bp (99%) 545/548bp	GGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAAC TGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAG GGAATCTTCCACAATGGACGCAAGYCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGG TCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGYCGGCGTGACGGTATCCA ACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCG GATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAAC CGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAG CGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGA CGCTGAGGCTCGAAAGCATGGGTA
IS 61	*Carnobacterium inhibens subsp. Gilichinskyi* *Carnobacterium divergens*	(99%) 396/398bp (100%) 398/398bp	TGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGA GTAACTGCTCATCCCCTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTCTTTA AGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGAGAACTTGAGTGCA GAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGG CGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTA GATAC
IS 269	*Carnobacterium inhibens subsp. Gilichinskyi Carnobacterium Maltaromaticum*	(99%) 394/396bp (100%) 396/396bp	GCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAAAGAAGAACAAGGATGAGAG TAACTGCTCATCCCCTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT AATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTCTTTAAG TCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGAGAACTTGAGTGCAGA AGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGATATGTGGAGGAACACCAGTGGCG AAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGA TA
IS 384	*L. plantarum* *L. pentosus*	(99%) 750/751bp (99%) 750/751bp	AGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTG GTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACC GCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCC CGCGGCGTATTAGCTAGATGGTGRGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGA GGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAAT CTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTA AAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGA AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTA TTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGA AGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGA AATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGA GGCTCGAAAGTATGGGTAGCAAACAGG
IS 352	*L. Paracasei* *L. casei*	(99%) 396/399bp (99%) 396/399bp	GAGTGAARAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAARAATGGTCGGCAGAGTAACTGT TGYCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG TAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGAT GTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGA CAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCG GCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTG GTAGT
IS 380	*L. Plantarum* *L. Paraplantarum* *L. pentosus* *L. fabifermentans*	(100%) 396/396bp	AGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTA TTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG CAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAG CCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGA ACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCT GGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCC A

IS 357	*L. pentosus* *L. plantarum* *L. paraplantarum*	(99%) 758/760bp (99%) 758/760bp (99%) 756/760bp	AGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTG GTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACC GCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCC CGCGGCGTATTAGCTAGATGGTGRGGTAACGGCTCACCATGGCRATGATACGTAGCCGACCTGAGA GGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAAT CTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTA AAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGA AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTA TTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGA AGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGA AATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGA GGCTCGAAAGTATGGGTAGCAAACAGGATTAGATAC
IS 361	*L. paracasei* *L. casei* *L. zeae*	(99%) 746/749bp (98%) 736/749bp (98%) 735/749bp	GTCGAACGAGTTCTCGTTGATGATYGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGCGGACG GGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACC GCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGAC CCGCGGCGTATTAGCTAGTTGGTGAGGTAAYGGCTCACCAAGGCGATGATACGTAGCCGAACTGAG AGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAA TCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTA AAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGYCGGCGTGACGGTATCCAACCAG AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTT ATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGG AAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTG AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGA GGCTCGAAAGCATGGGTAGCGAACA
IS 371	*L. pentosus* *L. plantarum* *L.paraplantarum* *L. plajomi* *L. xianfangensis*	(99%) 689/690 (99%) 688/690 (99%) 687/690 (99%) 685/690 (99%) 683/690	AGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTG GTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACC GCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCC CGCGGCGTATTAGCTAGATGGTGRGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGA GGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAAT CTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTA AAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGA AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTA TTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGA AGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGA AATGCGTAGATATATGGAAGAACACCAGTGG
IS 93	*L. plantarum* *L. paraplantarum* *L. pentosus*	(98%) 546/556bp	GTCCCGCGGCGTATTAGCYAGATGGTGRGGTAACGGCYCACCATGGCAATGATACGYAGCCGACCT GAGAGGGTAATCGGCCACATYGGGACYGAGACACGGCCCAAACYCCTACGGGAGGCAGCAGTAGG GAATCTYCCACAATGGACGAAAGYCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTC GTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACC AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGA TTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCG AAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG GTGAAATGCGTAGATATATGGAAGAKACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGAC GCTGAGGCTCGAAAGTATGGGTAGCAAACAG

APPENDIX 6 – DATA FROM THE EZYMATIC PROTEIN HYDROLYSIS

Table A6.1: *The overall resulting amount of each fraction from each hydrolysis in grams (g) (two parallels per product + enzyme). Result from finished hydrolysis-run of MDCR with enzymes; Alc (A), Cor (C) and Fla (F) and its total lipid-, sediment- (solids) and aqueous-phase + lyophilized product is listed below. All samples of the same enzyme + product was pooled together for further experiments (homogenized). The std.-deviation between parallel series are listed in parenthesis for the total resulting values.*

Parallel 1	MDCR_A	MDCR_C	MDCR_F
Sediment-phase (g)	203.26	228.71	335.27
Lipid-phase (g)	45.97	34.94	29.44
Aqueous-phase (g)	1009.90	992.10	784.40
Lyophilized aqueous-phase (g)	74.15	79.53	60.01
parallel 2			
Sediment-phase (g)	183.04	235.68	331.07
Lipid-phase (g)	50.25	34.29	32.34
Aqueous-phase (g)	1115.70	1076.79	884.80
Lyophilized aqueous-phase (g)	79.20	79.31	60.83
total (pooled) dividend			
Sediment-phase (g)	386.30 (14.3)	464.39 (4.9)	666.34 (2.9)
Lipid-phase (g)	96.22 (3.03)	69.22 (0.46)	61.78 (2.05)
Aqueous-phase (g)	2125.60 (74.8)	2068.9 (59.9)	1669.2 (71.0)
Lyophilized aqueous-phase (g)	153.35	158.84	120.84

Table A6.2: *The overall resulting amount of each fraction from each hydrolysis in grams (g) (two parallels per product + enzyme). Result from finished hydrolysis-run of BC with enzymes; Alc (A), Cor (C) and Fla (F) and its total lipid-, sediment- (solids) and aqueous-phase + lyophilized product is listed below. All samples of the same enzyme + product was pooled together for further experiments (homogenized). The std.-deviation between parallel series are listed in parenthesis for the total resulting values.*

Parallel 1	BC_A	BC_C	BC_F
Sediment-phase (g)	129.75	125.53	184.85
Lipid-phase (g)	N/D	N/D	N/D
Aqueous-phase (g)	1126.00	1089.80	1034.10
Lyophilized aqueous-phase (g)	46.73	66.27	52.54
parallel 2			
Sediment-phase (g)	127.12	131.28	180.53
Lipid-phase (g)	N/D	N/D	N/D
Aqueous-phase (g)	1158.58	1164.29	1103.77
Lyophilized aqueous-phase (g)	52.00	63.01	58.87
total (pooled) dividend			
Sediment-phase (g)	256.87 (1.8)	256.81 (4.1)	365.38 (3.0)
Lipid-phase (g)	N/D	N/D	N/D
Aqueous-phase (g)	2284.58 (23.0)	2254.09 (52.7)	2137.87 (49.3)
Lyophilized aqueous-phase (g)	98.73	129.28	111.41

Table A6.3: The overall resulting amount of each fraction from each hydrolysis in grams (g) (two parallels per product + enzyme). Result from finished hydrolysis-run of WP with enzymes; Alc (A), Cor (C) and Fla (F) and its total lipid-, sediment- (solids) and aqueous-phase + lyophilized product is listed below. All samples of the same enzyme + product was pooled together for further experiments (homogenized). The std.-deviation between parallel series are listed in parenthesis for the total resulting values.

Parallel 1	WP_A	WP_C	WP_F
Sediment-phase (g)	150.73	135.43	206.35
Lipid-phase (g)	N/D	N/D	N/D
Aqueous-phase (g)	1056.50	1089.30	986.30
Lyophilized aqueous-phase (g)	68.05	68.54	46.37
parallel 2			
Sediment-phase (g)	136.94	143.78	218.27
Lipid-phase (g)	N/D	N/D	N/D
Aqueous-phase (g)	1149.99	1135.21	1059.17
Lyophilized aqueous-phase (g)	70.60	66.64	43.20
total dividend			
Sediment-phase (g)	287.67 (9.7)	279.21 (5.9)	424.62 (8.4)
Lipid-phase (g)	N/D	N/D	N/D
Aqueous-phase (g)	2206.49 (66.1)	2224.51 (32.5)	2045.47 (51.5)
Lyophilized aqueous-phase (g)	138.65	135.18	89.57

Table A6.4: The relative weight (in grams) of recovered peptide fractions after lyophilization, compared to the total raw-material (wet weight). Note that some sample-loss occurred through lyophilization and the actual recovered lyophilized fraction may vary with +/- 5 g. The estimated loss in BC_A was dramatically higher compared to other samples with a deviation at roughly 30g from the expected yield.

#	MDCR_A	MDCR_C	MDRC_F	BC_A	BC_C	BC_F	WP_A	WP_C	WP_F
Tot. Raw-material	998.92	997.93	995.75	1000.86	1000.50	1000.93	250.54	250.95	250.66
Lyophilized fraction	153.35	158.84	120.84	98.73	129.28	111.41	138.65	135.18	89.57
Recovered material (%)	15.035	15.92	12.14	9.86	12.92	11.13	13.86	13.518	8.9

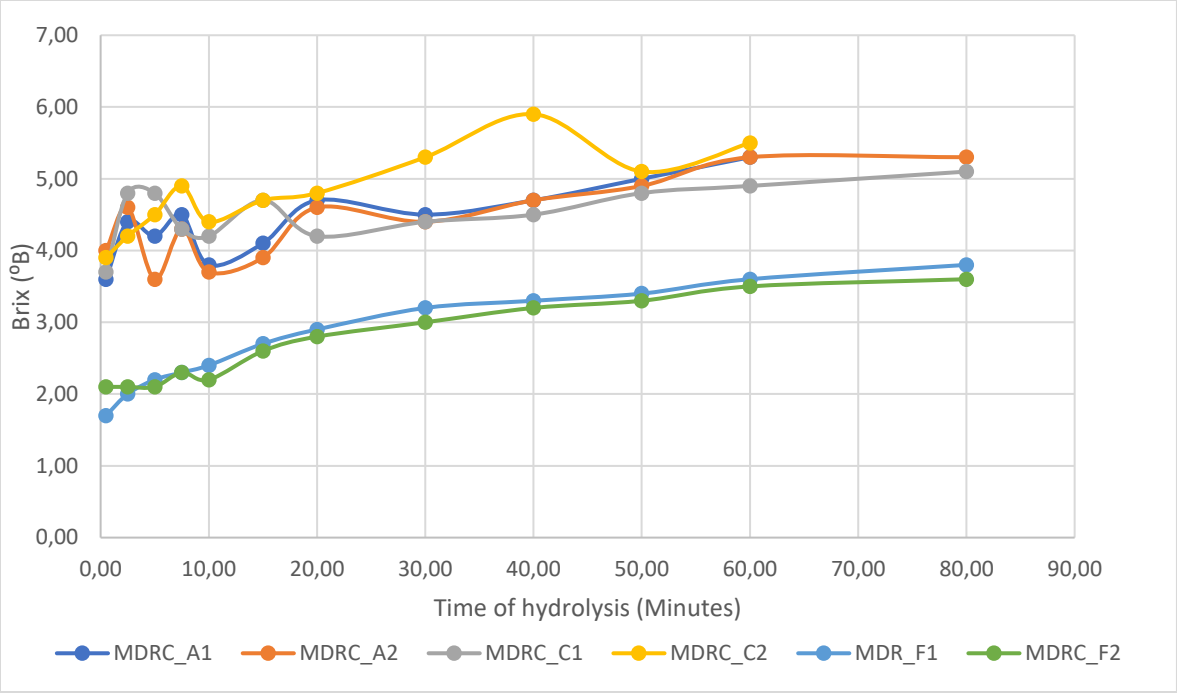


Figure A6.4.1: *The figure shows the degree of brix (°B) measurements over the course of hydrolysis for MDCR with enzymes; Alc (A), Cor (C) and Fla (F) (shown in duplicate series).*

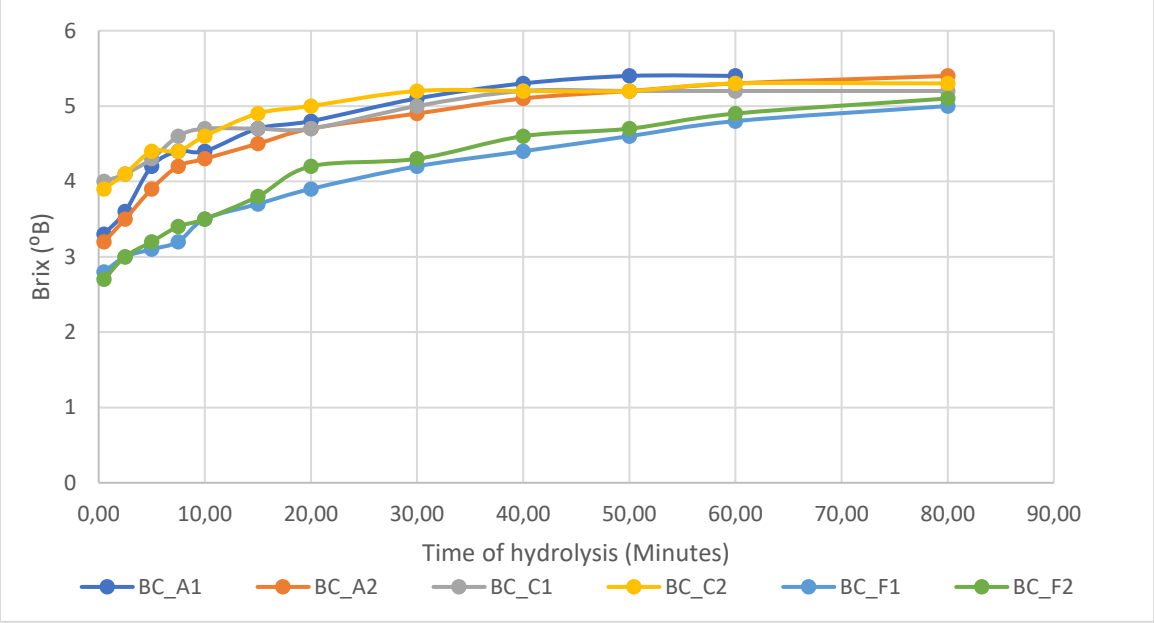


Figure A6.4.2: *The figure shows the degree of brix (°B) measurements over the course of hydrolysis for BC with enzymes; Alc (A), Cor (C) and Fla (F) (shown in duplicate series).*

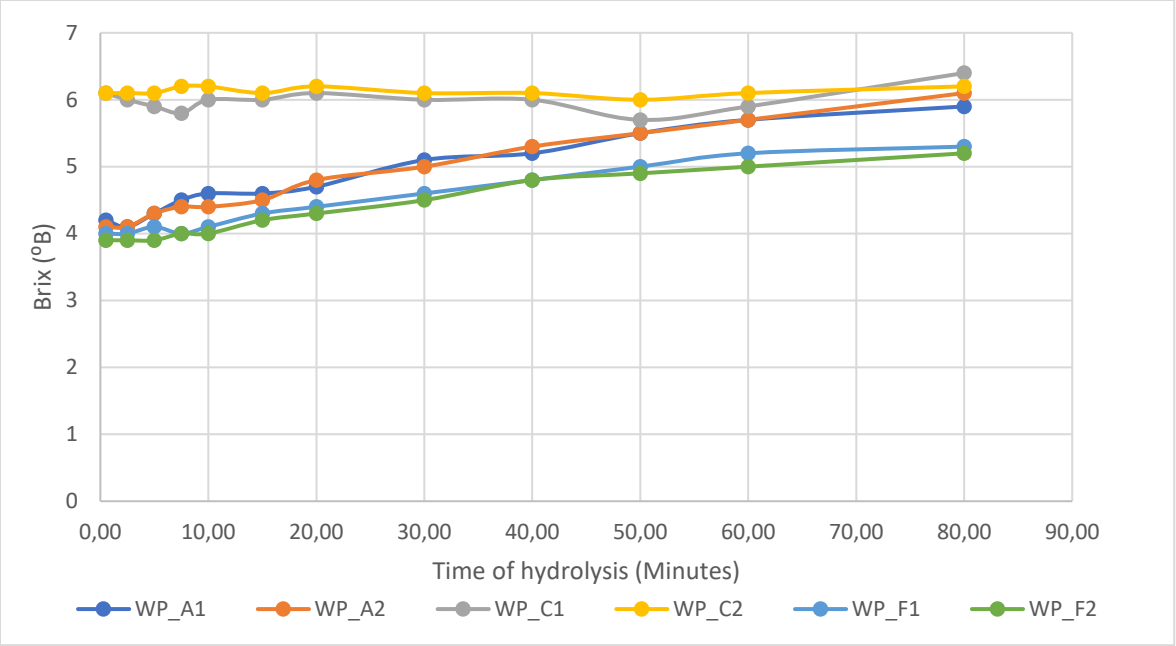
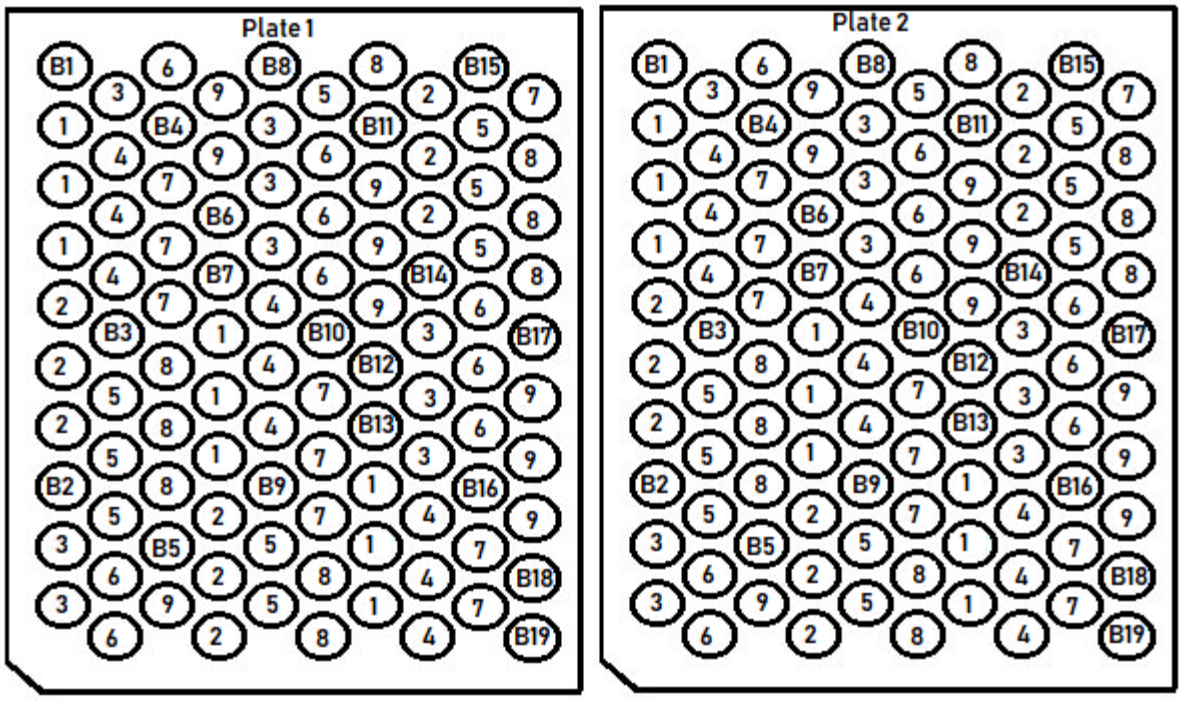


Figure A6.4.3: *The figure shows the degree of brix (°B) measurements over the course of hydrolysis for WP with enzymes; Alc (A), Cor (C) and Fla (F) (shown in duplicate series).*

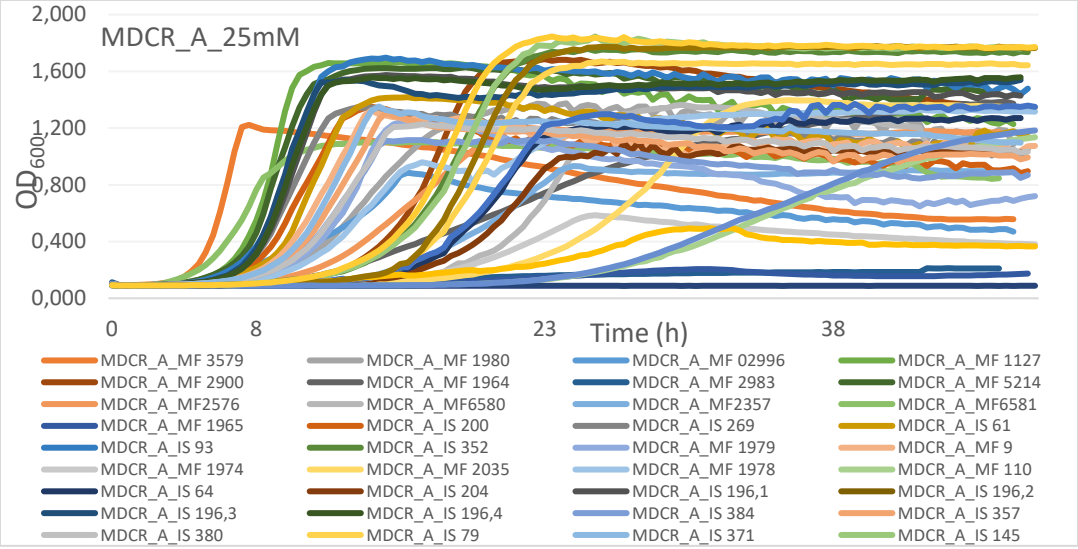
APPENDIX 7 – GRAPHS FROM BIOSCREEN-C TRIAL A, B, C & D

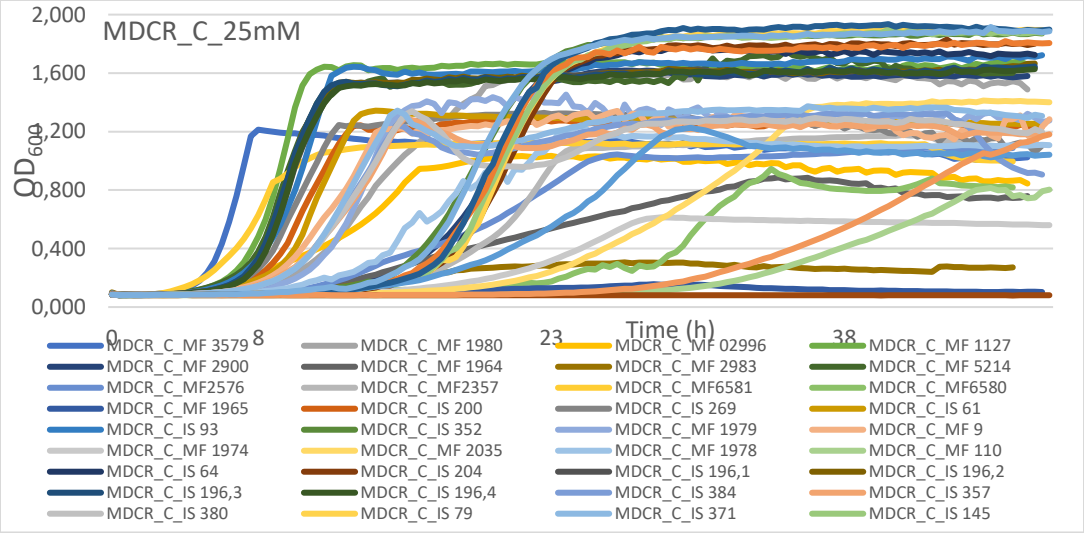
Picture A7.0: *Shows the used plate-setup for Bioscreen-C trial A, B, C, D. Each plate setup consists of nine hydrolysate media in triplicate series with two corresponding blank samples per hydrolysate media. The numbers 1 to 9 each symbolizes a different media, whereas B1-B19 are the different Blank samples. Each plate allows for screening of three strain at the same time, with each Bioscreen-C run containing six strains in total.*

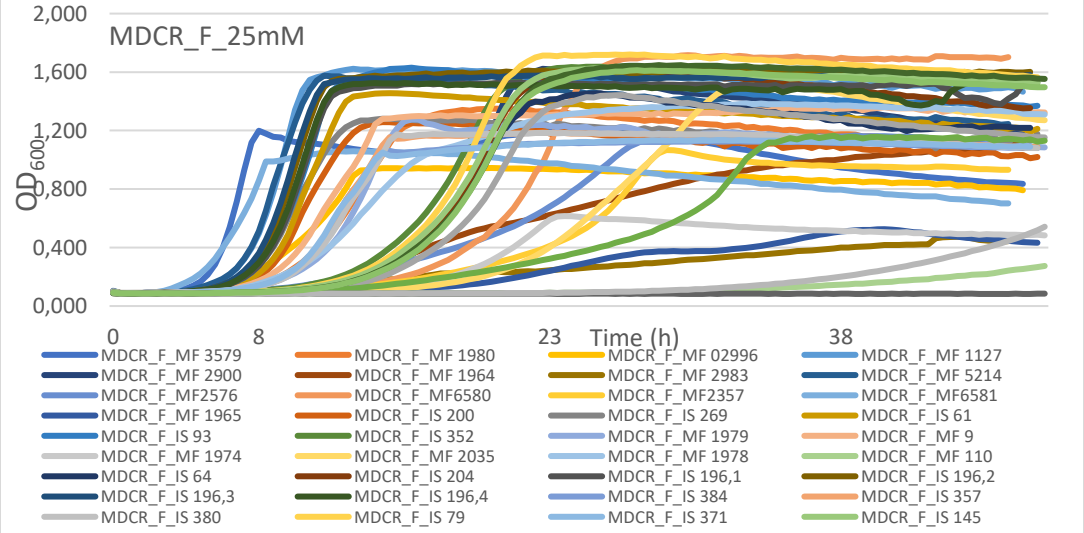


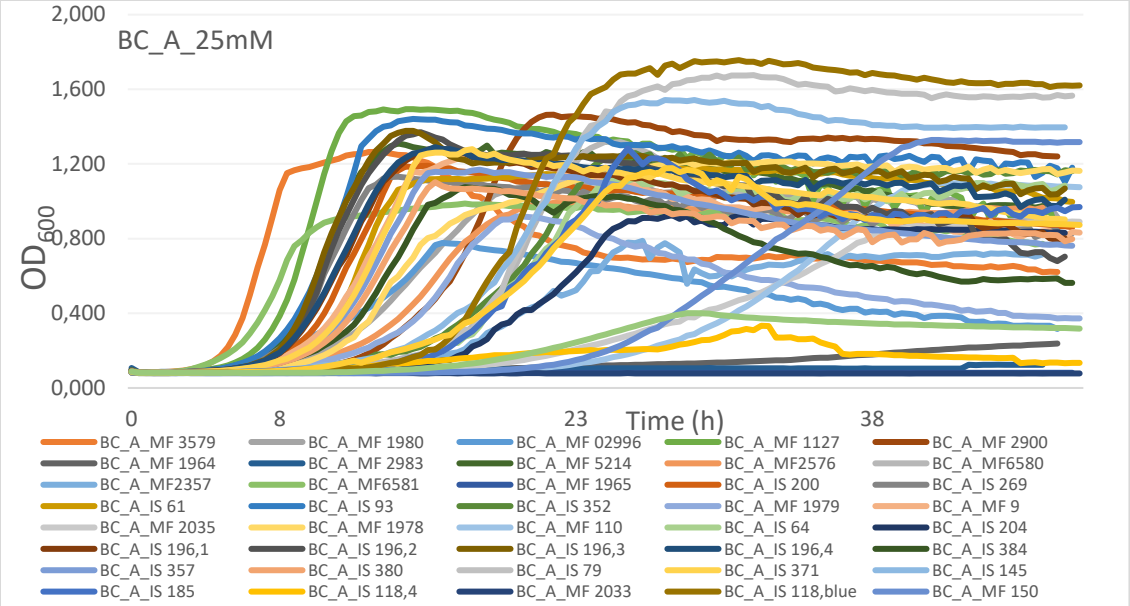
- **B1, B10:** MDCR_A
- **B2, B11:** BC_A
- **B3, B12:** WP_A
- **B4, B13:** MDCR_C
- **B5, B14:** BC_C
- **B6, B15:** WP_C
- **B7, B16:** MDCR_F
- **B8, B17:** BC_F
- **B9, B18, B19:** WP_F
- **1:** MDCR_A
- **2:** BC_A
- **3:** WP_A
- **4:** MDCR_C
- **5:** BC_C
- **6:** WP_C
- **7:** MDCR_F
- **8:** BC_F
- **9:** WP_F

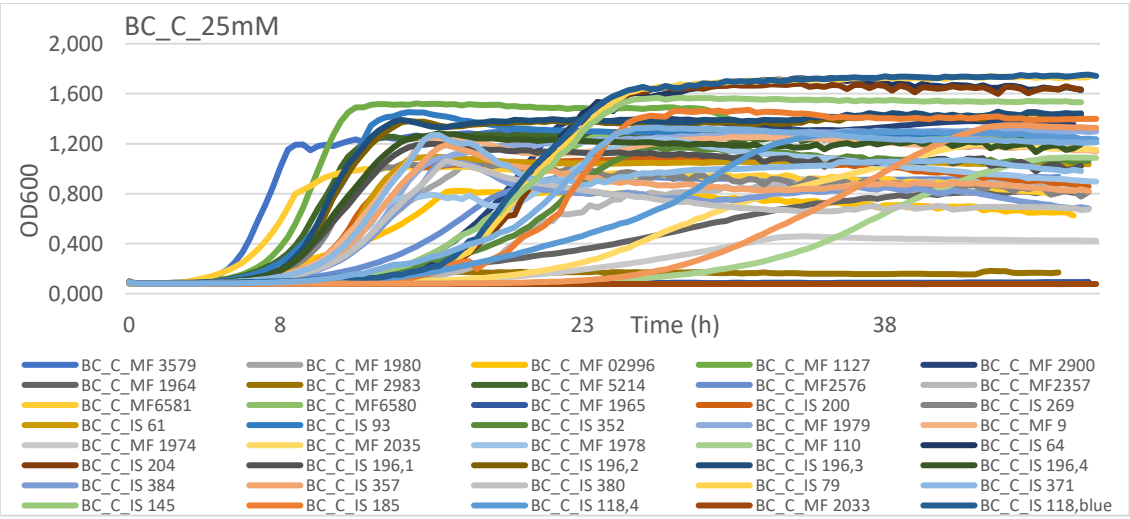
Figures A7.1: *Bioscreen-C Trial A results after pooling the triplicate series and trimming off deviating series. Bacterial growth was measured at 600nm (OD) over a period of 48 hours. Each series on the graph represents a different strain screened against the same SMAPT media. The following 11 graphs are from the same trial from all strains grown at 30^{o}C with 25 mM glucose SMAPT media, grown in triplicate series for each run. The last two graphs in the series are of basis media MAPT and M.R.S respectively (color of strain series may deviate between graphs).*

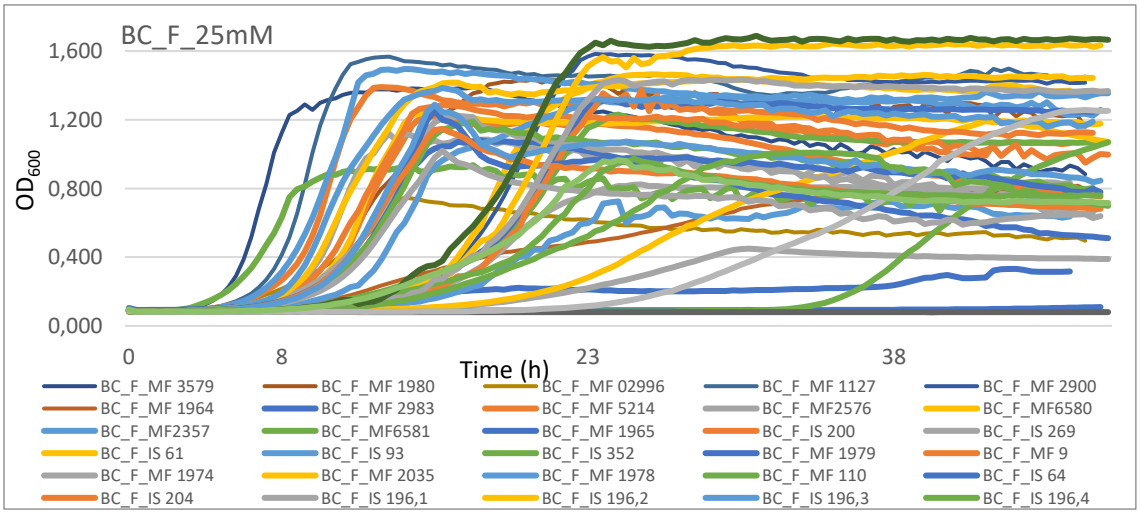

MDCR_A_25mM
2,000
1,600
1,200
0,800
0,400
0,000
OD600
0
8
23
38
Time (h)
MDCR_A_MF 3579
MDCR_A_MF 1980
MDCR_A_MF 02996
MDCR_A_MF 1127
MDCR_A_MF 2900
MDCR_A_MF 1964
MDCR_A_MF 2983
MDCR_A_MF 5214
MDCR_A_MF2576
MDCR_A_MF6580
MDCR_A_MF2357
MDCR_A_MF6581
MDCR_A_MF 1965
MDCR_A_IS 200
MDCR_A_IS 269
MDCR_A_IS 61
MDCR_A_IS 93
MDCR_A_IS 352
MDCR_A_MF 1979
MDCR_A_MF 9
MDCR_A_MF 1974
MDCR_A_MF 2035
MDCR_A_MF 1978
MDCR_A_MF 110
MDCR_A_IS 64
MDCR_A_IS 204
MDCR_A_IS 196,1
MDCR_A_IS 196,2
MDCR_A_IS 196,3
MDCR_A_IS 196,4
MDCR_A_IS 384
MDCR_A_IS 357
MDCR_A_IS 380
MDCR_A_IS 79
MDCR_A_IS 371
MDCR_A_IS 145

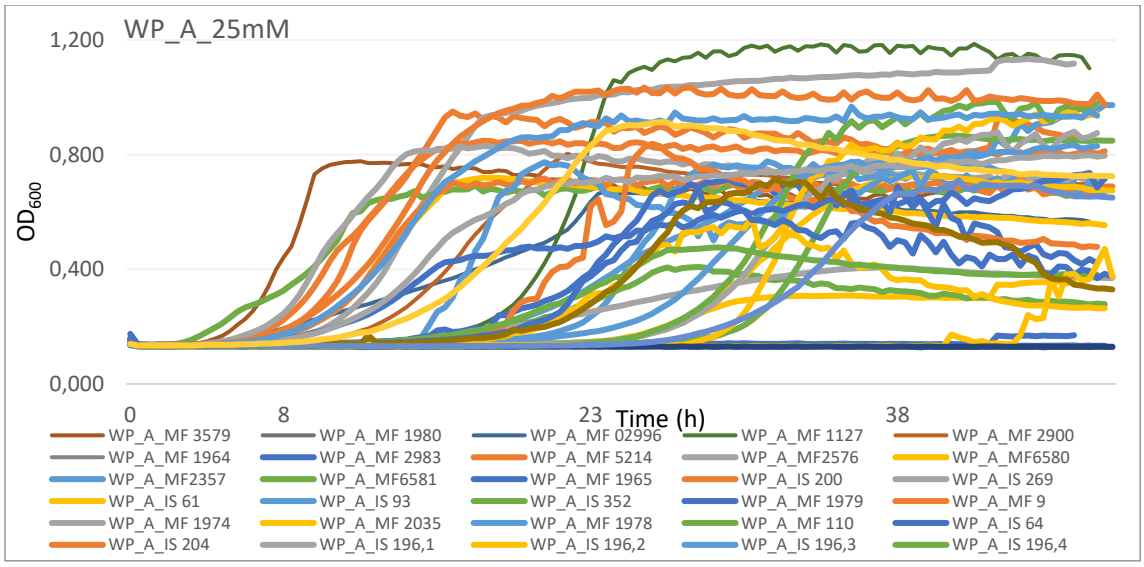

MDCR_C_25mM
2,000
1,600
1,200
0,800
0,400
0,000
OD600
0
8
23
38
Time (h)
MDCR_C_MF 3579
MDCR_C_MF 1980
MDCR_C_MF 02996
MDCR_C_MF 1127
MDCR_C_MF 2900
MDCR_C_MF 1964
MDCR_C_MF 2983
MDCR_C_MF 5214
MDCR_C_MF2576
MDCR_C_MF2357
MDCR_C_MF6581
MDCR_C_MF6580
MDCR_C_MF 1965
MDCR_C_IS 200
MDCR_C_IS 269
MDCR_C_IS 61
MDCR_C_IS 93
MDCR_C_IS 352
MDCR_C_MF 1979
MDCR_C_MF 9
MDCR_C_MF 1974
MDCR_C_MF 2035
MDCR_C_MF 1978
MDCR_C_MF 110
MDCR_C_IS 64
MDCR_C_IS 204
MDCR_C_IS 196,1
MDCR_C_IS 196,2
MDCR_C_IS 196,3
MDCR_C_IS 196,4
MDCR_C_IS 384
MDCR_C_IS 357
MDCR_C_IS 380
MDCR_C_IS 79
MDCR_C_IS 371
MDCR_C_IS 145

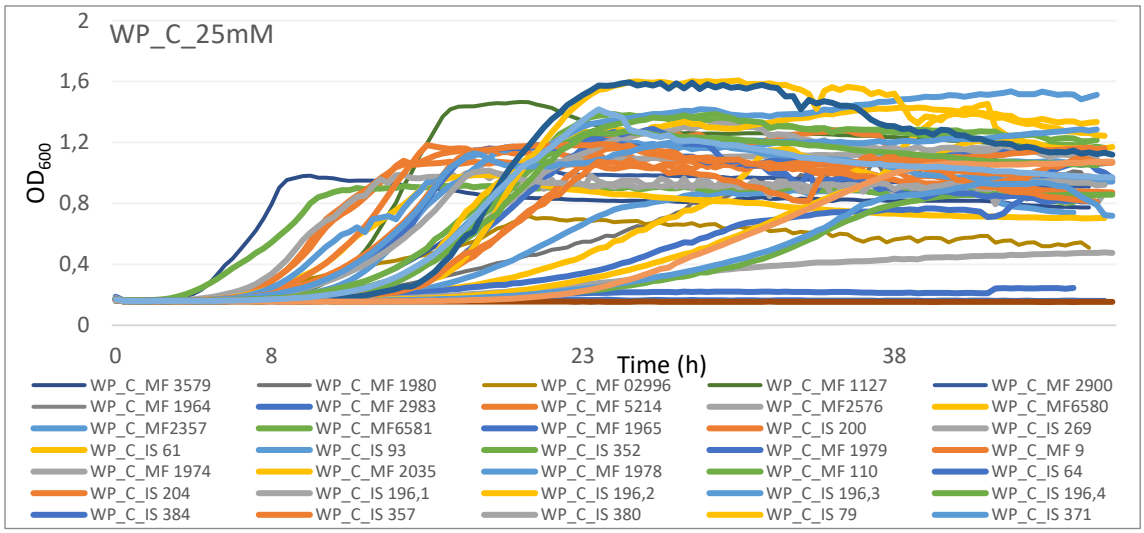

MDCR_F_25mM
2,000
1,600
1,200
0,800
0,400
0,000
OD600
0
8
23
38
Time (h)
MDCR_F_MF 3579
MDCR_F_MF 1980
MDCR_F_MF 02996
MDCR_F_MF 1127
MDCR_F_MF 2900
MDCR_F_MF 1964
MDCR_F_MF 2983
MDCR_F_MF 5214
MDCR_F_MF2576
MDCR_F_MF6580
MDCR_F_MF2357
MDCR_F_MF6581
MDCR_F_MF 1965
MDCR_F_IS 200
MDCR_F_IS 269
MDCR_F_IS 61
MDCR_F_IS 93
MDCR_F_IS 352
MDCR_F_MF 1979
MDCR_F_MF 9
MDCR_F_MF 1974
MDCR_F_MF 2035
MDCR_F_MF 1978
MDCR_F_MF 110
MDCR_F_IS 64
MDCR_F_IS 204
MDCR_F_IS 196,1
MDCR_F_IS 196,2
MDCR_F_IS 196,3
MDCR_F_IS 196,4
MDCR_F_IS 384
MDCR_F_IS 357
MDCR_F_IS 380
MDCR_F_IS 79
MDCR_F_IS 371
MDCR_F_IS 145

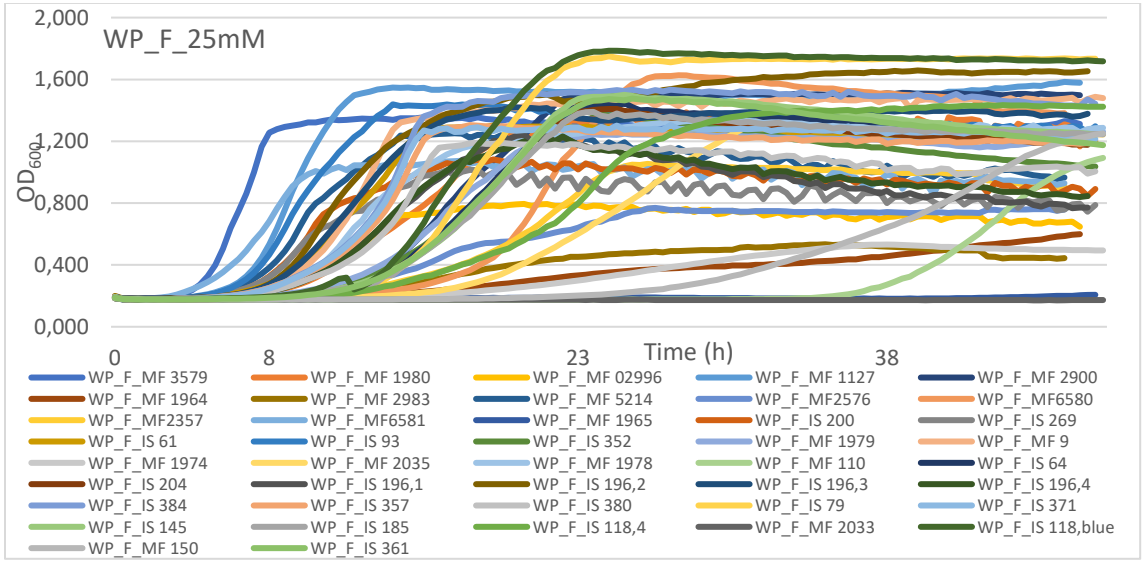

BC_A_25mM
OD600
2,000
1,600
1,200
0,800
0,400
0,000
0
8
23
38
Time (h)
BC_A_MF 3579
BC_A_MF 1980
BC_A_MF 02996
BC_A_MF 1127
BC_A_MF 2900
BC_A_MF 1964
BC_A_MF 2983
BC_A_MF 5214
BC_A_MF2576
BC_A_MF6580
BC_A_MF2357
BC_A_MF6581
BC_A_MF 1965
BC_A_IS 200
BC_A_IS 269
BC_A_IS 61
BC_A_IS 93
BC_A_IS 352
BC_A_MF 1979
BC_A_MF 9
BC_A_MF 2035
BC_A_MF 1978
BC_A_MF 110
BC_A_IS 64
BC_A_IS 204
BC_A_IS 196,1
BC_A_IS 196,2
BC_A_IS 196,3
BC_A_IS 196,4
BC_A_IS 384
BC_A_IS 357
BC_A_IS 380
BC_A_IS 79
BC_A_IS 371
BC_A_IS 145
BC_A_IS 185
BC_A_IS 118,4
BC_A_MF 2033
BC_A_IS 118,blue
BC_A_MF 150

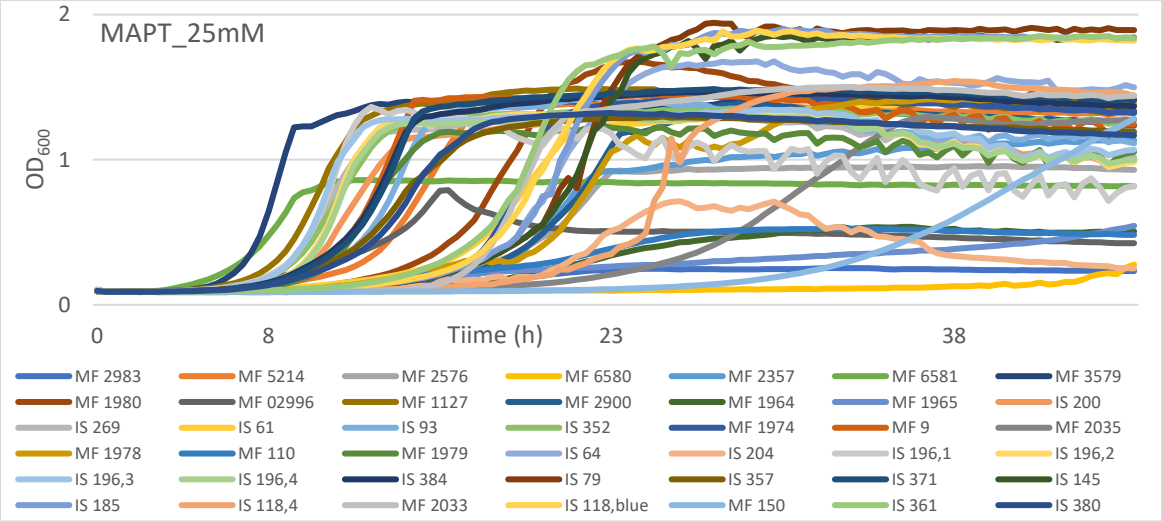

BC_C_25mM
OD600
2,000
1,600
1,200
0,800
0,400
0,000
0
8
23
38
Time (h)
BC_C_MF 3579
BC_C_MF 1980
BC_C_MF 02996
BC_C_MF 1127
BC_C_MF 2900
BC_C_MF 1964
BC_C_MF 2983
BC_C_MF 5214
BC_C_MF2576
BC_C_MF2357
BC_C_MF6581
BC_C_MF6580
BC_C_MF 1965
BC_C_IS 200
BC_C_IS 269
BC_C_IS 61
BC_C_IS 93
BC_C_IS 352
BC_C_MF 1979
BC_C_MF 9
BC_C_MF 1974
BC_C_MF 2035
BC_C_MF 1978
BC_C_MF 110
BC_C_IS 64
BC_C_IS 204
BC_C_IS 196,1
BC_C_IS 196,2
BC_C_IS 196,3
BC_C_IS 196,4
BC_C_IS 384
BC_C_IS 357
BC_C_IS 380
BC_C_IS 79
BC_C_IS 371
BC_C_IS 145
BC_C_IS 185
BC_C_IS 118,4
BC_C_MF 2033
BC_C_IS 118,blue

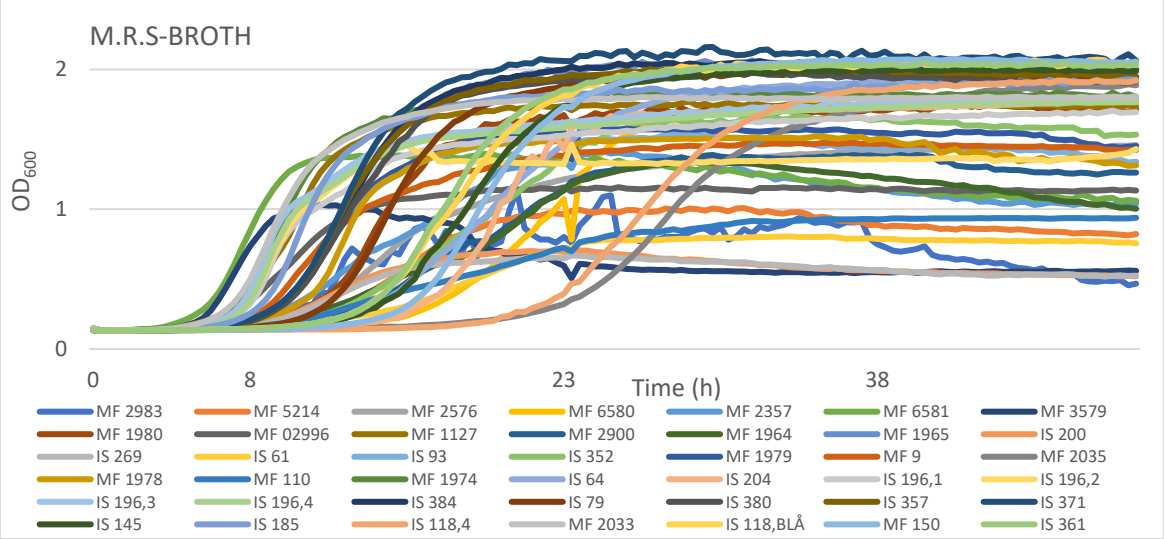

BC_F_25mM
OD600
1,600
1,200
0,800
0,400
0,000
0
8
23
38
Time (h)
BC_F_MF 3579
BC_F_MF 1980
BC_F_MF 02996
BC_F_MF 1127
BC_F_MF 2900
BC_F_MF 1964
BC_F_MF 2983
BC_F_MF 5214
BC_F_MF2576
BC_F_MF6580
BC_F_MF2357
BC_F_MF6581
BC_F_MF 1965
BC_F_IS 200
BC_F_IS 269
BC_F_IS 61
BC_F_IS 93
BC_F_IS 352
BC_F_MF 1979
BC_F_MF 9
BC_F_MF 1974
BC_F_MF 2035
BC_F_MF 1978
BC_F_MF 110
BC_F_IS 64
BC_F_IS 204
BC_F_IS 196,1
BC_F_IS 196,2
BC_F_IS 196,3
BC_F_IS 196,4


WP_A_25mM
1,200
0,800
0,400
0,000
OD600
0
8
23
38
Time (h)
WP_A_MF 3579
WP_A_MF 1980
WP_A_MF 02996
WP_A_MF 1127
WP_A_MF 2900
WP_A_MF 1964
WP_A_MF 2983
WP_A_MF 5214
WP_A_MF2576
WP_A_MF6580
WP_A_MF2357
WP_A_MF6581
WP_A_MF 1965
WP_A_IS 200
WP_A_IS 269
WP_A_IS 61
WP_A_IS 93
WP_A_IS 352
WP_A_MF 1979
WP_A_MF 9
WP_A_MF 1974
WP_A_MF 2035
WP_A_MF 1978
WP_A_MF 110
WP_A_IS 64
WP_A_IS 204
WP_A_IS 196,1
WP_A_IS 196,2
WP_A_IS 196,3
WP_A_IS 196,4

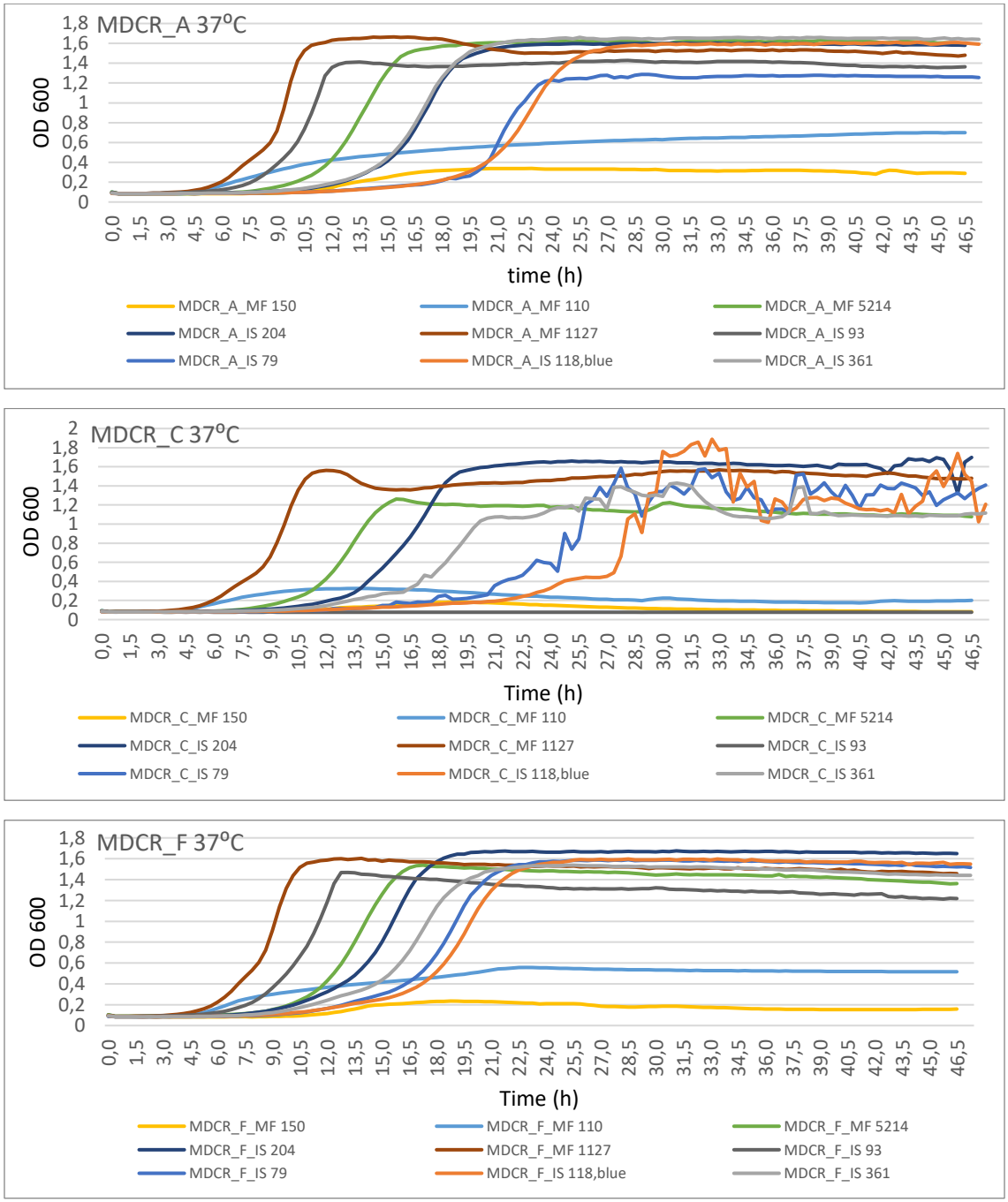

WP_C_25mM
2
1,6
1,2
0,8
0,4
0
OD600
0
8
23
38
Time (h)
WP_C_MF 3579
WP_C_MF 1980
WP_C_MF 02996
WP_C_MF 1127
WP_C_MF 2900
WP_C_MF 1964
WP_C_MF 2983
WP_C_MF 5214
WP_C_MF2576
WP_C_MF6580
WP_C_MF2357
WP_C_MF6581
WP_C_MF 1965
WP_C_IS 200
WP_C_IS 269
WP_C_IS 61
WP_C_IS 93
WP_C_IS 352
WP_C_MF 1979
WP_C_MF 9
WP_C_MF 1974
WP_C_MF 2035
WP_C_MF 1978
WP_C_MF 110
WP_C_IS 64
WP_C_IS 204
WP_C_IS 196,1
WP_C_IS 196,2
WP_C_IS 196,3
WP_C_IS 196,4
WP_C_IS 384
WP_C_IS 357
WP_C_IS 380
WP_C_IS 79
WP_C_IS 371

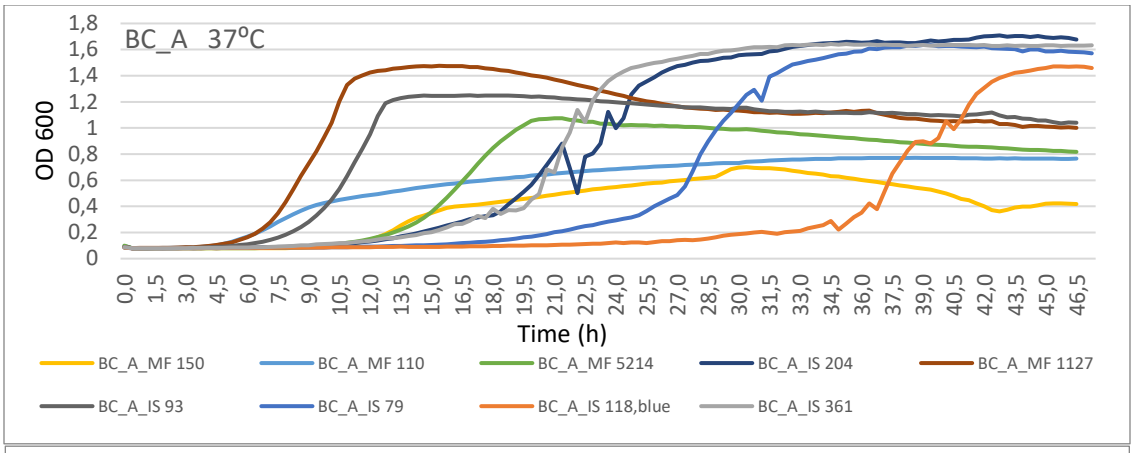

WP_F_25mM
2,000
1,600
1,200
0,800
0,400
0,000
OD600
0
8
23
38
Time (h)
WP_F_MF 3579
WP_F_MF 1980
WP_F_MF 02996
WP_F_MF 1127
WP_F_MF 2900
WP_F_MF 1964
WP_F_MF 2983
WP_F_MF 5214
WP_F_MF2576
WP_F_MF6580
WP_F_MF2357
WP_F_MF6581
WP_F_MF 1965
WP_F_IS 200
WP_F_IS 269
WP_F_IS 61
WP_F_IS 93
WP_F_IS 352
WP_F_MF 1979
WP_F_MF 9
WP_F_MF 1974
WP_F_MF 2035
WP_F_MF 1978
WP_F_MF 110
WP_F_IS 64
WP_F_IS 204
WP_F_IS 196,1
WP_F_IS 196,2
WP_F_IS 196,3
WP_F_IS 196,4
WP_F_IS 384
WP_F_IS 357
WP_F_IS 380
WP_F_IS 79
WP_F_IS 371
WP_F_IS 145
WP_F_IS 185
WP_F_IS 118,4
WP_F_MF 2033
WP_F_IS 118,blue
WP_F_MF 150
WP_F_IS 361

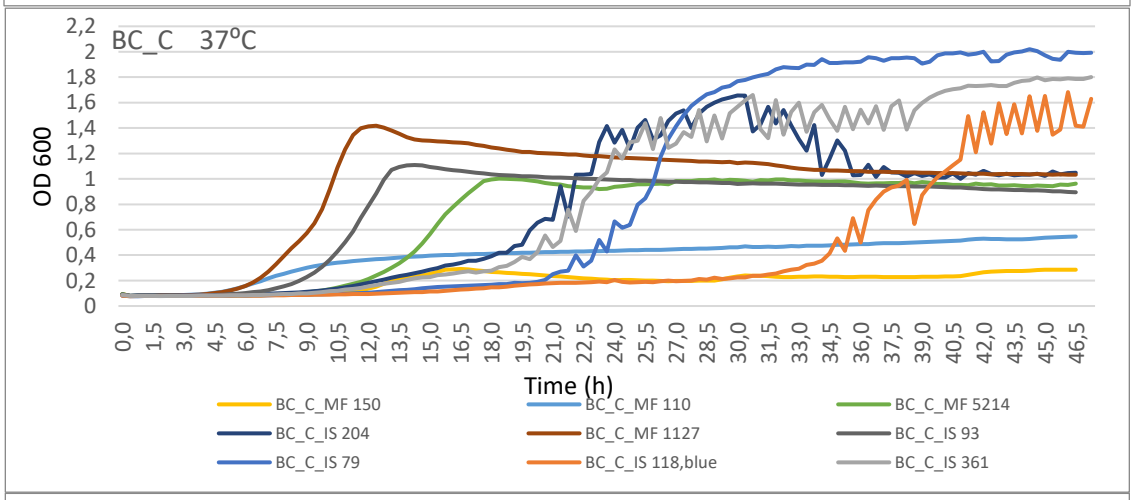

MAPT_25mM
OD600
2
1
0
0
8
Tiime (h)
23
38
MF 2983
MF 5214
MF 2576
MF 6580
MF 2357
MF 6581
MF 3579
MF 1980
MF 02996
MF 1127
MF 2900
MF 1964
MF 1965
IS 200
IS 269
IS 61
IS 93
IS 352
MF 1974
MF 9
MF 2035
MF 1978
MF 110
MF 1979
IS 64
IS 204
IS 196,1
IS 196,2
IS 196,3
IS 196,4
IS 384
IS 79
IS 357
IS 371
IS 145
IS 185
IS 118,4
MF 2033
IS 118,blue
MF 150
IS 361
IS 380

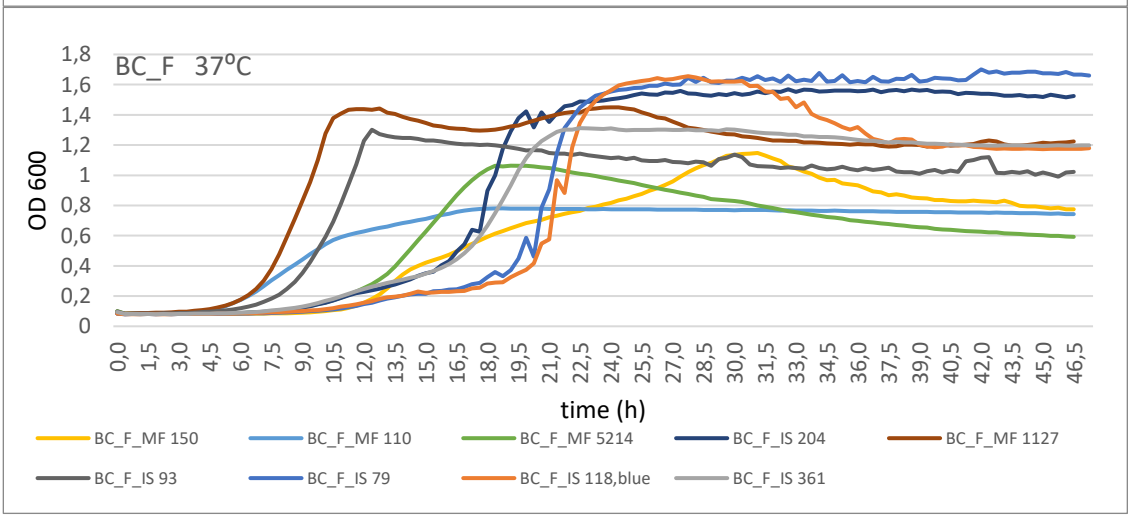

M.R.S-BROTH
OD600
2
1
0
0
8
23
Time (h)
38
MF 2983
MF 5214
MF 2576
MF 6580
MF 2357
MF 6581
MF 3579
MF 1980
MF 02996
MF 1127
MF 2900
MF 1964
MF 1965
IS 200
IS 269
IS 61
IS 93
IS 352
MF 1979
MF 9
MF 2035
MF 1978
MF 110
MF 1974
IS 64
IS 204
IS 196,1
IS 196,2
IS 196,3
IS 196,4
IS 384
IS 79
IS 380
IS 357
IS 371
IS 145
IS 185
IS 118,4
MF 2033
IS 118,BLÅ
MF 150
IS 361

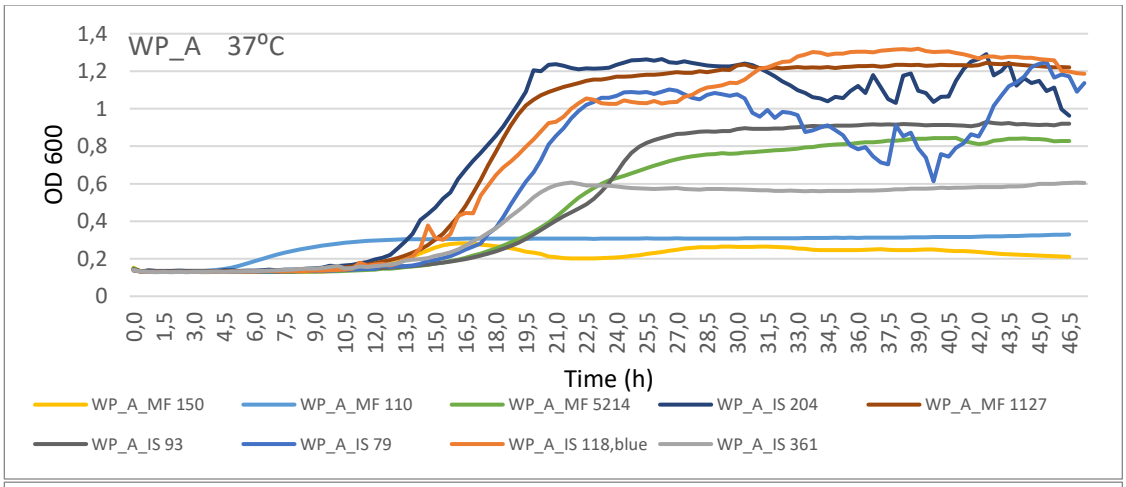
Figures A7.2: *Bioscreen-C Trial B results after pooling the triplicate series, trimming off deviating series. Bacterial growth was measured at 600nm (OD) over a period of 48 hours. Each series on the graph represents a different strain screened against the same SMAPT media. The following 11 graphs are from the same trial from all strains grown at 37°C with 25 mM glucose SMAPT media, grown in triplicate series for each run. The last two graphs in the series are of basis media MAPT and M.R.S respectively. (color of strain series may deviate between graphs).*

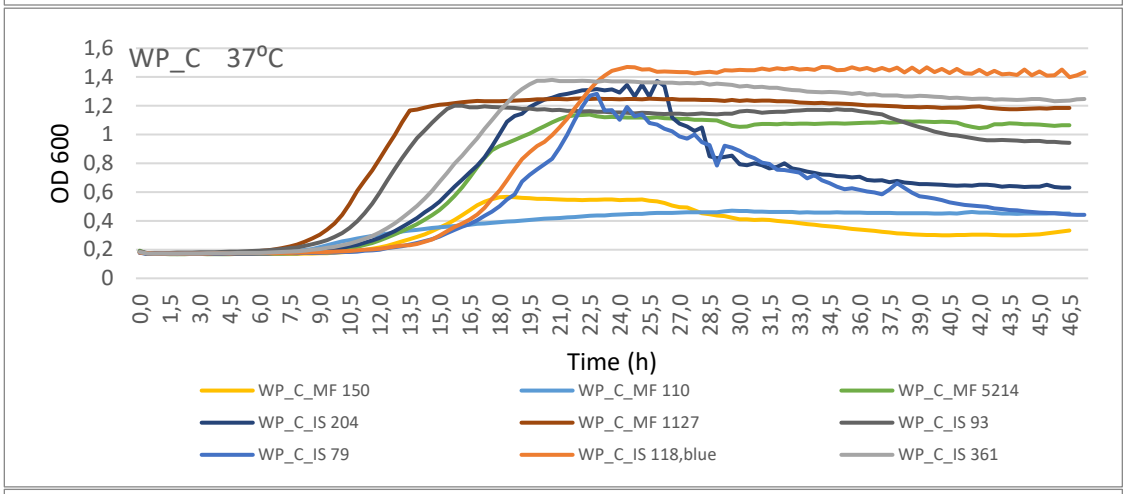


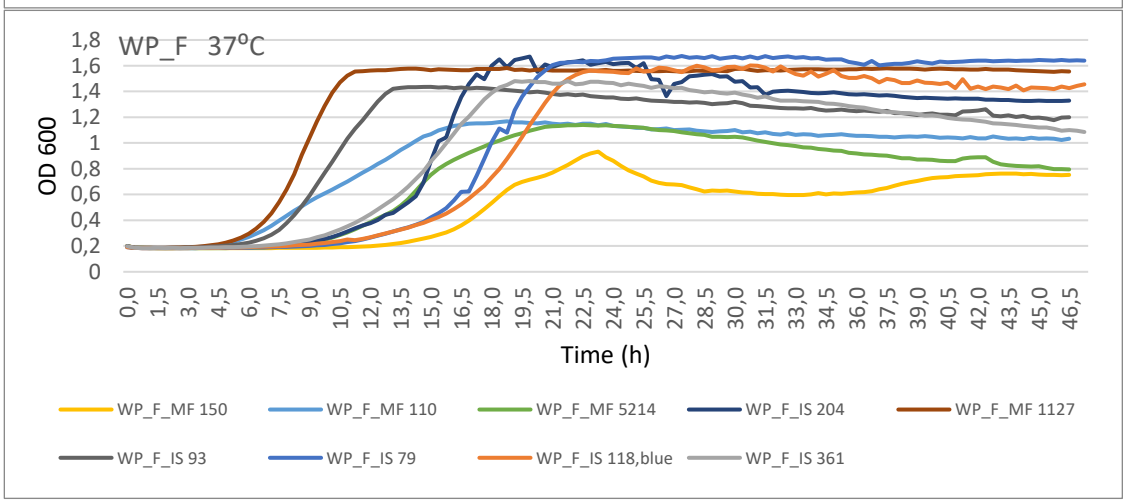

BC_A 37°C
OD 600
Time (h)
BC_A_MF 150
BC_A_MF 110
BC_A_MF 5214
BC_A_IS 204
BC_A_MF 1127
BC_A_IS 93
BC_A_IS 79
BC_A_IS 118,blue
BC_A_IS 361

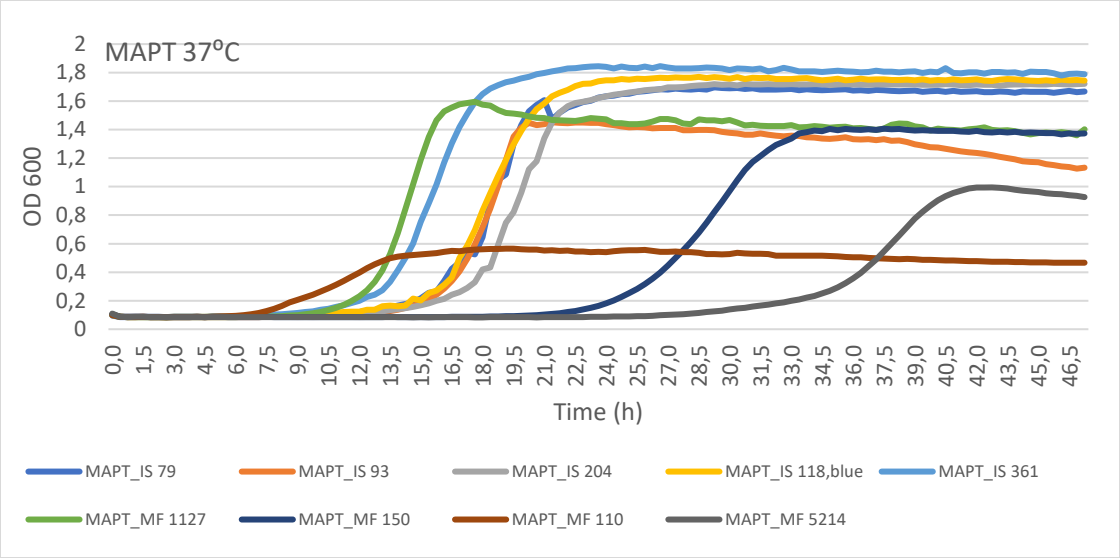

BC_C 37°C
OD 600
Time (h)
BC_C_MF 150
BC_C_MF 110
BC_C_MF 5214
BC_C_IS 204
BC_C_MF 1127
BC_C_IS 93
BC_C_IS 79
BC_C_IS 118,blue
BC_C_IS 361

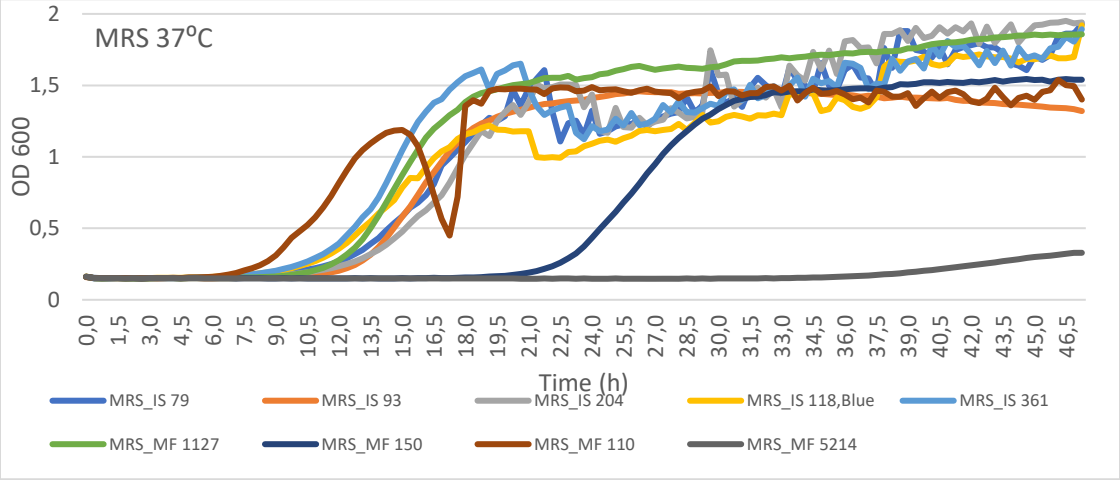

BC_F 37°C
OD 600
time (h)
BC_F_MF 150
BC_F_MF 110
BC_F_MF 5214
BC_F_IS 204
BC_F_MF 1127
BC_F_IS 93
BC_F_IS 79
BC_F_IS 118,blue
BC_F_IS 361


WP_A 37°C
OD 600
Time (h)
WP_A_MF 150
WP_A_MF 110
WP_A_MF 5214
WP_A_IS 204
WP_A_MF 1127
WP_A_IS 93
WP_A_IS 79
WP_A_IS 118,blue
WP_A_IS 361

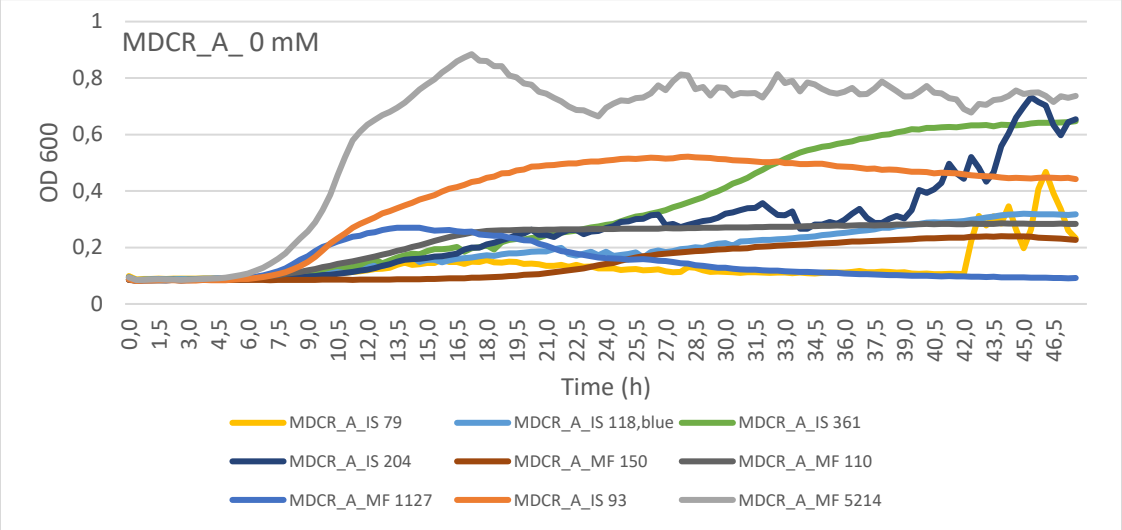

WP_C 37°C
OD 600
Time (h)
WP_C_MF 150
WP_C_MF 110
WP_C_MF 5214
WP_C_IS 204
WP_C_MF 1127
WP_C_IS 93
WP_C_IS 79
WP_C_IS 118,blue
WP_C_IS 361

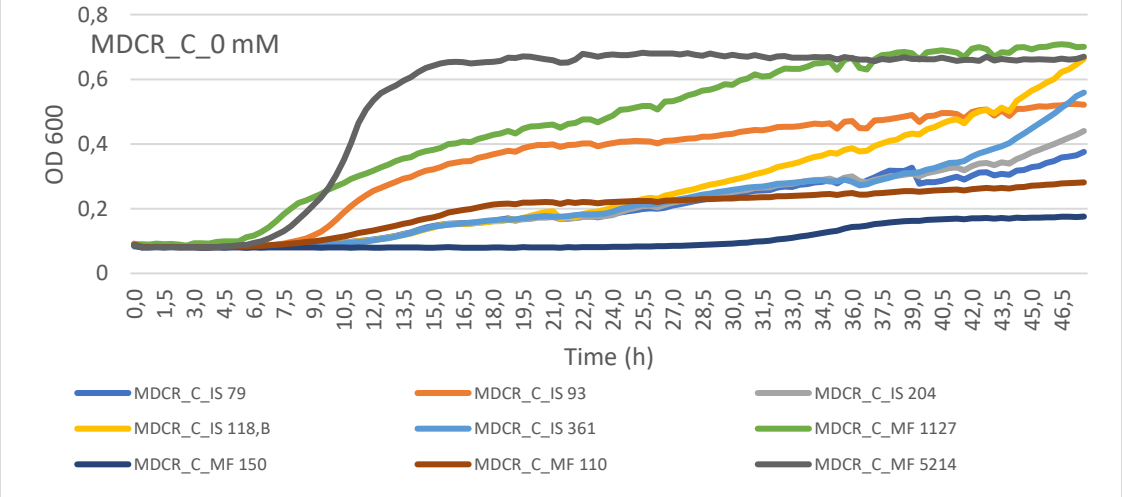

WP_F 37°C
OD 600
Time (h)
WP_F_MF 150
WP_F_MF 110
WP_F_MF 5214
WP_F_IS 204
WP_F_MF 1127
WP_F_IS 93
WP_F_IS 79
WP_F_IS 118,blue
WP_F_IS 361

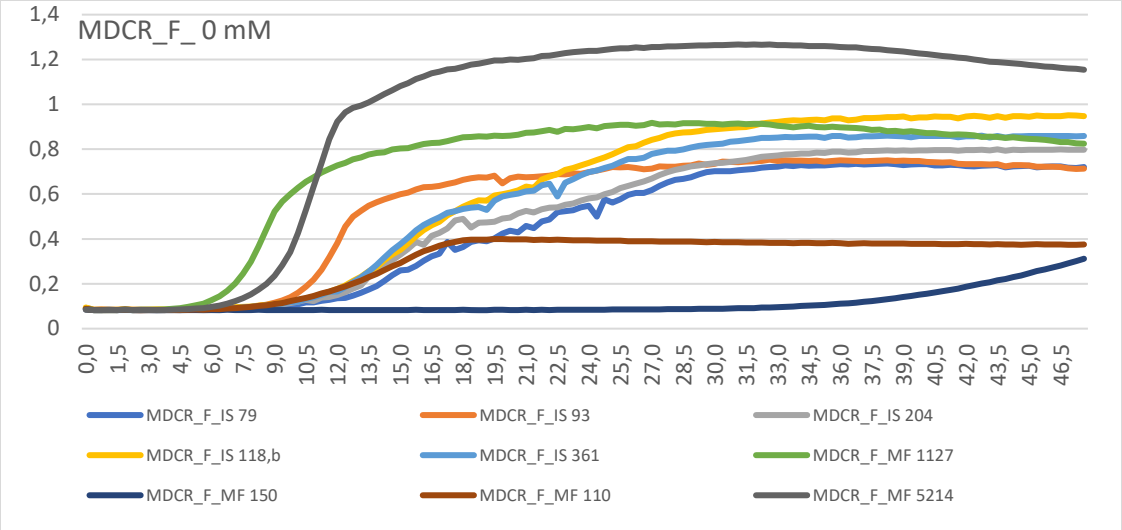

MAPT 37°C
OD 600
Time (h)
MAPT_IS 79
MAPT_IS 93
MAPT_IS 204
MAPT_IS 118,blue
MAPT_IS 361
MAPT_MF 1127
MAPT_MF 150
MAPT_MF 110
MAPT_MF 5214

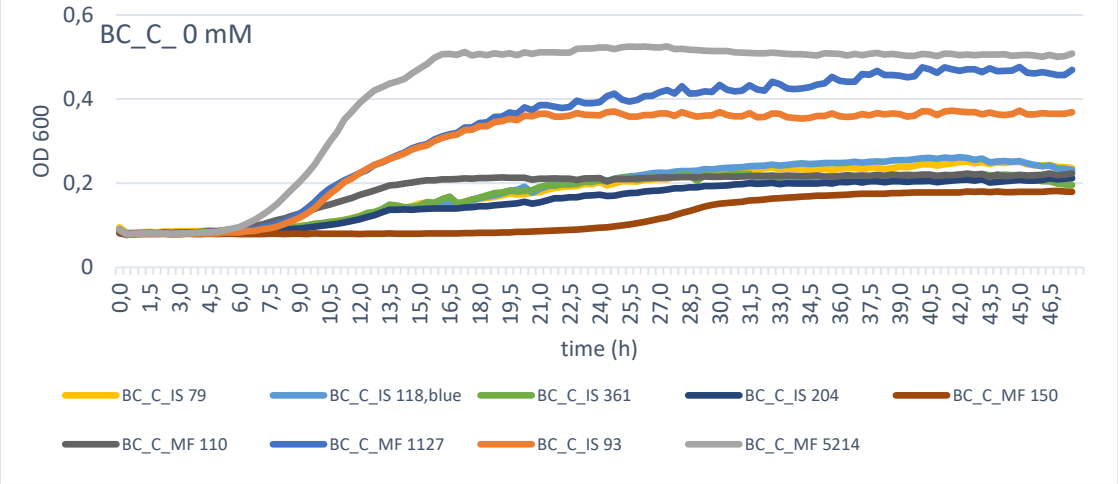

MRS 37°C
OD 600
Time (h)
MRS_IS 79
MRS_IS 93
MRS_IS 204
MRS_IS 118,Blue
MRS_IS 361
MRS_MF 1127
MRS_MF 150
MRS_MF 110
MRS_MF 5214

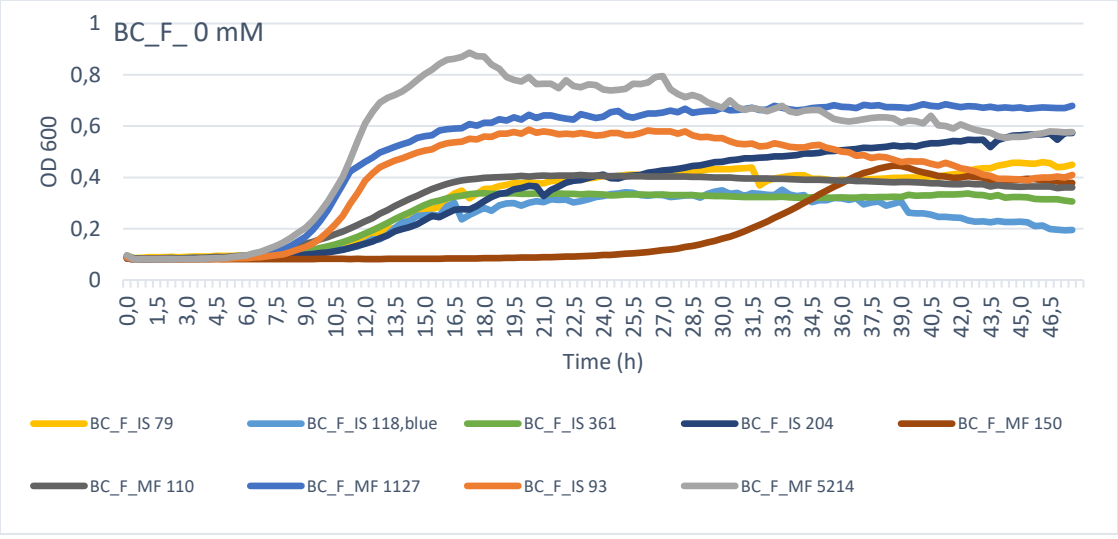
Figures A7.3: *Bioscreen-C Trial C results after pooling the triplicate series, trimming off deviating series. Bacterial growth was measured at 600nm (OD) over a period of 48 hours. Each series on the graph represents a different strain screened against the same SMAPT media. The following 10 graphs are from the same trial from all strains grown at 30°C with 0 mM glucose SMAPT media, grown in triplicate series for each run. The last two graphs in the series are of basis media MAPT (color of strain series may deviate between graph).*

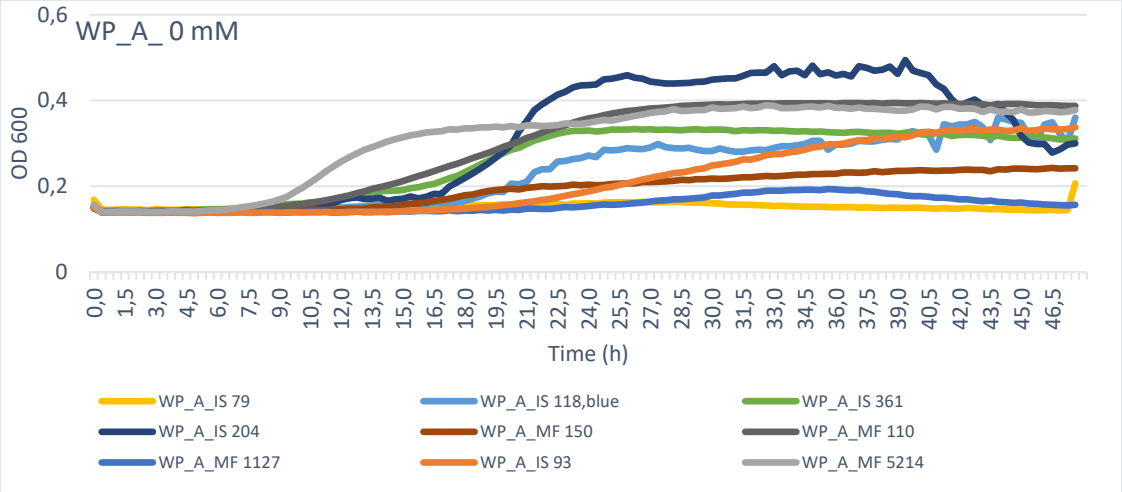


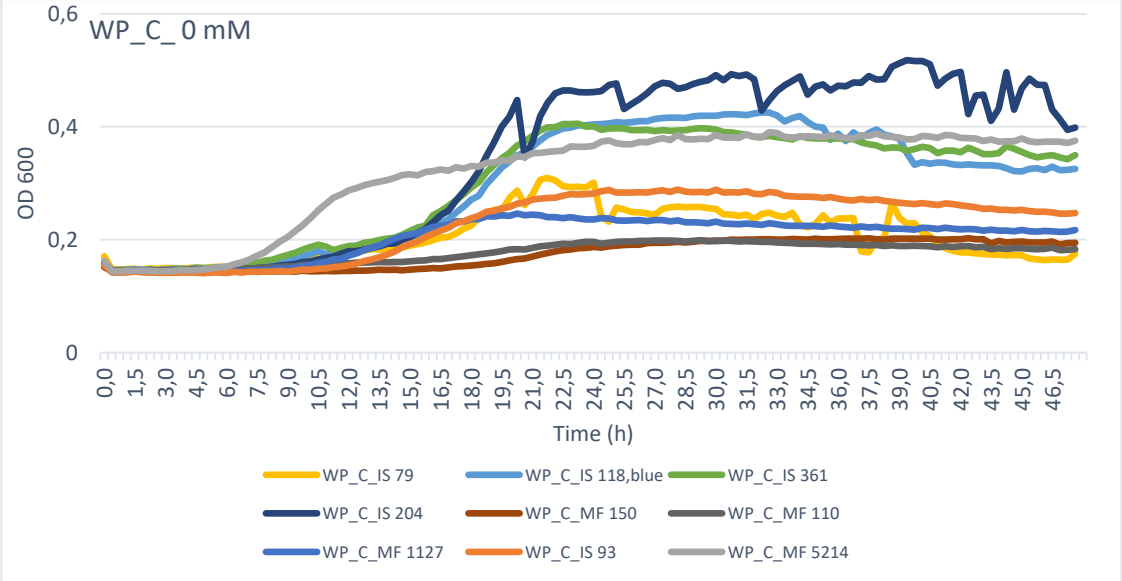


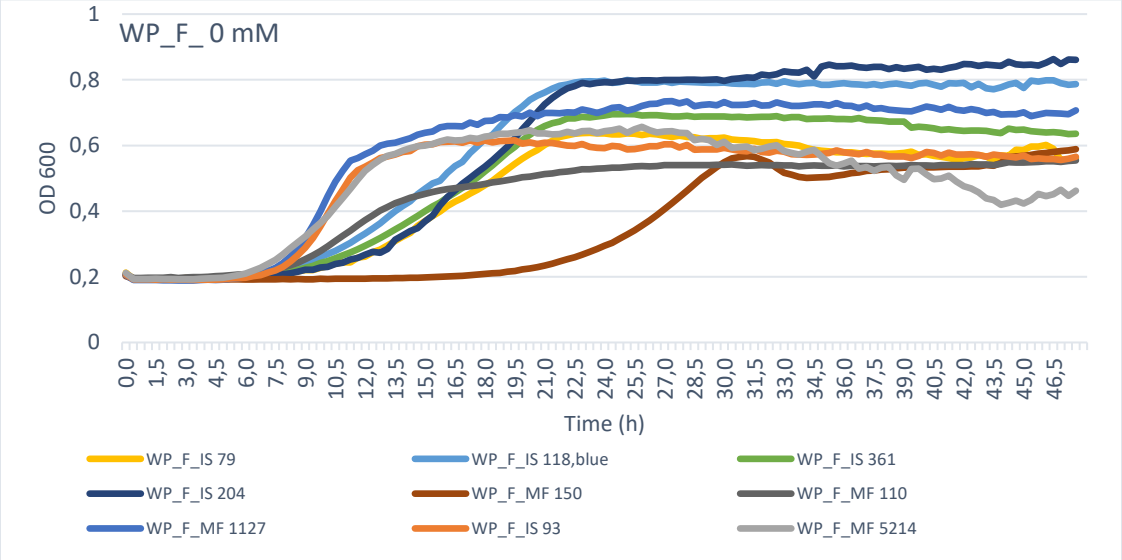

MDCR_F_ 0 mM
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0,0 1,5 3,0 4,5 6,0 7,5 9,0 10,5 12,0 13,5 15,0 16,5 18,0 19,5 21,0 22,5 24,0 25,5 27,0 28,5 30,0 31,5 33,0 34,5 36,0 37,5 39,0 40,5 42,0 43,5 45,0 46,5
MDCR_F_IS 79
MDCR_F_IS 93
MDCR_F_IS 204
MDCR_F_IS 118,b
MDCR_F_IS 361
MDCR_F_MF 1127
MDCR_F_MF 150
MDCR_F_MF 110
MDCR_F_MF 5214

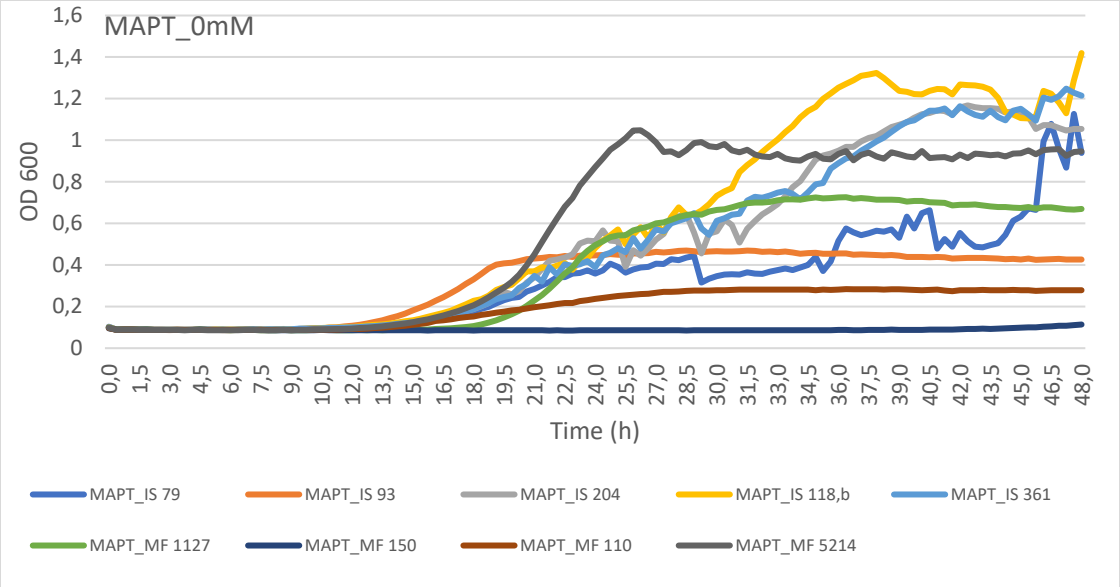

BC_C_ 0 mM
0,6
0,4
0,2
0
OD 600
0,0 1,5 3,0 4,5 6,0 7,5 9,0 10,5 12,0 13,5 15,0 16,5 18,0 19,5 21,0 22,5 24,0 25,5 27,0 28,5 30,0 31,5 33,0 34,5 36,0 37,5 39,0 40,5 42,0 43,5 45,0 46,5
time (h)
BC_C_IS 79
BC_C_IS 118,blue
BC_C_IS 361
BC_C_IS 204
BC_C_MF 150
BC_C_MF 110
BC_C_MF 1127
BC_C_IS 93
BC_C_MF 5214


BC_F_ 0 mM
1
0,8
0,6
0,4
0,2
0
OD 600
0,0 1,5 3,0 4,5 6,0 7,5 9,0 10,5 12,0 13,5 15,0 16,5 18,0 19,5 21,0 22,5 24,0 25,5 27,0 28,5 30,0 31,5 33,0 34,5 36,0 37,5 39,0 40,5 42,0 43,5 45,0 46,5
Time (h)
BC_F_IS 79
BC_F_IS 118,blue
BC_F_IS 361
BC_F_IS 204
BC_F_MF 150
BC_F_MF 110
BC_F_MF 1127
BC_F_IS 93
BC_F_MF 5214

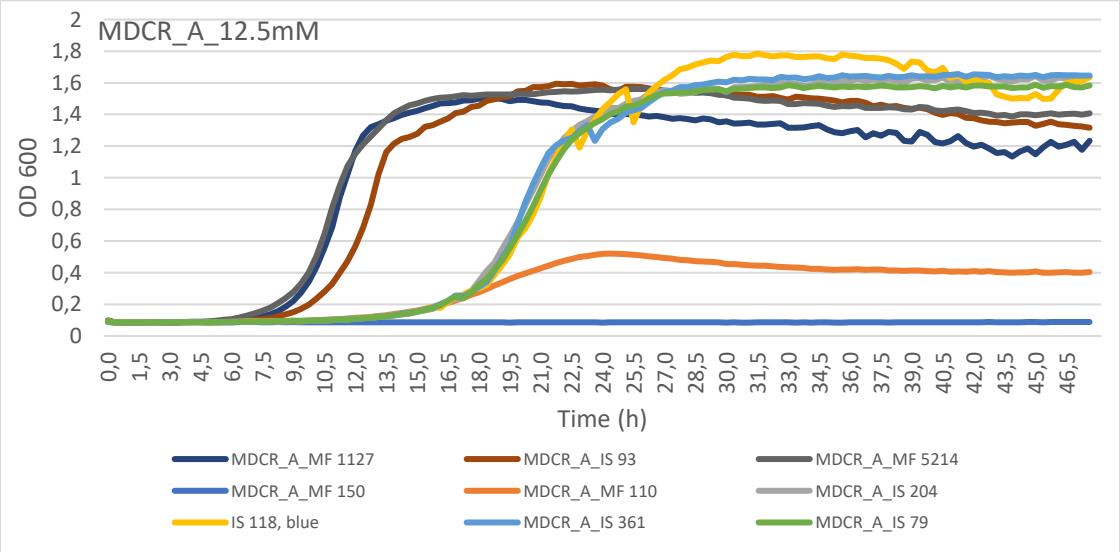

WP_A_ 0 mM
OD 600
Time (h)
WP_A_IS 79
WP_A_IS 118,blue
WP_A_IS 361
WP_A_IS 204
WP_A_MF 150
WP_A_MF 110
WP_A_MF 1127
WP_A_IS 93
WP_A_MF 5214

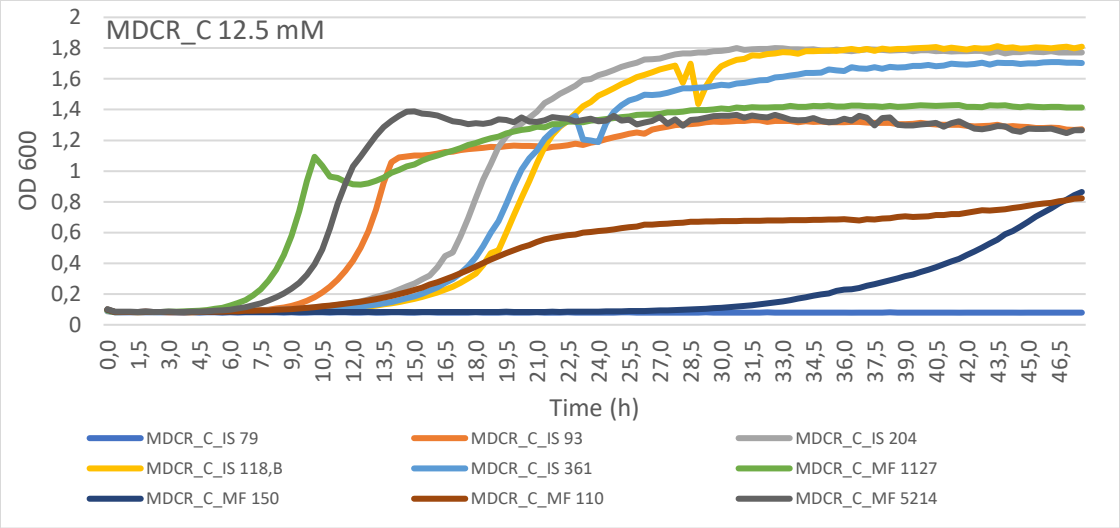

WP_C_ 0 mM
OD 600
Time (h)
WP_C_IS 79
WP_C_IS 118,blue
WP_C_IS 361
WP_C_IS 204
WP_C_MF 150
WP_C_MF 110
WP_C_MF 1127
WP_C_IS 93
WP_C_MF 5214

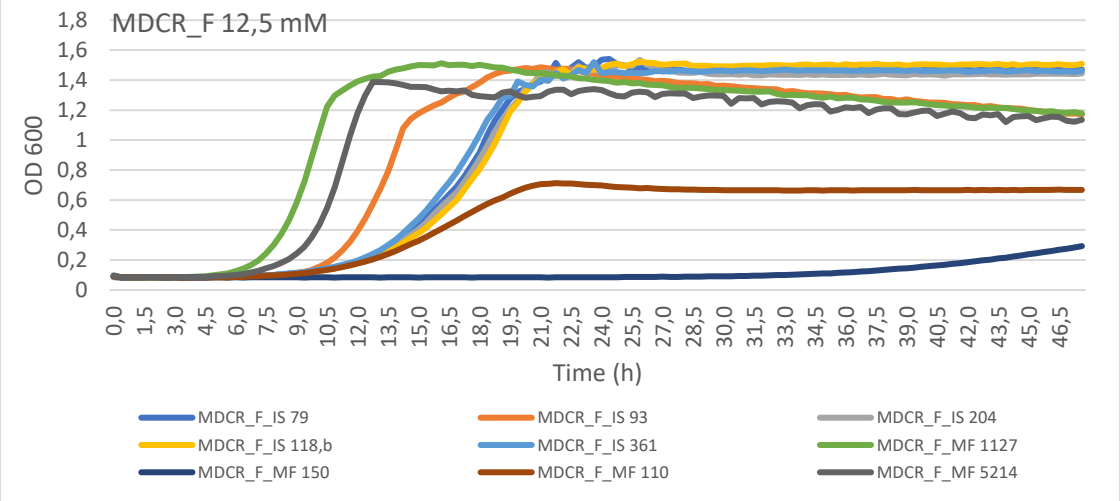

WP_F_ 0 mM
OD 600
Time (h)
WP_F_IS 79
WP_F_IS 118,blue
WP_F_IS 361
WP_F_IS 204
WP_F_MF 150
WP_F_MF 110
WP_F_MF 1127
WP_F_IS 93
WP_F_MF 5214

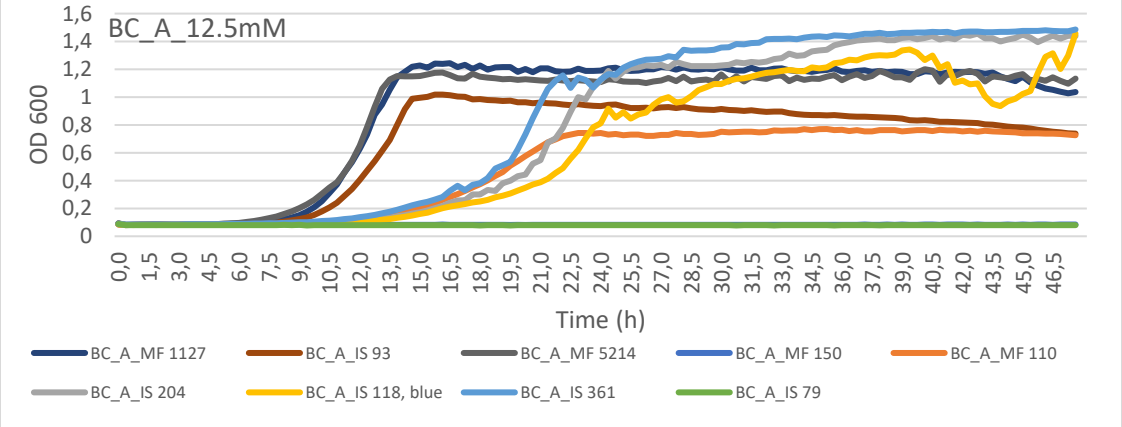


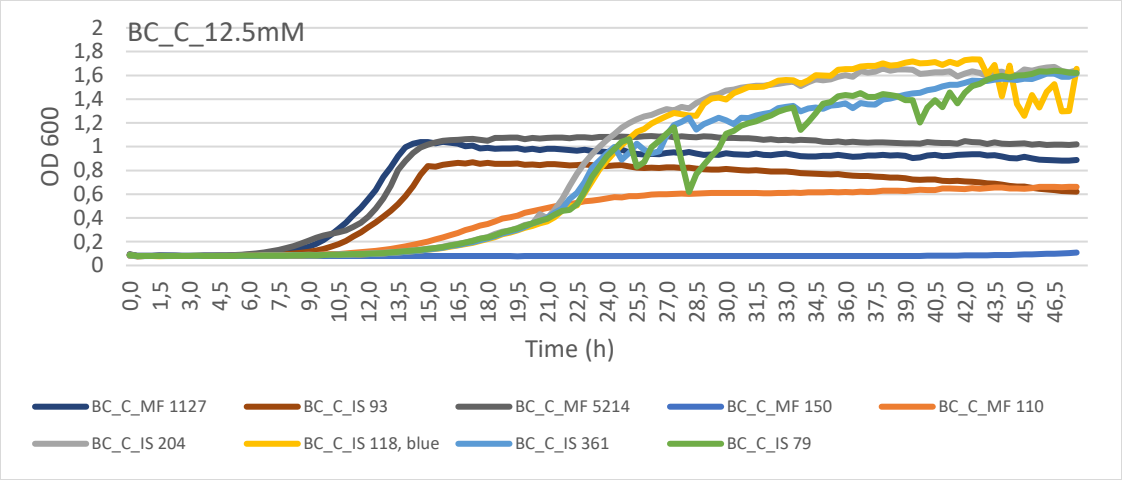
Figures A7.4: *Bioscreen-C Trial C results after pooling the triplicate series, trimming off deviating series. Bacterial growth was measured at 600nm (OD) over a period of 48 hours. Each series on the graph represents a different strain screened against the same SMAPT media. The following 10 graphs are from the same trial from all strains grown at 30°C with 12.5 mM glucose SMAPT media, grown in triplicate series for each run. The last two graphs in the series are of basis media MAPT. (color of strain series may deviate between graphs*

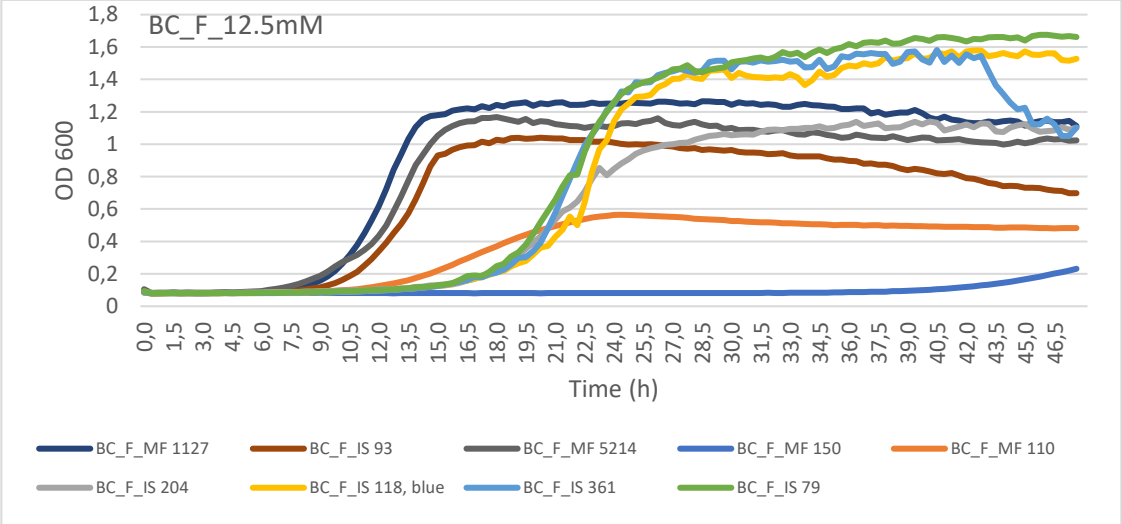


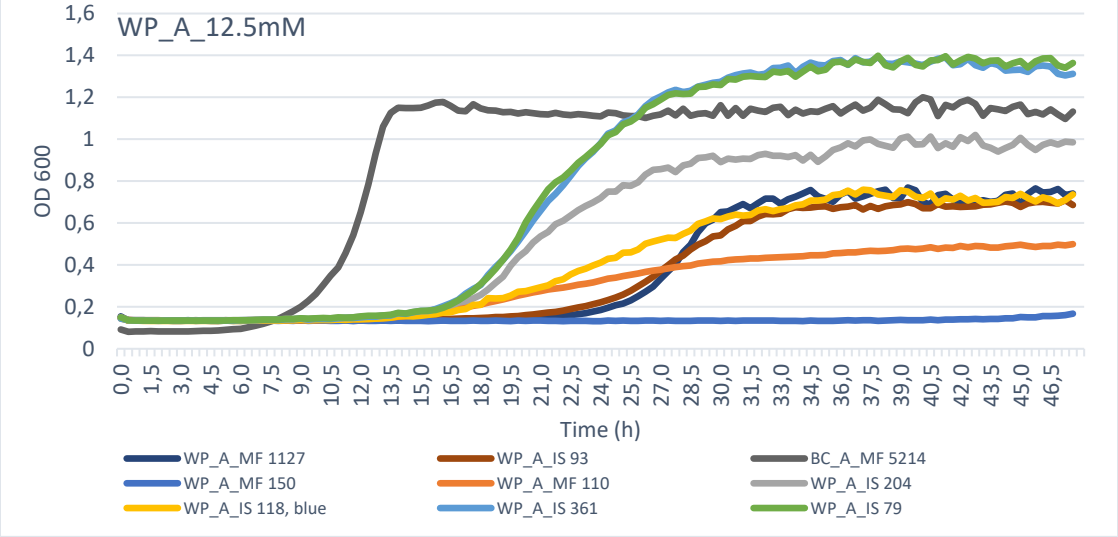

MDCR_C 12.5 mM
OD 600
Time (h)
MDCR_C_IS 79
MDCR_C_IS 93
MDCR_C_IS 204
MDCR_C_IS 118,B
MDCR_C_IS 361
MDCR_C_MF 1127
MDCR_C_MF 150
MDCR_C_MF 110
MDCR_C_MF 5214

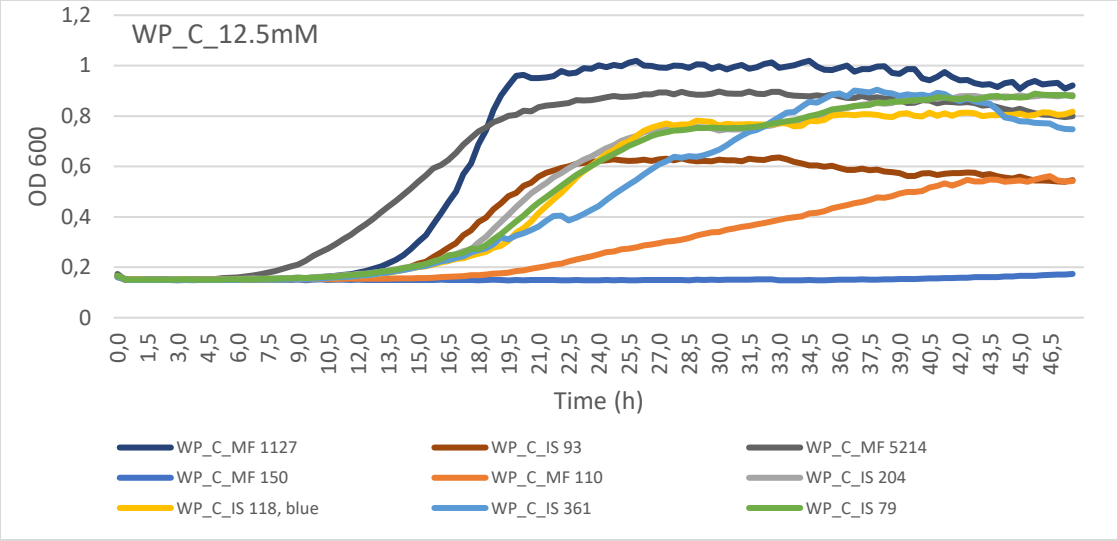

MDCR_F 12,5 mM
OD 600
Time (h)
MDCR_F_IS 79
MDCR_F_IS 93
MDCR_F_IS 204
MDCR_F_IS 118,b
MDCR_F_IS 361
MDCR_F_MF 1127
MDCR_F_MF 150
MDCR_F_MF 110
MDCR_F_MF 5214

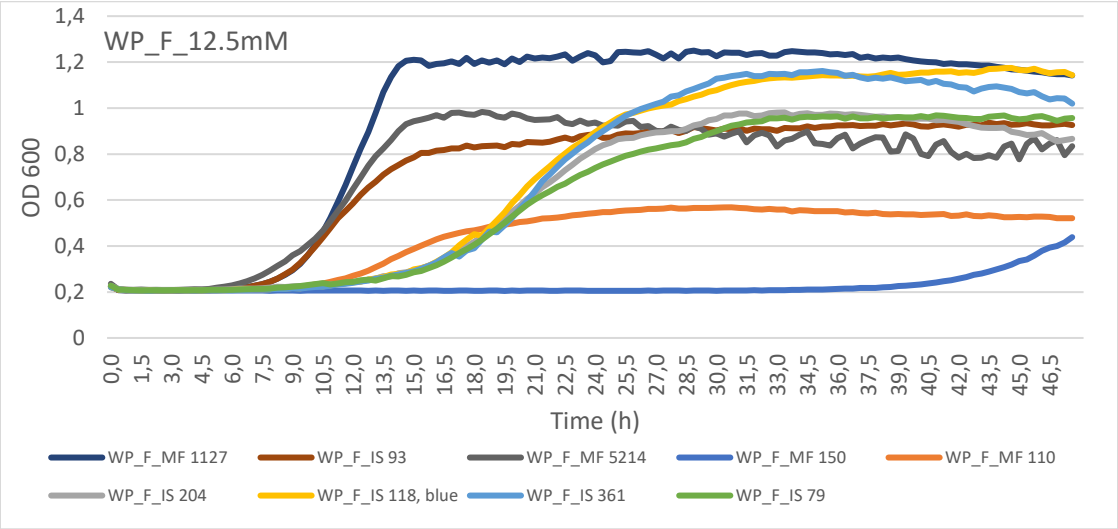

BC_A_12.5mM
OD 600
Time (h)
BC_A_MF 1127
BC_A_IS 93
BC_A_MF 5214
BC_A_MF 150
BC_A_MF 110
BC_A_IS 204
BC_A_IS 118, blue
BC_A_IS 361
BC_A_IS 79

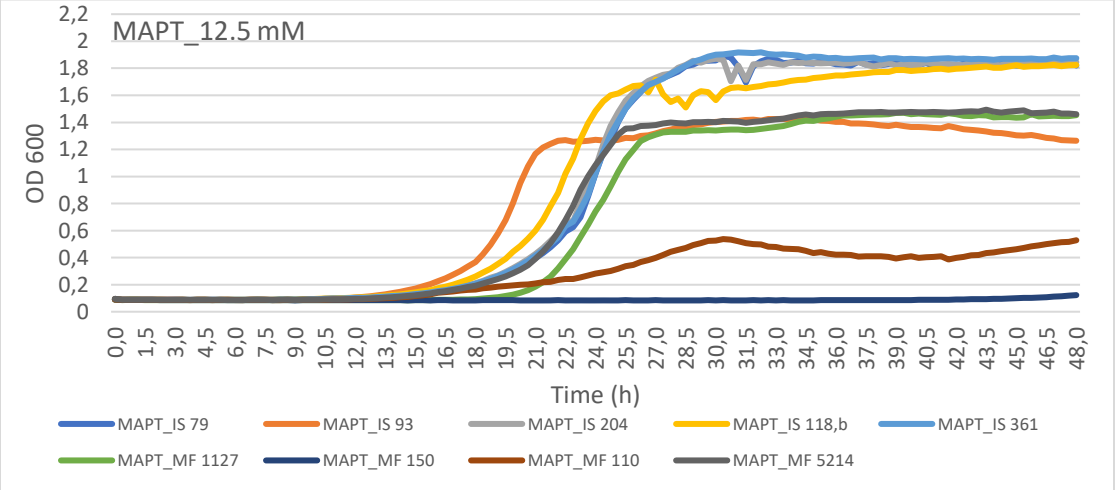

BC_C_12.5mM
OD 600
Time (h)
BC_C_MF 1127
BC_C_IS 93
BC_C_MF 5214
BC_C_MF 150
BC_C_MF 110
BC_C_IS 204
BC_C_IS 118, blue
BC_C_IS 361
BC_C_IS 79


BC_F_12.5mM
OD 600
Time (h)
BC_F_MF 1127
BC_F_IS 93
BC_F_MF 5214
BC_F_MF 150
BC_F_MF 110
BC_F_IS 204
BC_F_IS 118, blue
BC_F_IS 361
BC_F_IS 79

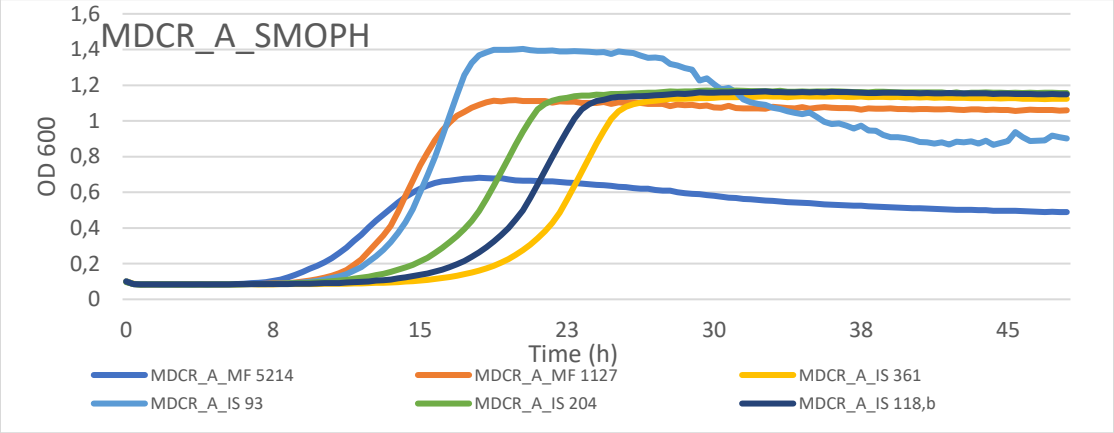

WP_A_12.5mM
OD 600
Time (h)
WP_A_MF 1127
WP_A_IS 93
BC_A_MF 5214
WP_A_MF 150
WP_A_MF 110
WP_A_IS 204
WP_A_IS 118, blue
WP_A_IS 361
WP_A_IS 79

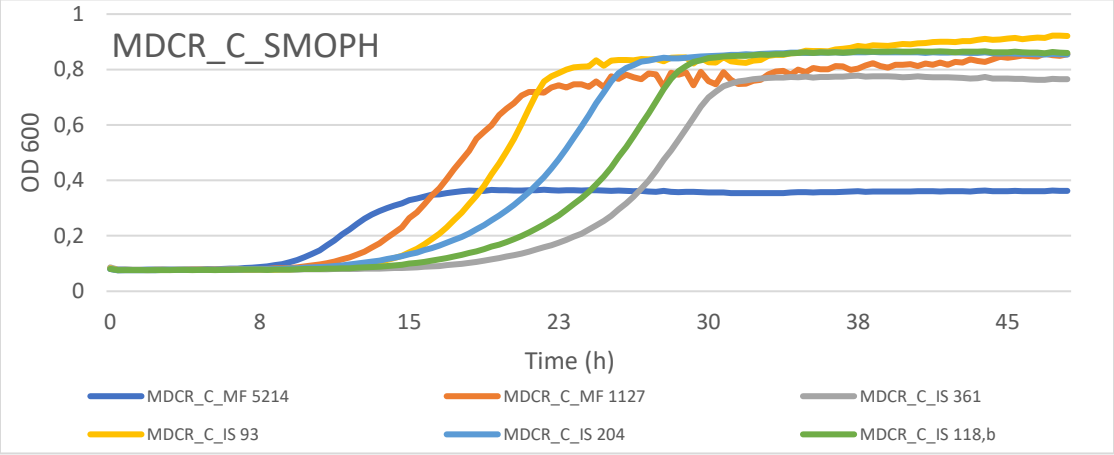

WP_C_12.5mM
OD 600
Time (h)
WP_C_MF 1127
WP_C_IS 93
WP_C_MF 5214
WP_C_MF 150
WP_C_MF 110
WP_C_IS 204
WP_C_IS 118, blue
WP_C_IS 361
WP_C_IS 79

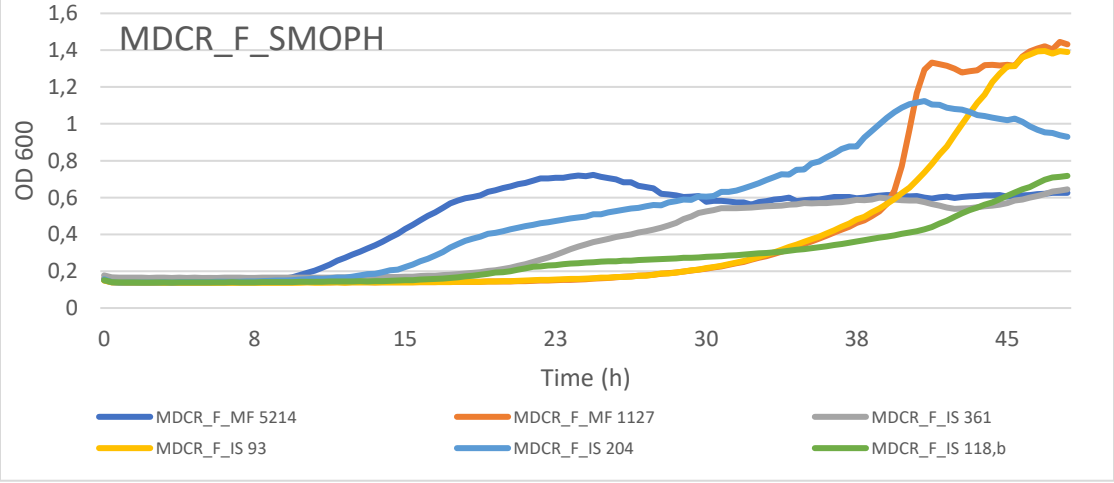

WP_F_12.5mM
OD 600
Time (h)
WP_F_MF 1127
WP_F_IS 93
WP_F_MF 5214
WP_F_MF 150
WP_F_MF 110
WP_F_IS 204
WP_F_IS 118, blue
WP_F_IS 361
WP_F_IS 79

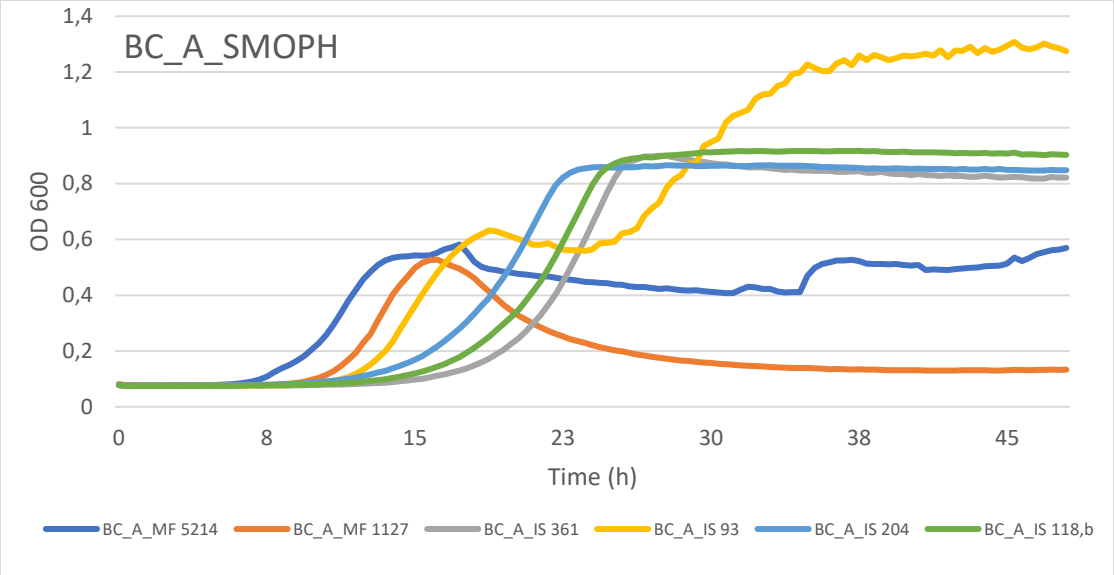

MAPT_12.5 mM
OD 600
Time (h)
MAPT_IS 79
MAPT_IS 93
MAPT_IS 204
MAPT_IS 118,b
MAPT_IS 361
MAPT_MF 1127
MAPT_MF 150
MAPT_MF 110
MAPT_MF 5214

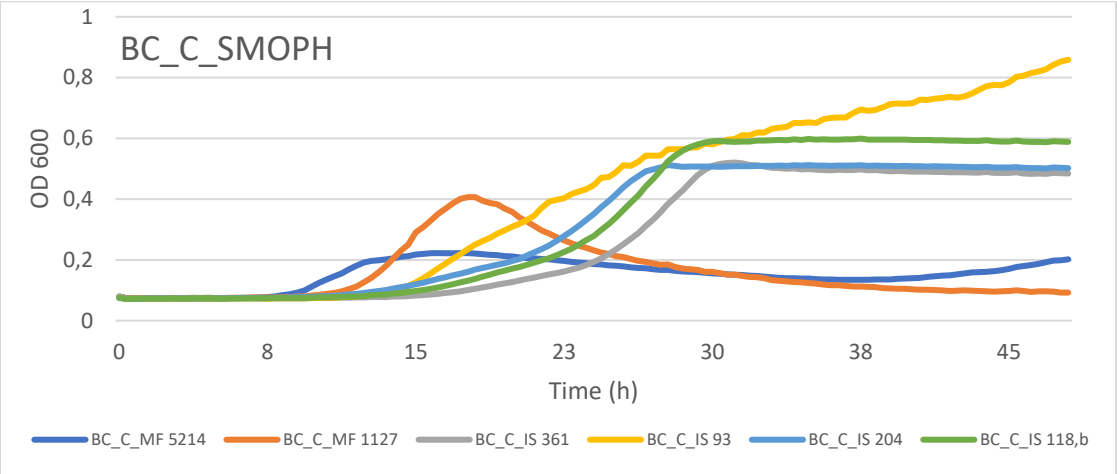
Figures A7.5: *Bioscreen-C Trial D results after pooling the triplicate series, trimming off deviating series. Bacterial growth was measured at 600nm (OD) over a period of 48 hours. Each series on the graph represents a different strain screened against the same SMOPH media. The following 10 graphs are from the same trial from all strains grown at 30°C with 12.5 mM glucose SMOPH media, grown in triplicate series for each run (color of strain series may deviate between graphs). Results from SMOPH 0mM is not listed.*

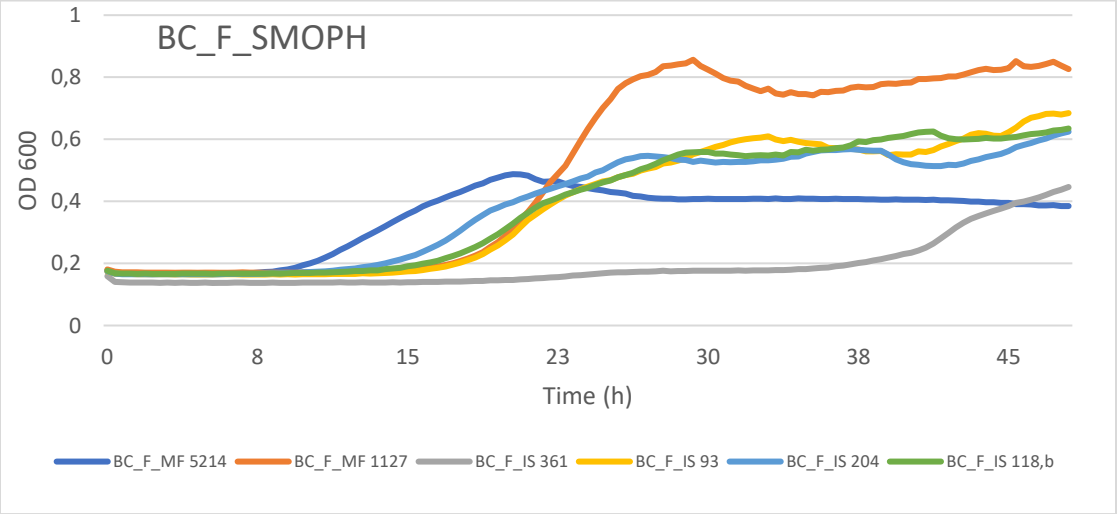


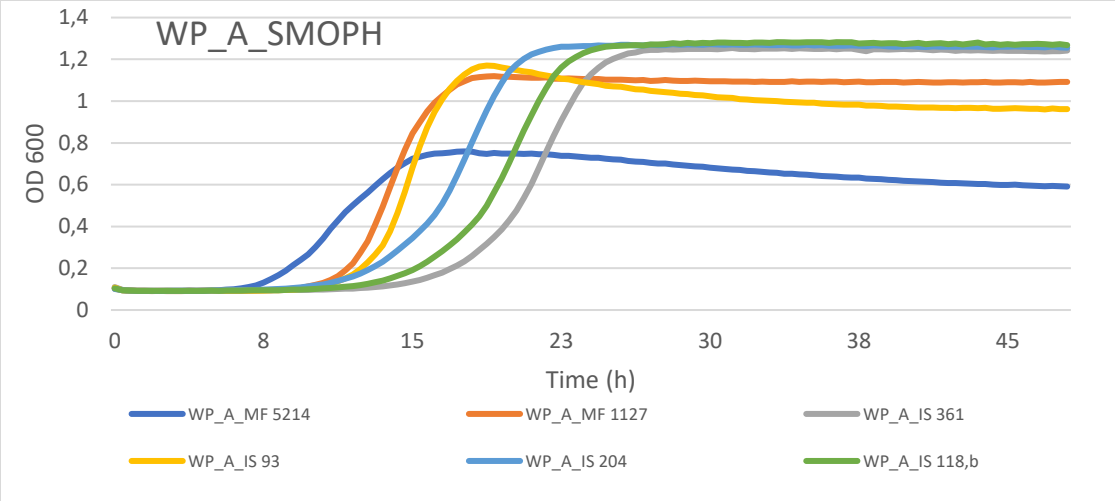


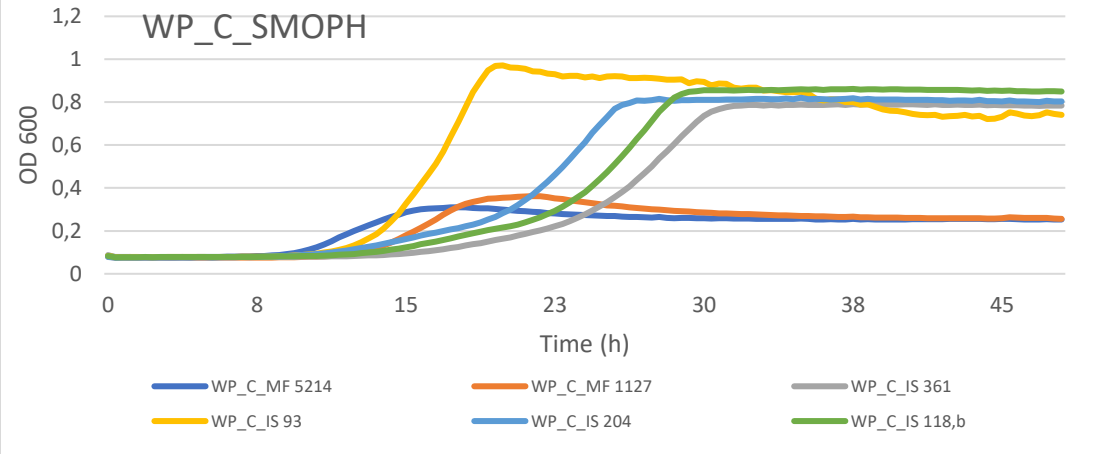


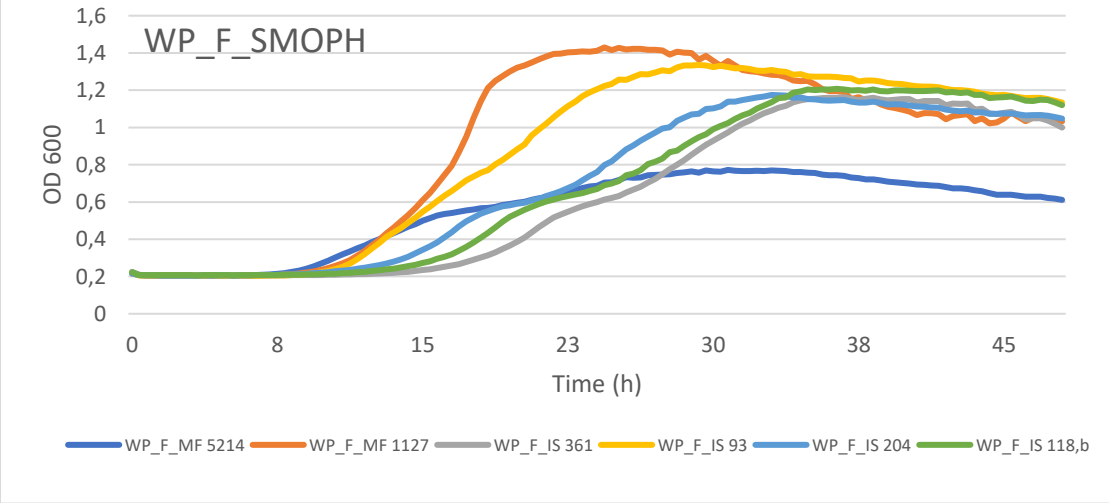

BC_A_SMOPH
OD 600
Time (h)
BC_A_MF 5214
BC_A_MF 1127
BC_A_IS 361
BC_A_IS 93
BC_A_IS 204
BC_A_IS 118,b


BC_C_SMOPH
OD 600
Time (h)
BC_C_MF 5214
BC_C_MF 1127
BC_C_IS 361
BC_C_IS 93
BC_C_IS 204
BC_C_IS 118,b


BC_F_SMOPH
OD 600
Time (h)
BC_F_MF 5214
BC_F_MF 1127
BC_F_IS 361
BC_F_IS 93
BC_F_IS 204
BC_F_IS 118,b


WP_A_SMOPH
OD 600
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
8
15
23
30
38
45
Time (h)
WP_A_MF 5214
WP_A_MF 1127
WP_A_IS 361
WP_A_IS 93
WP_A_IS 204
WP_A_IS 118,b


WP_C_SMOPH
OD 600
1,2
1
0,8
0,6
0,4
0,2
0
0
8
15
23
30
38
45
Time (h)
WP_C_MF 5214
WP_C_MF 1127
WP_C_IS 361
WP_C_IS 93
WP_C_IS 204
WP_C_IS 118,b


WP_F_SMOPH
OD 600
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
8
15
23
30
38
45
Time (h)
WP_F_MF 5214
WP_F_MF 1127
WP_F_IS 361
WP_F_IS 93
WP_F_IS 204
WP_F_IS 118,b

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway