Functional screening of beneficial strains and consortia to maximize healthy fermentation potential

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Legumes and cereals are globally consumed plant-based sources known for their health benefits, including low-fat and high protein content. However, they also contain antinutritional elements. This thesis, conducted as part of the EU Horizon project "Healthferm," aimed to screen food fermentation lactic acid bacteria for optimal fermentation and health-promoting effects.

In this study, oat flour (OatF-F), oat protein concentrate (OatF-C) and faba bean protein concentrate (Faba-C) based media were used. Overall, the strains demonstrated good bacterial growth in all media, notably, Faba-C supported favorable growth. Additionally, the impact of overnight incubation without shaking on strain growth was examined.

The study found that the growth rate was higher in OatF-F. Moreover, the growth rate of strains in media not incubated overnight with shaking was generally higher. Reductions in pH were observed with selected strains in all media.

In the scale-up fermentation, the lowest pH values after 24 hours were observed in OatF-F (3.5±0.02), OatF-C (3.4±0.03), and Faba-C (4.4±0.03).

Rhamnose was not detectable in any of the samples, and raffinose was not detected in Faba-C. Complete degradation of raffinose was achieved in OatF-C. Complete hydrolysis of sucrose was observed in Faba-C. Faba-C exhibited a higher concentration of glucose (1.49 g/L), but complete hydrolysis was observed.

Keywords: Plant-based, sustainable, fermentation, screening, lactic acid bacteria, legume, cereal

PREFACE

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Content

Abbreviations 3

1 Introduction 4

1.1 Plant-based foods 5

1.1.1 Nutritional compounds in plant-based foods 6

1.2 Fermentation 8

1.2.1 Lactic acid bacteria fermentation 8

1.2.2 Effect of lactic acid bacteria fermentation on antinutritional factors 9

1.3 Faba bean 10

1.3.1 Antinutritional factors and FODMAP content of faba bean 12

1.4 Oat 14

1.4.1 Antinutritional factors and β-Glucan content of oat 16

1.5 Aim 18

2 Materials and Methods 19

2.1 Raw Materials 19

2.2 Strains 19

2.2.1 Lactic Acid Bacteria 19

2.3 Media 20

2.4 Analysis 22

2.4.1 API-ZYM test 22

2.4.2 Functional Screening of Strains 23

2.4.3 Biolector protocol for monitoring fermentative performance of selected strains 24

2.4.4 Scale-up fermentation experiment 24

2.4.4 Sugar composition analysis 25

2.5 R Studio-Assisted Investigation of Growth Kinetics and pH Profiling of Lactic Bacteria in Cereal and Legume-Based Media 25

2.6 Statistical Analysis 25

3 Results 26

3.1 Enzymatic Profiling of Bacterial Strains using API-ZYM 26

3.2 Screening of Bacterial Growth in Cereal and Legume- based Media with Bioscreen® 28

3.2.1 Screening results of lactic acid bacteria strains in OatF-F using Bioscreen® 28

3.2.2 Screening Results of Bacterial Strains in OatF-C Medium with Bioscreen® 31

3.2.3 Screening Results of Bacterial Strains in Faba-C Medium with Bioscreen® 33

3.2.3 Screening of Bacterial Strains in Media without Overnight Shaking using Bioscreen®..35

3.3 Bacterial Growth Rate Calculation Using R Studio..37

3.3.1 Bacterial growth rate calculation on media with and without shaking overnight using R studio 42

3.4 Biolector-based analysis of bacterial biomass and pH profiles in cereal and legume-based media...44

3.4.1 Bacterial biomass profiling in cereal and legume-based media....................44

3.4.2 pH profiling in cereal and legume-based media...50

3.5 Scale-up fermentation 55

3.6 Characterization of Sugar Compositions in Fermented Samples Post Biolector Cultivation..62

4 Conclusion..67

5 References 70

Abbreviations

Antinutritional factors	ANF
Carrying capacity of population	K
Colony forming unit	cfu
Faba bean protein concentrate medium	Faba-C
Fermentable oligosaccharides, disaccharides, monosaccharides and polyols	FODMAPs
Gram	g
Growth rate	r
Hour	h
Lactic acid bacteria	LAB
Liter	L
Midpoint of growth	t_mid
Mililiter	ml
Milligram	mg
Nanometer	nm
Oat flour medium	OatF-F
Oat protein concentrate medium	OatF-C
Optical density	OD
Potential of hydrogen	pH
Raffino-oligosaccharide	ROS
Standard deviation	SD
Subspecies	subsp.

1 Introduction

The shift towards plant-based foods has been a growing trend in recent years, driven by a number of factors including increasing health concerns, environmental sustainability, and ethical considerations. With the rise of information and technology, people are becoming more aware of the impact their diets have on the world and are looking for alternatives to the traditional animal-based diets that have dominated for centuries (Manus et al., 2021). Indeed, the increase in alternative plant-based products can reduce malnutrition and can contribute to improving food production sustainability and a reduced environmental impact (Loveday, 2019; Aiking and DeBoer, 2020). However, consumers still need to be informed about the characteristics of plant-based alternatives, while industries should find new products to meet the acceptability of consumers. Thus, research on fermented plant-based products as an alternative is expanding. The growing consumer interest in the health benefits of food is driving the food industry towards the use of alternative protein sources (Montemurro et al., 2023).

One of the primary reasons for this shift is the recognition that a plant-based diet can have a positive impact on health. A diet rich in fruits, vegetables, whole grains, and legumes has been shown to lower the risk of chronic diseases such as heart disease, type 2 diabetes, and certain cancers. In addition, plant-based diets tend to be lower in saturated fat and higher in fiber, which can help improve overall health and reduce the risk of obesity and other health problems (Le and Hekmat, 2020; Tong et al., 2020).

Another important factor driving the shift towards plant-based diets is the impact of animal agriculture on the environment. Livestock production is a major contributor to greenhouse gas emissions, deforestation, and water pollution. By choosing plant-based foods, individuals can reduce their carbon footprint and help protect the planet for future generation (Clark and Tilman, 2017; Manus et al., 2021)

The ethical treatment of animals is also a significant concern for many people who are shifting to a plant-based diet. The inhumane conditions in which many animals are raised for food production, including confinement, mutilation, and the use of antibiotics, have led many people to seek out alternative food sources. By choosing plant-based options, consumers can feel good about their food choices and know that they are helping to reduce animal suffering (Whybrow and Macdiarmid, 2018).

Antinutritional factors are naturally occurring substances in plant-based foods that can interfere with the digestion and absorption of essential nutrients, leading to a reduced availability of these nutrients to the body. Legumes and cereals are two of the most important food sources for humans and are widely consumed globally. Despite their high nutritional content, both legumes and cereals contain antinutritional factors that can affect their nutritional value and safety for consumption.

Legumes, such as beans, lentils, and chickpeas, are a rich source of protein, carbohydrates, and fiber (Samtiya et al., 2020). However, they also contain a range of antinutritional factors, including lectins, protease inhibitors, and phytates (Granito and Alvarez, 2006). Lectins are proteins that bind to carbohydrates and can interfere with the absorption of nutrients in the small intestine (Muramoto, 2017). Protease inhibitors are substances that can reduce the activity of digestive enzymes, leading to reduced protein digestion and absorption (Yasmin et al., 2018). Phytates are compounds that can bind to minerals, such as calcium, iron, and zinc, reducing their availability for absorption in the body (Al Hasan et al., 2016). Heat treatment is a common method used to reduce the levels of antinutritional factors in legumes. Cooking, sprouting, and fermentation can also be used to reduce lectins, protease inhibitors, and phytates in legumes (Samtiya et al., 2020). Fermentation is a widely used method for reducing antinutritional factors in these foods, as well as improving their nutritional value and safety for consumption (Curiel et al., 2015). In a study by Santos et al. (2019), it was found that fermentation reduced the levels of lectins in soybeans by up to 80%, which improved their nutritional value and safety for consumption. In addition to reducing antinutritional factors, fermentation can also improve the flavor and texture of legumes and cereals (Das and Tamang, 2021). This can make these food sources more appealing and increase their acceptability, particularly in regions where legumes and cereals are staples of the diet.

1.1 Plant-based foods

Food is pivotal to human survival. Although food and beverage producers try to produce enough food for everyone as the world population increases, an important part of the world population is still undernourished and even starvation-related deaths are experienced (Zeray et al., 2019). The generation and elimination of malnutrition solutions is the main objective however is also one of the most difficult tasks. Produced foods should also have an elevated level of nutritional value and offer consumers health advantages (Popova and Mihaylova, 2019; Manus et al., 2021).

People have a tendency to alter their dietary habits as the world's population grows because of rising greenhouse gas emissions, higher water and energy use, and moreover declining arable areas (Poutanen et al., 2022). Globally, food production accounts for 30% of greenhouse gas emissions and 70% of water footprints (Smetana et al., 2015). The need for a sustainable food system is therefore imperative. Plant-based meals that are alternatives to their animal counterparts now start to emerge. Thanks to its benefits, such as having a lower impact on global warming, using less land for agriculture, and producing significantly more food per unit of production, plant food sources provide an alternative to animal production (Clark and Tilman, 2017; Manus et al., 2021). Moreover, the planetary health diet, which encourages the consumption of plant-based foods, was recently proposed by the EAT Lancet Commission as a theoretical model for a nutritious and sustainable diet (Willett et al., 2019). In addition, Global Panel on Agriculture and Food Systems for Nutrition, 2020, stresses the importance of achieving the Sustainable Development Goals through climate-resilient food systems that provide healthy nutrition.

1.1.1 Nutritional compounds in plant-based foods

The importance of pulses and cereal-based diets are being recognized recently (Sadh et al., 2022). *Leguminosae* is a group of the largest flowering plant family with 650 genera in its family. Legumes are grown in many species and varieties for human nutrition (Duranti, 2006). Pulse and cereal-based foods added to the diet contain quality nutritional elements with beneficial effects on human health (Tharanathan and Mahadevamma, 2003; Sadh et al., 2022). They are regarded as advantageous due to their low-fat content, high protein content, dense dietary fiber content, and phosphate content. Numerous studies have found that consuming foods high in fiber and protein can help people control their appetites, improve their lipid profiles by reducing levels of low-fat lipoprotein cholesterol, and perhaps reduce their risk of developing cardiovascular disease (Blackburn, 1995; Ha et al., 2014; Havemeier et al., 2017; Le and Hekmat, 2020; Tong et al., 2020). Due to this, there has been a rise in interest in grains and legumes for food applications in recent years.

Plants contain components such as vitamins, minerals, antioxidants, dietary fibers that have positive effects on health. In addition to these excellent sources on the other hand, they also contain anti-nutrient factors (ANFs) such as phytic acid, lectins, saponins and protease inhibitors and can cause adverse effects on nutrition and lead to some diseases (Filannino et al., 2018).

The aforementioned antinutrients are produced by plants as secondary metabolites as part of their physiologically active defensive systems (Shanthakumari et al., 2008; Gemede and Ratta, 2014; Rizzello et al., 2017). Antinutritional factors hinder effective nutrient utilization, reducing the amount of nutrients that are absorbed, including minerals, proteins, and vitamins (Alemayehu et al., 2021) and may cause toxicity (Jain et al., 2009; Gobbetti et al., 2019). Antinutrient elements can enter the body through diet and cause certain unpleasant symptoms. Some of these symptoms include nausea, bloating, headache, inadequate absorption of some nutrients (Gobbetti et al., 2019; Popova and Mihaylova, 2019) or the more severe existence of digestive issues (Filannino et al., 2018).

Technological processes such as extrusion, cooking, air classification, wetting, and pulling are preferred to reduce the concentrations of ANFs (Jezierny et al., 2010; Coda et al., 2015). Additionally, biological processes like fermentation are also reported as efficient and advantageous (Rizzello et al., 2014; Coda et al., 2015; Gobbetti et al., 2019).

FODMAPs stands for fermentable oligosaccharides, disaccharides, monosaccharides, and polyols, are defined as carbohydrates such as easily fermentable fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactose, fructose, and polyols such as mannitol and sorbitol. Due to the presence of these fermentable carbohydrates in many grains and pulses, these foods are regarded as sources of FODMAPs (Pejcz et al., 2021). The role of FODMAPs on health is proportional to the amount of ingestion of foods having FODMAPs and may exhibit positive or negative effects. Sufficient intake of FODMAP may have a positive effect on health by providing prebiotic effects. Furthermore, many FODMAP compounds are part of dietary fiber (Granito and Alvarez, 2006; Makki et al., 2018), on the other hand, excessive consumption can lead to intestinal problems by causing sugar accumulation and flatulence issues in the intestines (Loponen and Ganzle, 2018).

Pulses, legumes, and cereals have a prominent place in human nutrition. As a result, several ways have been suggested to either lower or entirely eliminate the FODMAPs or ANFs. However, the effectiveness of these processes varies according to the type of FODMAP or ANFs and food composition. Raffinose from FODMAPs are classified as causes of intestinal distension and can be eliminated by conventional processes such as soaking or heat treatment, but dietary fibers are not affected by heat treatment and remain stable. It has been suggested that the application of the fermentation process will provide beneficial results (Granito et al., 2002; Prajapati and Nair, 2008; Rizzello et al., 2010; Curiel et al., 2015; Verni et al., 2017).

1.2 Fermentation

The origin of the word fermentation comes from the verb fervere, which means boiling in Latin (Stanbury et al., 2015). This process is one of the oldest food preservation methods that humanity has used for centuries. Humanity developed a variety of fermented foods and drinks via the fermentation process, including wine, beer, and bread. At the time, the function of microorganisms in fermentation was unclear (Tamang et al., 2016).

Fermentation is a multidisciplinary concept and can be defined by different disciplines from different perspectives (Faniyi and Oyatokun, 2021). Fermentation refers to a biological process in which certain microorganisms convert especially carbohydrates, proteins, fats, organic acids, or other organic compounds into simpler components or produce new compounds. In terms of food science, bacteria, yeasts, molds, or a combination of them, are the most prevalent or often employed microorganism types in fermentation (Stanbury et al., 2015). Fermentation improves the shelf life of food (Marco et al., 2017). During fermentation, highly diverse secondary metabolites, including many vitamins, minerals and antioxidants, are synthesized and these metabolites have a positive effect on the nutritional value of food and improve nutritional quality (Palla et al., 2020; Montemurro et al., 2021).

1.2.1 Lactic acid bacteria fermentation

Lactic acid bacteria (LAB) are characterized as gram-positive, catalase-negative, non-spore-forming, aero-tolerant, and fermentative microorganisms with tolerance to acid, appearing as rods, coccus, or cocobacillus (Leroy and DeVuyst, 2004; Cook and Bamforth, 2019). Additionally, they are primarily mesophilic while having strains that can grow at temperatures ranging from 4°C to 45°C. pH levels between 4.0 and 4.5 are optimum for growth, however some strains can tolerate a wide range of pH. In order for them to grow, purine, pyrimidine, certain amino acids and vitamin B must be supplemented to the media or previously formed in the environment (Cook and Bamforth, 2019).

LAB are ubiquitous in nature. This is an indicator of the ability of LAB to adapt to different environments. Although some strains exhibit pathogenic properties, they are considered Generally Recognized as Safe (GRAS) (Cook and Bamforth, 2019). Among plants, both homo-fermentative and hetero-fermentative species of Lactobacillus, Weissella, Enterococcus and Pediococcus genera were isolated more frequently, but the most abundant species was found to be Lactobacillus plantarum (Di Cagno et al., 2013).

Although many LAB species are isolated, the number of species used in the industry is very limited (Yazar and Tavman, 2021). By producing lactic acid and numerous other antimicrobial metabolites through lactic acid fermentation, they may survive and prevent other microorganisms or their metabolites from proliferating in low pH environments. Lactic acid fermentation takes place under microaerophilic or relatively anaerobic conditions and occurs by converting the sugar present in the medium into lactic acid and other metabolites. Foods fermented by LAB such as Pediococcus, Lactobacillus, Lactococcus, Streptococcus, Leuconostoc etc. form lactic acid as the main metabolite (Collado-Fernandez, 2003; Cichońska and Ziarno, 2021).

Mital et al. (1973), described α-galactosidase synthesized by lactobacilli from cell extracts and stated that this enzyme is active in a wide range such as pH 4.5-8.0, but function optimally between pH 5.2-5.9. It has been shown that the enzyme can degrade other oligosaccharides containing α (1 → 6) -linked galactose, including melibiose. α-galactosidase enzyme activity is mostly specific to *Lactobacillus plantarum* and *Lactobacillus casei* subsp. *casei* strains. The enzyme activity of these strains is categorized as moderate-high. β-galactosidase activity is mostly associated with the species *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. These strains are also capable of hydrolyzing raffinose, stachyose and verbascose complex carbohydrates (RFO), which are raffinose group oligosaccharides (Cichońska and Ziarno, 2021).

1.2.2 Effect of lactic acid bacteria fermentation on antinutritional factors

Fermented foods have a major place in human nutrition (Samtiya et al., 2021). In particular, the health promoting effects of plant-based diets can be further improved through fermentation. Such effects are a consequence of lactic acid fermentation, which is more frequently carried out by LAB (Filannino et al., 2018). In particular, lactic acid fermentation is widely employed to enhance the nutritional content and sensory qualities of fruits and vegetables (Buckenhüskes, 1997; Steinkraus, 1996). Particularly in non-European nations, this sort of fermentation is commonly used on crops like soybeans, chickpeas, and beans (Coda et al., 2015; Humblot and Guyot, 2008). In addition, fermentation is acknowledged and applied as an effective process to increase the nutritional value of legumes. LAB demonstrate proteolytic activity depending on strain-related activity with bacterial fermentation (Gobetti et al., 1996). It has been claimed that fermentation may be utilized to lower the amount of antinutritional substances such phytates, tannins, proteinase, or trypsin inhibitors to improve protein digestibility (Coda et al., 2011) as well as to boost the bioavailability of nutrients for human health

(Starzyńska-Janiszewska et al., 2010; De Pasquale et al., 2021; Patterson et al., 2017). The application of fermentation to legumes (Coda et al., 2015) and cereals (Rizzello et al., 2010; 2012) has been examined and it has been observed that the composition of antinutritional factors such as α-galactosides, phytic acid, tannins, and trypsin inhibitors, especially in legumes, can be partially or completely eliminated by lactic acid fermentation (Coda et al., 2015; Curiel et al., 2015).

FODMAPs, on the other hand, are characterized as small active molecules that are used and processed as substrates by bacteria in the large intestine yet only in limited amounts in the small intestine (Gibson and Shepherd, 2010). These substances are promptly digested by bacteria in the colon, generating short-chain fatty acids and gas despite having health-protective benefits. This has quite unpleasant side effects, especially for sensitive people (Nyyssölä et al., 2020). In order to reduce the high FODMAP content in foods, it should be reduced with the target aim and the processes that ensure the absorption of other nutrients by the body should be taken into account. As a way of doing this, the addition of exogenous enzymes during food processing can be regarded as a solution, or bio-processing processes such as germination or fermentation have been proposed to reduce FODMAP content (Loponen and Ganzle, 2018).

1.3 Faba bean

The FAO (Food and Agriculture Organization) 2022-2031 strategic framework was approved at the 42nd session of the FAO conference, and in order to support sustainable development, "transition to efficient, inclusive, durable, sustainable agri-food systems for better production, better nutrition, a better environment, and a better life, without leaving anyone behind" (FAO, 2021). It was determined to use cross-sectional accelerators to speed up invention to accomplish this aim. Researchers seek novel strategies to speed up invention (FAO, 2021). More sustainable food production, processing, and food chain innovations are required in the agricultural sector. The Faba bean (*Vicia Faba L.*) is more intriguing than other legumes due to its effectiveness for the environment (Augustin and Cole, 2022). Faba bean is the second most grown legume in the world. It is used for both animal feed and human nutrition. However, in addition to providing nourishment, it also has an ecological benefit due to its ability to bind nitrogen, which can lower the need for mineral fertilizers that include nitrogen (Köpke and Nemecek 2010). It has the ability to increase product efficiency and reduce environmental footprints. It is an ecologically sustainable legume due to its high nitrogen-fixing capacity (Tadele, 2020; Augustin and

Cole, 2022). Faba bean is still underutilized in Western countries, but it has a long history in the Middle East and is part of culinary culture. As a sustainable source of protein, its use is becoming increasingly widespread in Europe and Australia (Tijhuis et al., 2012; Augustin and Cole, 2022).

Faba beans (*Vicia faba L.)* belong to the *Fabaceae* family. The faba bean is a rich source of lysine-rich proteins, complex carbohydrates, fiber, and bioactive substances that promote good health, such as antioxidants, phenolic compounds, and -aminobutyric acid (Dhull et al., 2021). Moreover, it contains considerable amounts of vitamins, minerals and carotenoids which emphasizes the high nutritional quality of this Faba bean (Randhir et al., 2004). Faba beans may have a protein content between 20-41% depending on the type of source. High protein contents are more common in fraction and isolate forms (Dhull et al., 2021; Shi and Nickerson, 2022). Considering Faba beans as such the protein content is almost twice the protein content of cereal grains. The protein composition is 60% Globulin, 20% Albumin, 15% Glutelins and 8% Prolamins. Prolamins make up the majority of the storage proteins in grains, globulins constitute an important part of the storage proteins of legumes (Rahate et al., 2021). Legumes and cereal protein composition are complementary. While legumes are rich in lysine content, they exhibit low percentages in terms of methionine, cysteine, and tryptophan amino acids. Grains have the opposite composition, thus complementing human amino acid requirements (Rahate et al., 2021).

Gu et al. (2020) found in their research that faba beans contain more protein and dietary fiber than other legumes tested. In comparison to extrudates from the other bean flours examined, faba bean flour extrudates contained greater levels of protein and dietary fiber. This study has been an important study that reveals the protein quality and functional properties of faba beans. In addition, Margier et al (2018) reported that faba beans contain more vitamin C than other legumes. Based on beans in general, they exhibit 31-34% protein, 44-47% carbohydrate, 8% dietary fiber and 3.5-4% ash nutritional content. However, the nutrient content varies greatly depending on whether it is fraction, isolate or flour type (Hall et al., 2017; Rahate et al., 2021).

Table 1. Nutritional composition of different fractions of faba beans (Coda et al., 2015).

Nutrient Ingredient	Bean Flour	Protein-Rich Fraction	Starch-Rich Fraction
Protein (% Dm)	32–36	50–52	15–17
Starch (% Dm)	40–44	-	63–66
Dietary Fiber (% Dm)	7–7.5	10–10.5	4–5
Ash Content (% Dm)	3–4	5–5.5	2–2.5
Oil (% Dm)	1.5–2	2–2.5	0.5–1
Moisture (%)	9–10	7–8	8–9

1.3.1 Antinutritional factors and FODMAP content of faba bean

Faba beans contain antinutritional factors that may negatively affect their biological value such as raffinose family oligosaccharides (raffinose, stachyose, verbascose), tannins, trypsin inhibitors, protease inhibitors, lectins and favism inducing factors such as vicine and convicine (Liener, 1990; Revilla, 2015; Valente et al., 2019). Fermentable oligosaccharides (raffinose, stachyose and verbascose) can be found in faba beans and can cause gastrointestinal disorders (gas production, bloating) (Toklu et al., 2021). These ANFs and FODMAPs have been reported to reduce the nutritional value of beans, reduce digestibility, and even lead to pathological conditions (Gupta, 1987; Xu et al., 2017b). Faba beans contain phytic acid as found in various other legumes. Faba beans have a phytic acid content of approximately 0.69g/100g (Vasić et al., 2012; Rahate et al., 2021). It has been stated that phytic acid found in faba beans both reduces bioavailability from minerals and adversely affects protein absorption due to its binding and formation of mineral-protein complexes (Vasić et al., 2012).

Faba beans also contain concentrated tannins. Tannin content has been reported to be approximately 0.75-1.92 % (Aw and Swanson, 1985; Betancur-Ancana et al., 2010; Rahate et al., 2021). These substances have the ability to inhibit the proteolytic activities of some enzymes, another antinutritional factor that may negatively affect the digestion of proteins. In particular, the Leguminosae family exhibits rich content of protease inhibitors (Sohonie and Ambe, 1955).

Another antinutritional factor identified in faba beans is lectin (hemagglutinin). The lectin isolated from Faba beans was named “favin” (Rahate et al., 2021). After entering the digestive system, lectins bind to epithelial cells of the small intestine and interfere with metabolism in the gastrointestinal tract. It may also cause changes in cell morphology (Vascancelos and Oliviera, 2004).

Vicine (2,6-diamino-4,5-hydroxyprimidine-5- [β-D-glucopyranoside]) and convicine (2,4,5-trihydroxy-6-aminopyrimidine-5- [β-D-glucopyranoside]) are antinutritional factors unique to the genus *Vicia*, to which Faba beans are associated. Vicine and convicine are glycosides of pyrimidine and have been widely described in the *Vicia* genus. Responses to these antinutritional factors occur with microbial β-glucosidases during seed formation or in the large intestine during digestion (Rizzello et al, 2016). Vicine and convicine in high concentration cause severe hemolytic anemia called Favism (Singh et al., 2013). Symptoms begin 6 to 24 hours after ingestion in sensitive individuals who are deficient in glucose-6-phosphate dehydrogenase. Favism involves the destruction of red blood cells, which causes acute hemolytic anemia (Rizzello et al., 2016; Sharan et al., 2021; Rahate et al., 2021).

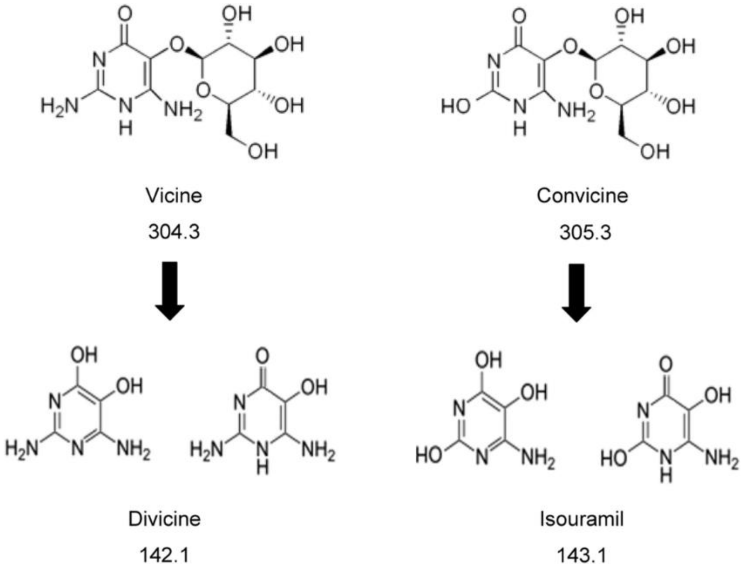

Figure 1. Molecular structures of vicine, convicine and their respective aglycones, divicine and isouramil (Rizzello et al., 2016).

Studies on legumes fermentation for the reduction or elimination of antinutritional factors in faba beans have gained momentum in recent years. However, there is little information in the literature on carefully selected starter cultures or their consortia and faba bean fermentation.

1.4 Oat

Several foods and beverages have been produced by fermenting cereals traditionally or by adding microorganisms over the years (Nout, 2009). Oats, as other cereals, are members of the *Pomaceae* family (Arendth and Zanini, 2013). However, oats are sub-classified into the *Aveneae* tribe while the other cereals belong to the *Triticeae* tribe (Tanner et al., 2019). Oats are widely produced around the world with an annual yield of more than 24 million tons (Arendth and Zanini, 2013; Angelov et al., 2018). It has mostly been employed in animal feeding, but in recent years, its positive effects on human health have drawn more attention (Wronkowska et al., 2022; Alemeyahu et al., 2022). The global oat protein market is forecasted to grow at a compound annual growth rate of 1.22% during the forecast period (2019–2024) (Research and Markets, 2022). Oat is a grain that may be used for a variety of purposes (Streimikyte et al., 2022). It is exploited in the manufacturing of products including bread, baby food formulas, and breakfast cereals (Bocchi et al., 2021). Additionally, oats and other oat components are frequently employed in the manufacturing of products including thickeners, sweetener (xylitol derived from oat hulls), fermented drinks and vegetable proteins (Streimikyte et al., 2022). It attracts increasing scientific attention with its rich content of β-glucans and phenolic compounds, its health benefits are being investigated thoroughly and its usage areas in the food industry are becoming widespread (Butt et al., 2008; Streimikyte et al., 2022). Obtaining functional foods by fermentation of oats has been practiced for a long time. By fermentation, the bioaccessibility of the nutrients in the oat content can be enhanced, and the protein digestibility can be amplified (Bocchi et al., 2021).

Oats is a corrugated caryopsis. Caryopsis is firmly covered in a hull that serves as protection. Caryopsis can be divided into 3 main components: the bran, germ, and starchy endosperm. Before further usage, the husk is often removed from the grain. The hull constitutes approximately 30-40% of the total grain by weight (Arendt and Zanini, 2013).

Oat flour is a significant component of oats. Oat flour can be manufactured from baked, dehulled oat grain in a variety of ways, including milling, or it can be acquired as a byproduct from the production of oat bran (Butt et al., 2008; Menon et al., 2016). Oats are an important crop, especially in Northern countries. Statistically, Finland's annual oat production has reached 1.2 million tons by 2022 (FAO, 2022).

Regarding its nutritional value, oat grain exhibits strong functional qualities (Angelov et al., 2018). According to Ramos-Diaz et al. (2022), it is particularly high in β-glucans, as well as other dietary fibers, proteins, phenolic compounds, unsaturated fatty acids, vitamins, and minerals (Nionelli et al., 2014; Shah et al., 2016). It contains higher amounts of fiber, calcium, iron, and protein compared to other cereal products (Flander et al., 2007; Sangwan et al., 2014; Shah et al., 2016). Additionally, oats have been cited as a unique source of polyphenols (avenanthramides) (Peterson, 2001; Clemens and van Klinken, 2014).

In terms of protein, oats have a balanced composition (Gühnmann et al., 2019). It contains a higher quality protein fraction than other grains. It has a greater protein quality in terms of amino acid composition due to its high Lysine content and low Prolamin content. Oat grains possess heterogeneous protein distributions that increase from the interior to the periphery (Beloshapka et al., 2016). Protein content in oats ranges from 11 to 15% (Sukhdeepkaur et al., 2019). The primary source of protein in oat grain is Globulins. In the oat grain, Globulins account for 70–80% of the total proteins (Peterson and Brinegar, 1986; Nieto-Nieto et al., 2015). Having followed it, Albumins comprise around 12%, while Prolamins contribute 4–14% (Ma and Harwalker, 1984).

Additionally, oats have a substantial portion of nutritional fiber (Nionelli et al., 2014; Shah et al., 2016; Ramos-Diaz et al., 2022). Based on the genotype, oats have a fiber content of 10.1% to 12.1%. The main dietary fiber components of oats have been reported as (1 → 3) -(1 → 4)- β - D-Glucan and Arabinoxylan (AX) (Haskå et al., 2008; Ordaz-Ortiz and Saulnier, 2055; Shewry et al., 2010; Flander, 2012). In oats, β-glucan is an important carbohydrate fraction that promotes health (Ramos-Diaz et al., 2022). β -glucan content ranging from 1.8-8% (by dry weight) has been reported in oats (Welch, 1995; Welch et al., 2000; Flander et al., 2007; Flander, 2012).

The flour fraction of oats also shows nutritional and functional properties similar to oat grain. Protein, dietary fiber, vitamins, minerals, and notably starch, are found in significant amounts (Rowal et al., 2023~~).~~ In a more recent study, oat grains were analyzed to contain 9.70-17.30% protein, 5.20-12.40 g100^{-1} lipids, 2.70-3.50 g100^{-1} β–glucans, 4.50-12.30 mgkg^{-1} Vitamin E and 13.66-30.17 g100^{-1} and 11.53-20.07 g100^{-1} total and soluble fiber, respectively (Sterna et al., 2016) (Table 2).

Table 2. Biochemical composition of oat grains (Boukid, 2021; Sterna et al., 2016; Hüttner et al., 2010).

Nutrients	**Oat**
Protein content, %	9.70-17.30
Carbohydrates, g100^{-1}	69-76
Total Dietary Fiber, g100^{-1}	13.66-30.17
Soluble Dietary Fiber, g100^{-1}	11.53-20.07
Lipid, g100^{-1}	5.20-12.40
Vitamin E, mgkg^{-1}	4.50-12.30
β–glucans, g100^{-1}	2.70-3.50
Ash, g100^{-1}	1-2

1.4.1 Antinutritional factors and β-Glucan content of oat

Oat β -glucan exhibits excellent functional properties (Arendt and Zanini, 2013). The dietary fiber in oats, particularly β-glucans, is primarily responsible for the beneficial effects on health (Bocchi et al., 2021). Due to the positive effects of dietary fiber on preventing obesity, providing energy control, regulating satiety (Zduńczyk et al., 2006), and preventing various diseases (Tapola et al., 2005; Berg et al., 2003; Nionelli et al., 2014; Saka et al., 2021) it is advised that foods rich in dietary fiber be consumed frequently and added to the daily diet. Food and Agriculture Organization of the United Nations (FAO, 2006) and the European Food Safety Authority (EFSA, 2010) have recommended a daily intake of soluble fiber (β -glucan) of at least 3 g. Numerous human intervention trials and research have shown such health-improving effects, and these findings have led to the approval of health claims for oat products (European Commission, 2011). Based on these studies, the European Commission has permitted Article 14(1)(a), a health claim for foods that provide at least 1g β -glucan per measured serving (3g/day): "Oat β -glucan has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor for the development of coronary heart disease." (European Commission, 2011). Another related health claim is explained in Article 13 (1). This claim includes foods containing 4g of β -glucan/30g of available carbohydrates from oats or barley in a given serving as part of the meal. These foods may carry a health claim that "consumption of β -glucans from oats or barley as part of a meal contributes to a reduction in blood sugar increases after the meal" (European Commission, 2012).

Oats are excellent sources of soluble fibers such as β -glucan, proteins, minerals, and vitamins, as well as a source of antinutritional factors. These components can reduce the bioavailability of health-promoting nutrients in oats by generating insoluble complexes.

Trypsin inhibitors and phytates found in grains such as oats, similar to those found in legumes, have an impact that restricts mineral absorption and protein digestion. Most of the phytate found was identified in the soluble fiber fraction (Nkhata et al., 2018). Egli et al (2003), reported low levels of phytase activity in oats. Oats also include saponins, tannins, polyphenolic substances, and phytic acid (Sukhdeepkaur et al., 2019).

Raffinose family oligosaccharides (RFO) or α-galactosides, known as FODMAPs, are a group of storage carbohydrates found in pulses and cereals, including oats. These carbohydrates play essential roles in protecting the plant's physiological functions. Derived from sucrose, they are characterized by α $(1 \rightarrow 6)$ galactose bonds, with raffinose, stachyose, and verbascose being the most common forms of RFO. Additionally, oats contain non-digestible fructans, another type of storage carbohydrate found in various plants, providing energy to plants during adverse conditions (Verspreet et al., 2013).

Studies conducted by Dodevska et al. (2013) and Biesiekierski et al. (2011) have identified the presence of fructans in oat flakes, with fructan levels reported as 0.35% and 0.32%, respectively. Another study by Ispiryan et al. (2020) revealed that oat exhibits moderate levels of GOS, with a content of 0.33 g/100 g dry matter. This integrated research demonstrates the presence of both RFO and fructans in oats, highlighting their significance in the composition of this cereal grain.

1.5 Aim

This study was a part of the EU Horizon project Healthferm: Plant-based Fermented Foods for Healthier and More Sustainable Diets. The overall objectives of the project were exploring the interactions between food fermentation microbiomes, fermented grain-based food, and the human gut microbiome, and developing novel foods using the generated knowledge, microbial resources, and fermentation technology.

The primary aim of this master’s thesis was to screen existing various food fermentation LAB strains for optimal fermentation and health improving effects in cereal and legume-based media that was prepared from oat flour, oat protein concentrate and faba protein concentrate. The evaluation of fermentative performance, growth kinetics, acidification, metabolite production and FODMAPs was conducted in consortia and individual strains by using high throughput microreactor Biolector.

Another aim of this study is to gain insights into the growth characteristics and kinetics of the selected strains under various conditions, specifically focusing on the utilization of overnight shaking and not shaken media. By examining the growth patterns and comparing the two approaches, valuable information will be obtained regarding the impact of shaking on the strains' growth behavior.

One additional objective of this study was to perform a scale-up fermentation experiment in order to gather valuable insights into the growth characteristics and behavior of selected strains under diverse conditions. By scaling up the cultivation volume and comparing the utilization of shaken and not shaken media, a thorough assessment of the growth patterns of these strains was accomplished.

2 Materials and Methods

2.1 Raw Materials

Faba bean (*V. faba* major) flour and oat (*Avena sativa*) flour were used as raw materials in this study. The analyzed composition of the raw materials is presented at Table 3.

Faba bean protein concentrate (Faba-C) was provided from Suomen Viljava OY while oat flour (OatF-F) and oat protein concentrate (OatF-C) was supplied from Raisio OYj.

Table 3. Chemical compositions of cereal and legume flours used in the study

Sample	**Naming system**	**Water (%)**	**Fat (%)**	**Ash (%)**	**NaCl (%)**	**Protein (%) ***
Faba bean protein concentrate	Faba-C	11,1	2,7	5,2	0,11	54,64
Oat flour	OatF-F	9,4	5,4	1,9	0,07	14,21
Oat protein concentrate	OatF-C	8,4	1,9	2,1	0,08	16,44

*Protein factor 6,25 was used to calculate protein composition

2.2 Strains

2.2.1 Lactic Acid Bacteria

Table 4. shows the strains included in this study. All 24 strains were obtained from the VTT Culture Collection (Espoo, Finland). Due to confidentiality reasons, the strains used in this study have been coded. The actual VTT codes corresponding to these strains will be disclosed upon publication of the research findings.

Table 4. Strains used as in the thesis

Strain Code	**Strain name**
A	*Lactiplantibacillus plantarum (Lb. plantarum)*
B	*Lactiplantibacillus paraplantarum (Lb. paraplantarum)*
C	*Lacticaseilactobacillus paracasei* subsp. *paracasei*
D	*Pediococcus pentosaceus (P. pentosaceus)*
E	*Lactococcus* sp.
F	*Lactococcus lactis* subsp. *lactis (Lc. lactis* subsp. *lactis)*
G	*Levilactobacillus brevis*
H	*Levilactobacillus brevis*
I	*Weissella confusa (W. confusa)*

Table 4. Strains used as in the thesis (continue)

J	*Lactococcus lactis* subsp. *lactis (Lc. lactis* subsp. *lactis)*
K	*Lacticaseilactobacillus paracasei* subsp. *paracasei*
L	*Lactiplantibacillus plantarum*
M	*Pediococcus parvulus (P. parvulus)*
N	*Lactiplantibacillus plantarum*
P	*Leuconostoc* sp.
R	*Lacticaseibacillus paracasei*
S	*Levilactobacillus brevis*
T	*Leuconostoc mesenteroides* subsp. *mesenteroides (Leu. mesenteroides* subsp. *mesenteroides)*
V	*Pediococcus pentosaceus (P. pentosaceus)*
X	*Leuconostoc mesenteroides* subsp. *mesenteroides (Leu. mesenteroides* subsp. *mesenteroides)*
Y	*Liquorilactobacillus nagelii*
Z	*Weissella confusa (W. confusa)*
AA	*Lactilactobacillus sakei*
AB	*Lacticaseilactobacillus paracasei* subsp. *paracasei*

TSCs cryopreservation beads (Technical Service Consultants Ltd., Lancashire, UK) were used to cryopreservation and stock the strains at -80°C. The strains were activated from frozen stocks (−80 °C) on MRS agar plates or broth for 48 h.

2.3 Media

de Man, Rogosa and Sharp (MRS) Agar and broth were prepared according to the instruction of the supplier (Oxoid Ltd., Hampshire, UK) (See Appendix 1).

Legume- and cereal-based media were prepared by adapting the method from Tinzl-Malang et al. (2015). 20 g of Faba-C, OatF-F and OatF-C were weighed in a glass flask and 180 ml of tap water was added the suspension was mixed until it was homogenous. Resulting suspension was incubated overnight in shaking condition at 200 rpm at 30 °C (Kühner Shaker, Switzerland). The suspension was centrifuged at 8.500 rpm for 20 min at 4 °C (Biofuge primo R, Heraeus). The supernatant was collected in a glass bottle. Its pH was measured and adjusted to 5.6-5.9 with NaOH 1M if necessary. The sterilized media was poured under laminar flow in previously sterilized centrifuge tubes and was centrifuged at 8.500 rpm for 20 min at 4 °C (Biofuge primo R, Heraeus). The supernatant was collected in a sterile glass bottle.

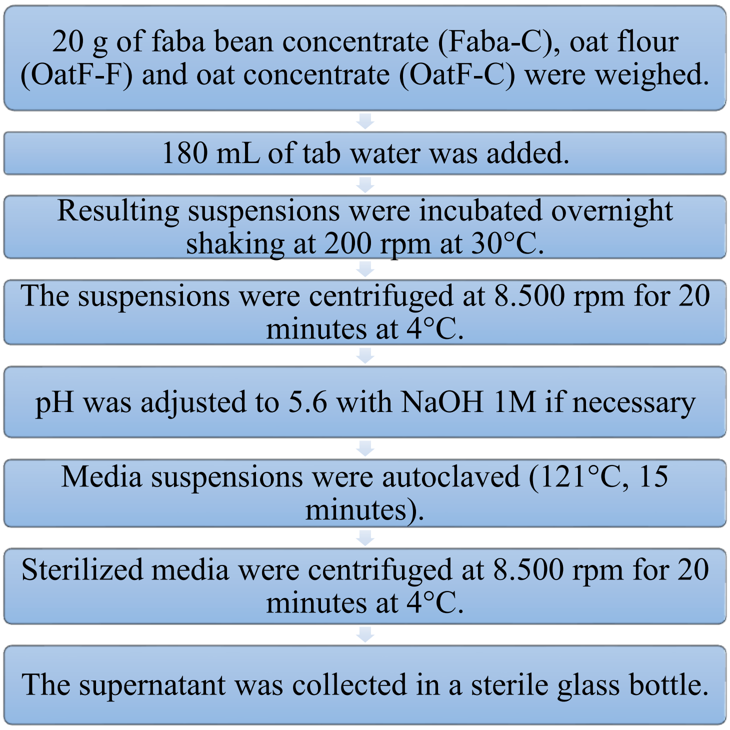

Figure 2. Preparation of cereal and legume-based media

2.4 Analysis

2.4.1 API-ZYM test

An API® ZYM (BioMerieux SA, France) test was performed according to the manufacturer's instruction. The API® ZYM method is a commercially available test method for identifying bacterial enzymatic activities (Gruner et al., 1992). Thus, API ZYM was used to evaluate the enzymatic profiles of the strains. Each strain tested was grown on MRS Agar for 48 h.

19 enzymatic activities such as alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, α-chymotrypsin, acid phosphatase, Phosphoamidase, α- galactosidase, ß-galactosidase, ß-glucuronidase, α-glucosidase, ß-glucosidase, N-acetyl-ß-glucosaminidase, α-mannosidase and α-fucosidase of strains were investigated using API® ZYM (BioMérieux, Marcy-l'Etoile, France).

Colony was removed from the surface of the plate and suspended in 2 ml of distilled water to obtain a very dense suspension. The tray provided by the kit was moistened with 5 ml distilled water. 65 µL of the bacterial suspensions were added to each of the 20 cupules in the API ZYM strip. The strip was placed in the tray previously moistened. The tray was covered with a lid to maintain a moist atmosphere and incubated at 30°C for 4 hours. After the incubation period, one drop of each of the API reagents A and B was added respectively to each of the cupules. Intensity of the colour reaction which developed within 5 minutes was graded from zero to 5 with reference to the API ZYM colour reaction chart.

2.4.2 Functional Screening of Strains

LAB strains were cultured in MRS broth for 72 hours, followed by homogenization via vortex. Subsequently, dilutions of 10-6 - 10-8 were prepared in cereal and legume-based media, according to the respective cell density. The prepared dilutions were inoculated onto MRS agar plates for enumeration, which were then incubated for 48 hours at 30°C. To assess the growth characteristics of the bacteria, a Honeycomb well plate was employed for Bioscreen® (Oy Growth Curves Ab Ltd., Turku, Finland) analysis. To serve as a control sample, sterile media was pipetted into the first row of the plate at a volume of 300 microliters/well. 10^{-2} and 10^{-3} dilutions of all the strains were utilized for each media. Each dilution was then pipetted in five replicates to other rows, with a volume of 300 microliters/well. Bioscreen® analysis was conducted for 72 hours, and the resulting data was collected and recorded on a computer.

An experiment was conducted by eliminating the overnight shaking step in the medium preparation process, and the effects were examined. For this purpose, OatF-C and Faba-C media were chosen. The impact of overnight shaking on bacterial growth within the media was investigated. The preparation of dilutions and pipetting were carried out in the same manner as described above.

2.4.3 Biolector protocol for monitoring fermentative performance of selected strains

The BioLector XT system (m2p-labs GmbH, Germany) was used for cultivation and MTP 48-well Flower Plate (m2p-labs GmbH) were used. This specific type of 48-well plate allows online monitoring of dissolved oxygen (DO) and pH.

All cultivations were performed at a shaking speed of 200 rpm at 30 °C and the relative humidity kept at 85%. The biomass was monitored online at 30 min intervals for 3 days by scattered light measurement at 620 nm, and cell dry mass (CDM) was calculated from a pre-defined calibration curve. This thesis utilizes Biomass Gain 5 from the Biolector system according to the suggestion of the manufacturer.

For inoculations cells were grown for 48 h. Cells were harvested by centrifugation at 3000 rpm for 10 minutes. The resulting cell pellet was resuspended in sterile peptone saline (NEOGEN, Lansing, MI, USA) to an equivalent volume. OatF-F, OatF-C and Faba-C growth media were pipetted 2 ml in each well of microfluidic flower plate (MTP) in dublicate. The cells were then homogenized and 50 µl of the cell suspension was added to each well containing the media. The plate was sealed with special plate covers. Additionally, a plate count analysis was performed on MRSA plates for inocula obtained from the dilutions of 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} before the Biolector experiment. Biolector run was conducted for 72 h. Moreover, plate counting from the dilutions 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} using the spot technique was conducted after the Biolector run for all strains in each media.

2.4.4 Scale-up fermentation experiment

The Bioscreen experiment was initially conducted using a protocol with a volume of 300 µl. Overnight shaken and not shaken media, were used, and compared in the experiment. Four strains (A, L, N and R) that exhibited the best overall growth in the Bioscreen experiment were selected for further analysis. These strains were chosen based on their growth characteristics and potential relevance to the research objectives. Samples were collected at specific time points (0, 4, 8, and overnight 24) with a sample volume of 500 µl per time point to capture the growth dynamics of the selected strains. Dilutions were prepared up to 10^{-8} and plate counting was performed from 10^{-5}, 10^{-6},10^{-7} and 10^{-8}. pH measurements were conducted, and duplicate samples were taken to ensure reliability and reproducibility of the results.

2.4.4 Sugar composition analysis

Analysis of mono- and oligosaccharides of the fermented cereal and legume-based media with selected LAB strains were performed with HPLC anion exchange method using DIONEX ICS-3000 DC equipment and PA 20 IC (ion chromatography) column. Water as eluent at a flow rate of 0.38 ml/min. Samples for sugar composition analysis were obtained from plate wells following Biolector cultivation and subjected to centrifugation (Eppendorf 5417 R, 10,000 rpm,10 minutes). Supernatant was collected and subsequently filtered through a 0.22 µm filter into Eppendorf tubes. 1:1000 dilution was made in MilliQ water (Millipore AG, Zug, Switzerland) for all samples prevent saturation of the detector, avoid overloading the column, and ensure accurate quantification of the target sugars. A standard curve was prepared using D (+)-Raffinose Pentahydrate (Fluka Chemie GmbH, Sigma-Aldrich, Steinheim), D (+)-Sucrose (Fluka Chemie GmbH, Sigma-Aldrich, Steinheim), D (+)-Glucose (Fluka Chemie GmbH, Sigma-Aldrich, Steinheim) and L-rhamnose (Sigma Aldrich, St. Louis, MO, USA) standards at known concentrations to quantify the sugars in the samples.

2.5 R Studio-Assisted Investigation of Growth Kinetics and pH Profiling of Lactic Bacteria in Cereal and Legume-Based Media

The growth kinetics of LAB strains were evaluated using R Studio (Posit, Boston, MA, USA). Data analysis was performed using the "growthcurver" package in R Studio, which was used to calculate the growth parameters. Standard deviations (SD) between replicates were calculated using built-in functions in R Studio. Biomass and pH curves were plotted using the "growthcurver" package, along with additional packages such as ggplot2, gridextra, dplyr, and data.table. Functions such as summarizegrowth, data.frame, and ggplot were utilized in the data analysis process.

2.6 Statistical Analysis

Statistical analysis was performed using R Studio software, and the agricolae package was used to compare the mean values obtained by ANOVA. Tukey's comparison test was employed to identify significant differences between the means at a significance level of $\alpha=0.05$.

3 Results and Discussion

3.1 Enzymatic Profiling of Bacterial Strains using API-ZYM

The API ZYM system is a diagnostic test used to identify various enzymes produced by microorganisms. The test is performed using strips containing different substrates, each of which is used to detect the presence of a specific enzyme. The enzymes detected by the API ZYM system are those commonly found in a range of bacteria, including alkaline phosphatase, esterase lipase, leucine aminopeptidase, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, and α-mannosidase.

The results of the API ZYM test can be used to identify the genus and species of the microorganism being tested, as each microorganism produces a unique set of enzymes.

In the screening process, almost all strains tested demonstrated high activity of leucine aminopeptidase. Valine aminopeptidase activity was observed in all strains, except for T, X, E, and J, which generally exhibited weak or no activity. Cystine aminopeptidase activity was generally weak or absent in the screened strains. Trypsin and chymotrypsin activity was generally weak or absent in all screened strains. Phosphatase activity was moderate to high overall in all strains, with the exception of strains Y, T, and X. Similarly, phosphoamidase activity was moderate to high overall in all strains.

It has also been shown that LAB strains exhibit a high activity of enzymes such as α and β-galactosidases by Mital et al. (1973). Consistent with this statement, it has been determined that the selected strains for this study also exhibit moderate to high levels of these enzyme activities. α-galactosidase activity was found to be high or moderate in strains H, T, X, G, A, S, I, P, and V. However, strains Y, M, F, I, and K showed no activity of β-galactosidase, while other strains demonstrated moderate to high activity. The β-galactosidase activity was found to be high in strains derived from *Lb. plantarum* and *Leu. mesenteroides* species. Similarly, in this study, generally, *Lactobacillus* and *Leuconostoc* spp. exhibited this enzyme activity. These strains also have the ability to hydrolyze raffinose group oligosaccharides (Mital et al., 1973). The G strain and the S strain, both of which are classified as *Levilactobacillus brevis* species, demonstrated varying levels of β-glucuronidase activity. The G strain exhibited a high level of activity, while the S strain displayed a moderate level of activity, while no activity was observed in strains AA, D, J, and V. α-glucosidase activity was absent in strains AA, E, J, and P, weak in strains E and I, and moderate to high in other strains. Similarly, β-glucosidase activity was absent in strains AA, E, J, and P, weak in AB and A strains, and moderate to high in other strains. Strains B, C, D, G, L, and V showed low or no activity of β-glucosaminidase, while α-mannosidase and α-fucosidase showed no activity in all strains.

In order to obtain faba bean sourdough, 27 LAB including *Lc. lactis*, *Leu. mesenteroides*, *P. pentosaceus*, *Pediococcus* spp., and *Weisella* spp. were used in the study conducted by Verni et al., 2017. Similarly, in this thesis work, the enumeration of LAB on MRS agar was performed before and after fermentation. Additionally, pH determination was conducted after fermentation, following the similar approach of the study by Verni et al. (2017).

It was determined that the strains used in the study by Verni et al. (2017) could metabolize α-D-glucose, D-fructose, mannose, maltose, and L-lactic acid. Only 7 strains were observed to be capable of utilizing D-raffinose, as the α-galactosidase enzyme breaks down the alpha-galactosidic bond in D-raffinose. Considering that most of the strains used in this thesis work (H, T, X, G, A, S, I, P, and V) exhibited moderate to high levels of this enzyme activity, it can be concluded that they are capable of utilizing D-raffinose.

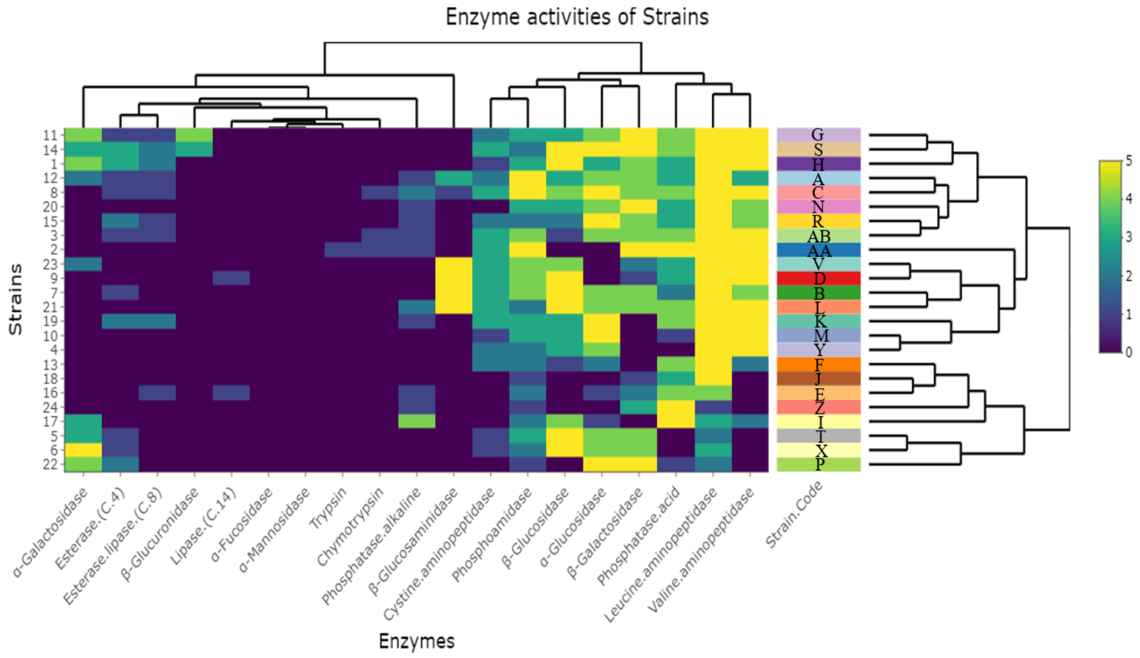

Figure 3. Heatmap showing Enzymatic Activity of Bacterial Strains

3.2 Screening of Bacterial Growth in Cereal and Legume- based Media with Bioscreen®

LAB naturally occur in environments, which highlights their ability to adapt to a specific environment (Cook and Bamforth, 2019). Lactic acid fermentation is defined as the enzymatic breakdown of energy-rich organic matter into less energy-containing simple compounds (Cichońska and Ziarno, 2021). In this experiment, growth data of selected LAB in cereal and legume-based media were obtained.

Growth curve prediction from optical density (OD) data is so commonly used. It is a useful method for estimating the concentration of bacteria in a media (Sutton, 2006). In bacterial cultures, the degree of turbidity or cloudiness, referred to as optical density, is an indicator of bacterial presence. It is a measurement of the light that is absorbed or scattered by bacterial cells in the media (Krishnamurthi et al., 2021). By monitoring the OD at set intervals, it is feasible to construct a growth curve depicting the pace of bacterial growth in the media. This data can be leveraged to identify the optimum growth conditions for a specific bacterial species.

Russo et al. (2016) reported initial inoculation levels of 8.9 log cfu/g for the production of functional food using oat flour. Meanwhile, Angelov et al. (2005) used initial inoculum levels of 7-8 log cfu/ml to produce oat-based fermented drink using *Lb. plantarum* and *Lb. paracasei* strains. In this study, relatively lower inoculum levels were maintained. Overnight grown cells were used as inoculum and number of viable cells ranged from 6.0-7.5 log cfu/ml. In the Bioscreen experiment, 10^{-2} and 10^{-3} dilutions were utilized from the overnight grown cells (indicated as -2 and -3 in this thesis) to ensure that bacterial cells were in the exponential growth phase, to prevent overcrowding of cells, and to obtain a quantifiable optical density.

3.2.1 Screening results of lactic acid bacteria strains in OatF-F using Bioscreen®

The initial OatF-F medium prepared by modifying the Tinz-Malang et al., 2015 protocol exhibited significant opaqueness and turbidity. Consequently, the initial optical densities were quite high during the Bioscreen assay, leading to optical densities surpassing 1.0.

It was observed that the strains used in this experiment generally completed the logarithmic phase within 10 hours. The F-coded strain, a member of the *Lc. lactis* subsp. *lactis* species, displayed superior bacterial growth compared to other strains when cultured in OatF-F medium. Both dilutions of this strain achieved higher final optical densities (OD). The initial OD for this strain was 1.36 (±0.03), which increased to 1.59

(±0.04) in the final measurement (Figure 4). The strain A, belonging to the *Lactiplantibacillus plantarum* species, showed the second-highest bacterial growth with an OD of 1.58 (±0.02). The 10^{-3} dilution of strain E (E -3), a *Lactococcus* species, exhibited the third-best bacterial growth, with an initial OD of 1.32 (±0.02) that increased to a final OD of 1.57 (±0.02). Similarly, the strain B belonging to the *Lactiplantibacillus paraplantarum* species also showed significant growth, with final ODs of 1.57 (±0.02) and 1.55 (±0.03) for its respective dilutions of 10^{-2} and 10^{-3}. Due to the high turbidity of the media, the initial ODs for all strains fell within the range of 1.3-1.4. The high turbidity of the medium has the potential to introduce inaccuracies in the measurements and should be carefully considered when interpreting the results. Overall, 10^{-2} dilutions showed shorter lag phase duration compared to 10^{-3} dilutions.

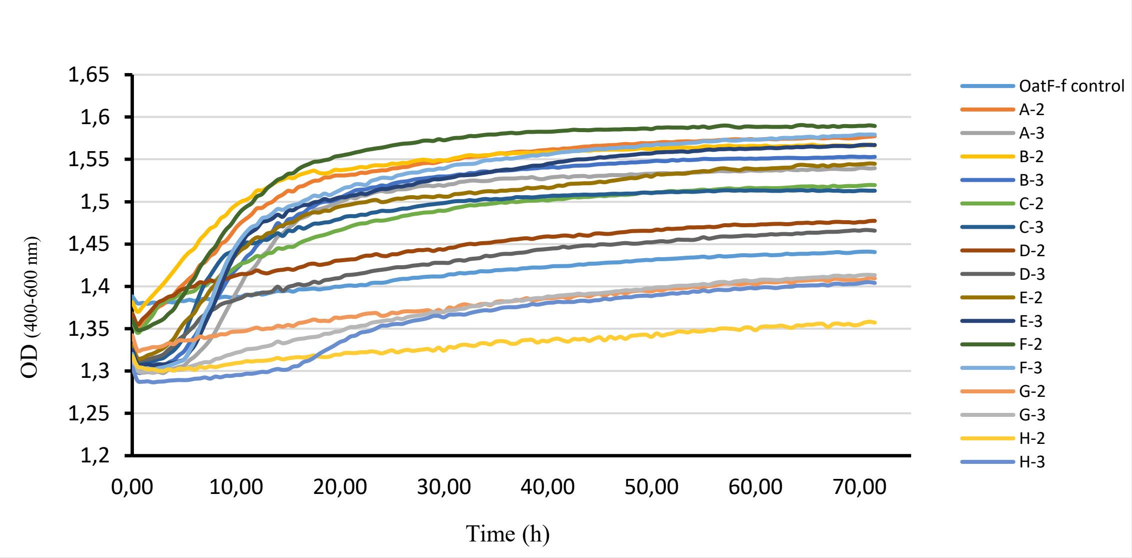

Figure 4. Growth curves of selected strains in turbid OatF-F 7.3 Medium

The I strain was excluded from the results due to significantly higher optical densities (ODs) exceeding 1. The L -2, belonging to the *Lactiplantibacillus plantarum* species, exhibited superior bacterial growth in OatF-F compared to other strains, with a final OD of 0.54 (±0.01). Similarly, the same dilution of N-coded strain also belonging to the *Lactiplantibacillus plantarum* species, showed the second-best bacterial growth, achieving a final OD of 0.46 (±0.01). Subsequently, the L -3 strain displayed noteworthy proliferation, with an initial OD of 0.12 (±0.03) that increased to a final OD of 0.45 (±0.01). Furthermore, the strain R, classified as *Lacticaseibacillus paracasei*, demonstrated significant growth with both 10^{-2} and 10^{-3} dilutions, resulting in a final OD of 0.37 (±0.04) (Figure 5).

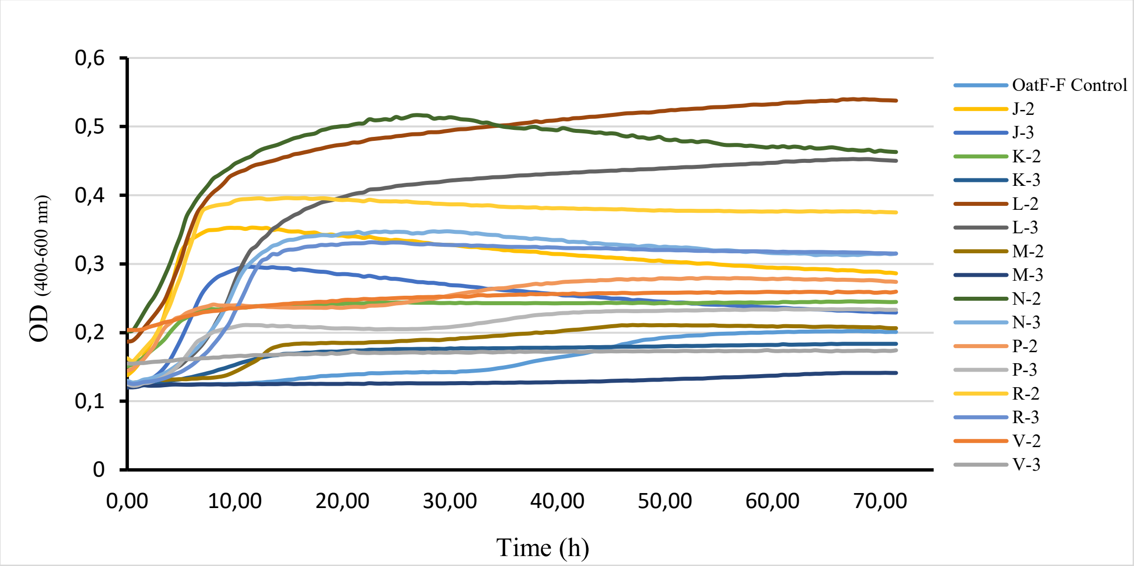

Figure 5. Growth curves of selected strains in clear OatF-F 3.3 Medium

In the study of Herrera-Ponce et al. (2014), it was determined that the maximal growth in simple oat medium was achieved by *Lb. acidophilus* with a value of 1.35 OD. In germinated oat medium, *Lb. casei* achieved the highest growth with a value of 0.65 OD. In this study, the highest OD in OatF-F medium was obtained with L strain (0.54±0.01) and in turbid medium, the highest OD was obtained with F strain (1.59±0.04). F strain belonged to *Lc. lactis* subsp. *lactis* species, while L strain belonged *to Lactiplantibacillus plantarum* species.

When analyzed in conjunction with API-ZYM results, it has been demonstrated that strain L displays robust enzymatic activity of leucine aminopeptidase, valine aminopeptidase, β-glucosidase, and β-glucosaminidase, along with moderate activity of α-glucosidase and β-galactosidase. Strain N exhibits strong activity of leucine aminopeptidase and β-galactosidase, moderate activity of valine aminopeptidase, α-glucosidase, and β-glucosaminidase, but shows low activity of phosphoamidase, phosphatase, and β-glucosidase. Strain R has been shown to possess strong leucine aminopeptidase and α-glucosidase activity, moderate activity of valine aminopeptidase and β-galactosidase, and low activity of β-glucosaminidase and β-glucuronidase.

Based on the findings from the strains that demonstrated optimal growth in OatF-F, it can be deduced that the activity of leucine aminopeptidase and β-galactosidase significantly contribute to their growth. Although α-glucosidase activity also plays a role, its impact is not as substantial as that of leucine aminopeptidase and β-galactosidase. The bacterial growth might have been positively impacted by the provision of essential nutrients for replication via sugar hydrolysis using the β-galactosidase enzyme.

3.2.2 Screening Results of Bacterial Strains in OatF-C Medium with Bioscreen®

The strain N, which belongs to the *Lactiplantibacillus plantarum* genus, showed superior bacterial growth compared to other strains in OatF-C, with both dilutions achieving higher final OD values. The initial OD for this strain was 0.20 (±0.003), which increased to 0.69 (±0.003) in the final measurement. The A-coded strain, also belonging to the *Lactiplantibacillus plantarum* genus, exhibited the second-best bacterial growth with a final OD of 0.54 (±0.04). The strain V, belonging to the *P. pentosaceus* species, showed the third-best bacterial growth, with a final OD of 0.52 (±0.04). The strain B, belonging to the *Lactiplantibacillus paraplantarum* species, followed with a final OD of 0.50 (±0.02).-The initial ODs for all strains were similar, and no significant difference was observed between the final ODs except for the strain N, which exhibited a final OD of 0.69 (±0.003). Overall, the bacterial strains showed better growth than OatF-F, reaching a final OD of almost 0.7.

The lag and exponential growth phases of the strains were studied to understand their growth behavior. The strain N demonstrated a lag phase duration of around 2.5 h, followed by an exponential growth phase that continued for approximately 26.5 h. Similarly, the strain A had a lag phase of 2.5 h, but its exponential growth phase lasted for approximately 33 h. The V strain showed a significantly shorter lag time of approximately 1 hour and exhibited rapid growth up to the 22nd hour, with a continuous increase in bacterial count even after the 40th hour. In Herrera-Ponce et al.'s (2014) study, it was observed that *Lb. rhamnosus* did not exhibit a lag phase in a simple oat-based medium and entered the stationary phase after 8 h. In this study, it was determined that particularly in the OatF-F medium, B, C, D, G, L, N, and P strains exhibited a very short lag phase. Specifically, a short lag phase was observed in the OatF-F medium, whereas longer lag phase durations were determined in the Faba-C medium (Figure 6a and 6b). These findings provide insights into the growth patterns of the strains and can inform their potential applications in various fields.

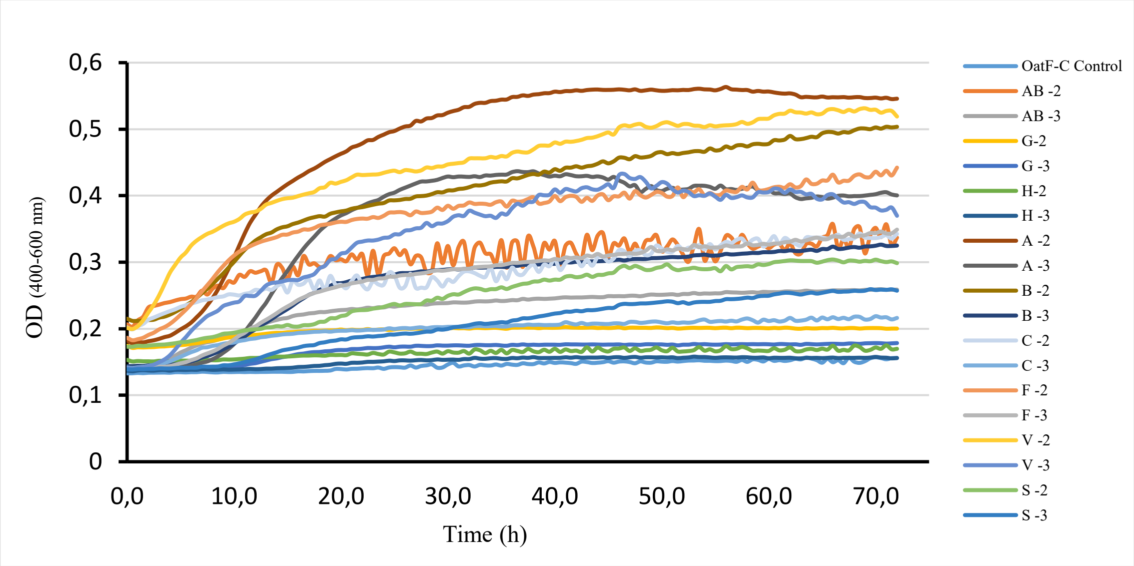

Figure 6a. Growth curves of first 9 selected strains in OatF-C medium

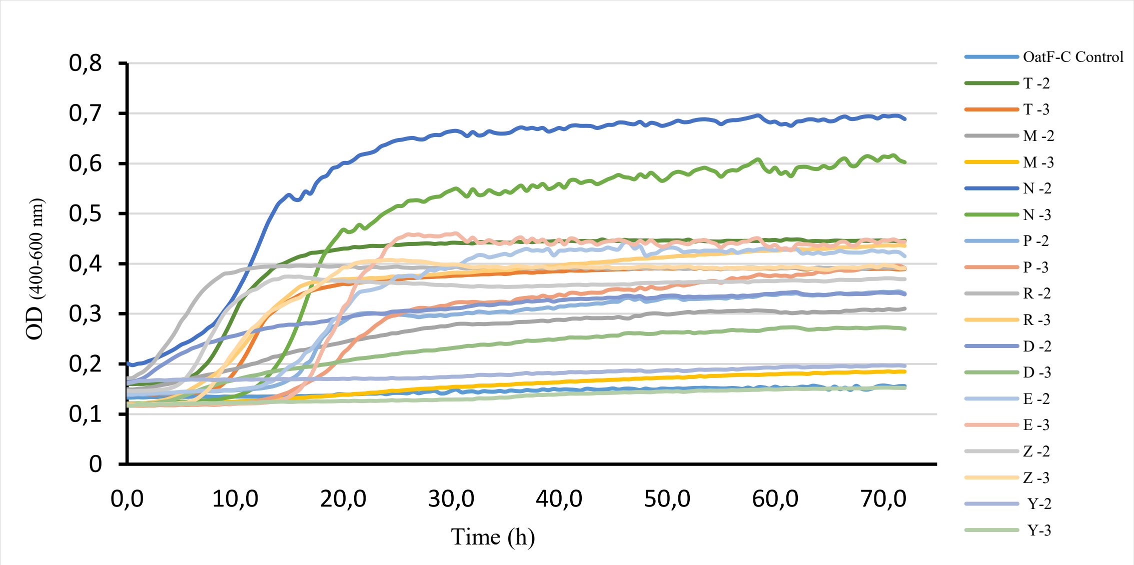

Figure 6b. Growth curves of remaining selected strains in OatF-C Medium

The API ZYM test results indicate that strain N has strong activity of leucine aminopeptidase and β-galactosidase, moderate activity of valine aminopeptidase, α-glucosidase, and β-glucosaminidase, but low activity of phosphoamidase, phosphatase, and β-glucosidase. The N strain demonstrates the presence of the β-galactosidase enzyme which is capable of breaking down sugars that contain β-galactosidic bonds, including lactose, into simpler sugars such as glucose and galactose through hydrolysis. This breakdown of lactose into simpler sugars provides the necessary nutrients for the growth of the N strain and can serve as a substrate for its growth. The increase in available nutrients within the medium has a positive impact on the growth of the N strain.

Similarly, the A-coded strain also exhibits strong leucine aminopeptidase activity, moderate levels of β-galactosidase and α-glucosidase activity, and low levels of valine aminopeptidase, phosphatase acid, β-glucosidase, and β-glucosaminidase activities. Both the N and A strains show moderate activity of the α-glucosidase enzyme, which is responsible for breaking down complex carbohydrates, particularly α-galactocytes, into glucose molecules that can be absorbed by the human body. By this enzyme, releasing glucose components that serve as nutrients for bacterial growth and reproduction.

The growth patterns observed in OatF-C suggest that the strains' growth is largely influenced by the activity of leucine aminopeptidase, followed by β-galactosidase, while the activity of α-glucosidase has a less pronounced impact.

Avena sativa L. is recognized as a high protein source due to its content of exogenous amino acids, including threonine, methionine, lysine, phenylalanine, valine, and leucine. (Rasane et al., 2015). The study found that the strains with the best growth also possessed enzymatic activity for valine and leucine aminopeptidase. This activity allows the strains to break down leucine and valine amino acids and use the resulting breakdown products as substrates for growth. As a result, these strains showed the best growth in OatF-F and OatF-C media due to their enzymatic activity.

3.2.3 Screening Results of Bacterial Strains in Faba-C Medium with Bioscreen®

The results of the cultivation experiments using Faba-C medium showed that the selected strains exhibited optimal bacterial growth in this specific medium, compared to OatF-F and OatF-C. Faba-C was found to have the highest protein content among the raw materials used, suggesting that the strains may thrive in a protein-rich environment. However, bacterial growth and viability are influenced by various factors, such as protein type and composition, availability of other nutrients, pH, temperature, and oxygen levels.

In Faba-C medium, the strain L, belonging to the *Lactiplantibacillus plantarum* species, exhibited the highest bacterial growth, surpassing an OD of 1 (±0.04) from an initial OD of 0.28 (±0.003). The strain N, also belonging to *Lactiplantibacillus plantarum*, showed the second highest growth with an initial OD of 0.29 (±0.01) and a final OD of 1.06 (±0.03), similar to OatF-C medium. Strain A, also belonging to *Lactiplantibacillus plantarum*, had a final OD value close to that of strain N, with an initial OD of 0.27 (±0.004) and a final OD of 0.96 (±0.05). Strain R, belonging to *Lacticaseibacillus paracasei*, showed good growth in Faba-C medium with a final OD of 0.95 (±0.02).

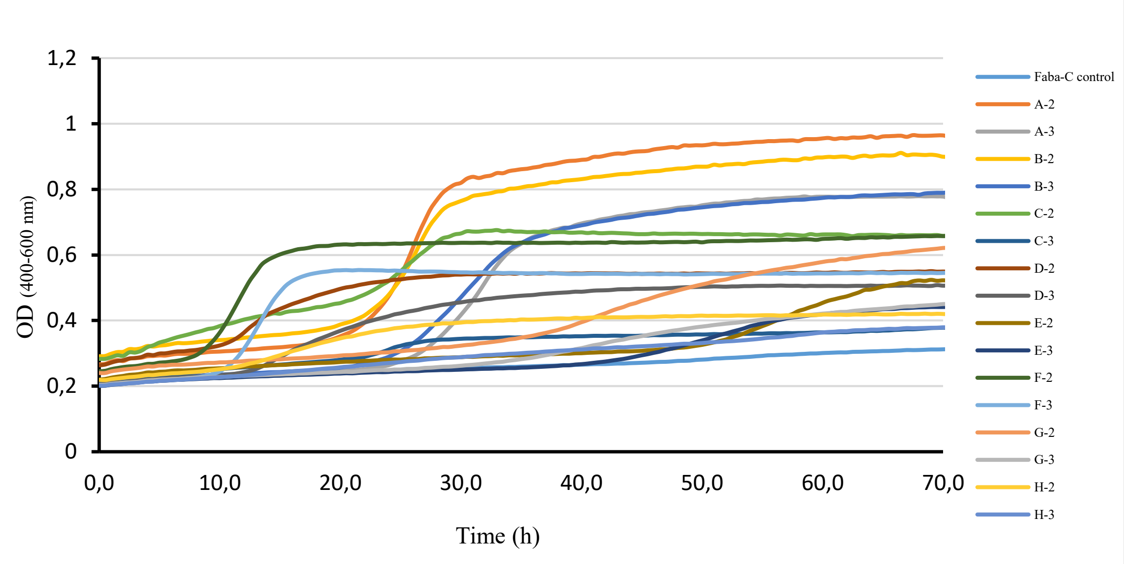

Figure 7a. Growth curves of first 8 selected strains in Faba-C Medium

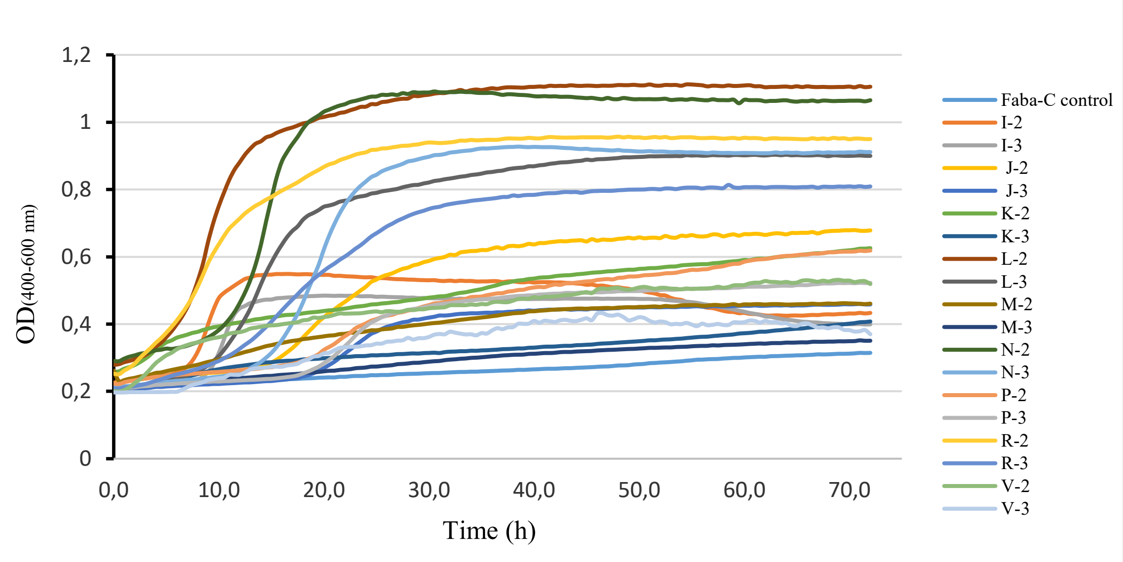

Figure 7b. Growth curves of remaining selected strains in Faba-C Medium

The strain L displayed favorable growth in Faba-C medium but did not show remarkable growth in OatF-C medium. Strain L was found to have strong activity of leucine aminopeptidase, valine aminopeptidase, β-glucosidase, and β-glucosaminidase, and moderate activity of α-glucosidase and β-galactosidase. Having strong leucine and valine aminopeptidase, indicates that the organism is capable of breaking down amino acids present in Faba-C and using the degradation products as a substrate for growth. In the case of Faba-C the main carbohydrates are sucrose and oligosaccharides such as raffinose, stachyose and verbascose which are composed of galactose, glucose and fructose units (Valente et al., 2019). Thus, strain L having strong β-glucosidase activity could hydrolyze the β-glucosidic bonds in oligosaccharides.

Based on the API ZYM results, strain N exhibited strong activity of Leucine aminopeptidase and β-galactosidase, moderate activity of valine aminopeptidase, α-glucosidase, and β-glucosaminidase, but low activity of phosphoamidase, phosphatase, and β-glucosidase. Strain A showed robust leucine aminopeptidase and phosphoamidase activity, and moderate activity in β-galactosidase and α-glucosidase. In Faba-C, β-galactosidase can also break down the β-glycosidic bonds in carbohydrates such as raffinose and stachyose, which are known to cause flatulence and gastrointestinal discomfort in some people. Strain N and A tested to secrete β-galactosidase enzyme can degrade the carbohydrates with β-glycosidic bonds in Faba-C.

Strain A showed a final OD value very close to that of strain N. The results showed that these two strains exhibited highly similar enzyme activities. This observation explains the good growth of these two strains in OatF-C and and similarly in Faba-C. However, the higher OD values and better bacterial growth achieved in Faba-C. This suggest that the composition of the medium favored their growth in Faba-C. The similar growth of these two strains in OatF-C and Faba-C may be due to their comparable enzyme activities.

From the observations of the strains that exhibited the best growth in Faba-C medium, it can be inferred that leucine aminopeptidase activity and β-galactosidase play a significant role in their growth, with α-glucosidase activity also having an impact, albeit not as substantial as Leucine aminopeptidase and β-galactosidase.

3.2.3 Screening of Bacterial Strains in Media without Overnight Shaking using Bioscreen®

In this experiment it was aimed to investigate the impact of overnight incubation with shaking during the medium preparation on nutrient release and subsequently on the growth kinetics of the bacterial strains. To achieve this, an experiment was conducted by eliminating the overnight shaking step in the medium preparation process, and the effects were examined. For this purpose, OatF-C and Faba-C media were chosen.

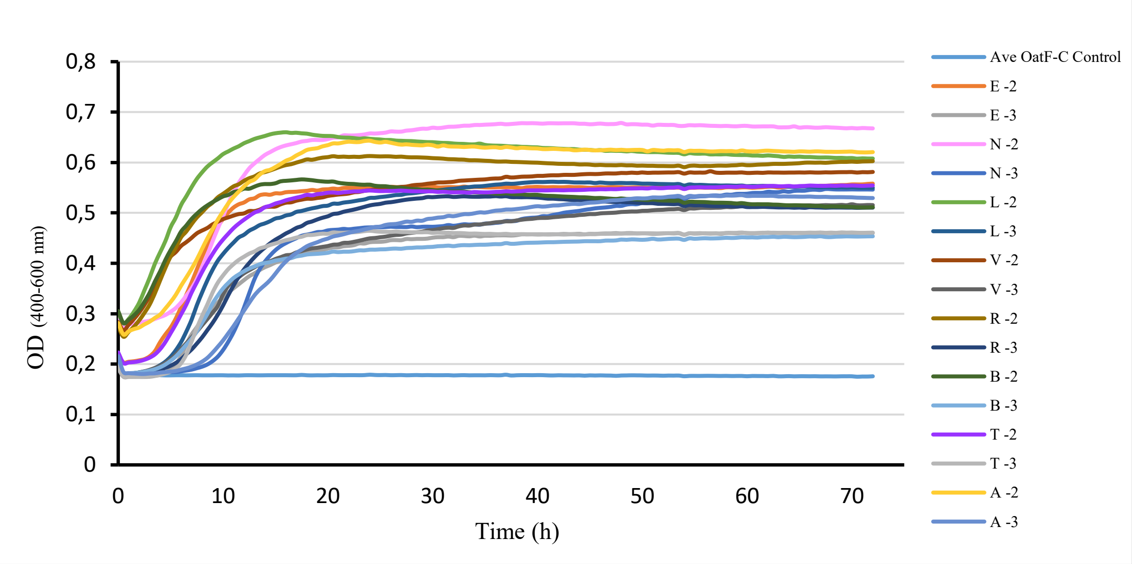

Figure 8. Growth curves of selected strains in OatF-C medium not shaken overnight

In the experiment involving OatF-C, it was observed that strain N exhibited the highest bacterial growth with a final OD of 0.69 (±0.001) when the medium was incubated overnight with shaking. Similarly, in the medium that was not shaken overnight (NS), strain N also showed the highest growth with a higher final OD value of 0.7 (±0.004). In contrast, in the medium that was incubated overnight with shaking, strain A was followed by the V-coded strain, while in the medium with no incubation, the R-coded strain followed the A strain with a final OD of 0.6 (±0.003). Notably, the OD achieved in the non-incubated medium was higher, reaching about 0.58 (±0.01), compared to the 0.52 OD (±0.003) achieved in the overnight incubated medium. Interestingly, the R strain did not exhibit substantial growth in the medium that was incubated with shaking overnight.

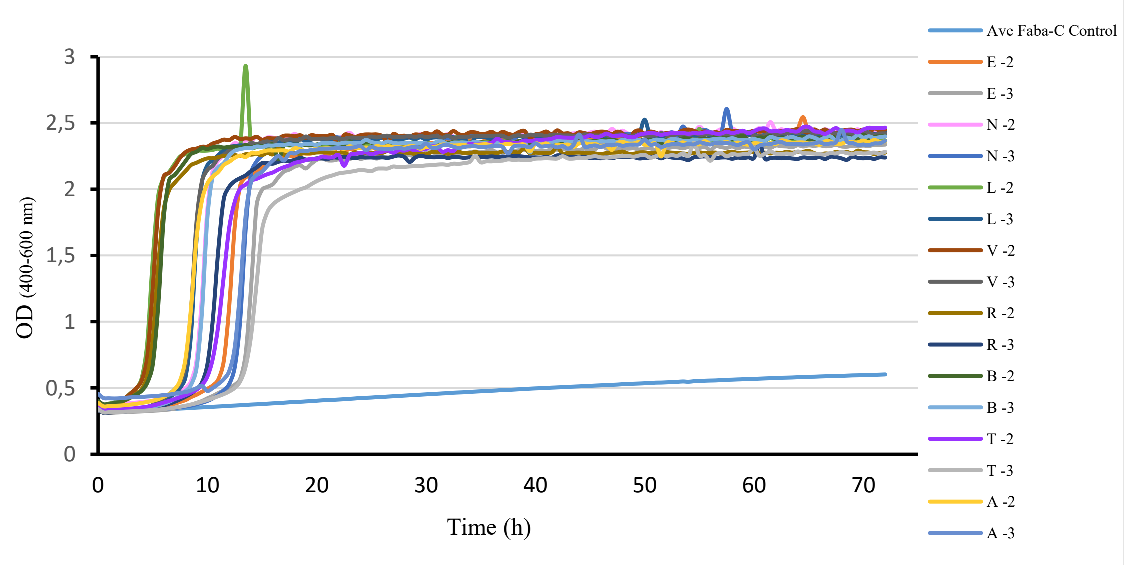

Figure 9. Growth curves of selected strains in Faba-C medium not shaken overnight

In the Faba-C medium that was not subjected to overnight shaking, the selected bacterial strains exhibited significant growth, with several strains achieving final OD exceeding 2.4 units. It was observed that the majority of the selected cultures completed the exponential growth phase rapidly within the first 10 hours.

In the experiment where Faba-C was incubated with shaking overnight, strain N showed the highest bacterial growth with final OD values of 2.44 (±0.04) and 2.43 (±0.02) for both two dilutions. The same strain, N, also showed the best growth in the OatF-C medium in an experiment that was not incubated overnight. However, in the medium that was incubated with shaking overnight, it was strain L that showed the best growth, followed by strain N with a final OD of 2.43 (±0.05). In contrast, in the media that were incubated overnight with shaking, strain N was followed by strain A, while in the media with no incubation, strain E was followed by strain L with a final OD of 2.41 (±0.03).

3.3 Bacterial Growth Rate Calculation Using R Studio

The growth rate of bacterial strains is a crucial parameter in microbiology research as it provides valuable insights into the dynamics of bacterial growth and proliferation. In this study, R Studio was utilized to calculate the growth rates of selected bacterial strains in response to three cereal and legume-based media. The growth rate offers a quantitative and objective measure of the rate at which the bacterial strains multiplied over time, providing valuable information on their growth performance.

A higher growth rate (r) indicates faster population growth, while a lower growth rate indicates slower growth. The growth rate can be positive or negative, with positive values indicating exponential growth and negative values indicating exponential decay.

The given results are obtained using the growthcurver package in R studio, which is used to determine the bacterial growth rate and time. The value of r represents the growth rate of the bacterial population, which is the rate at which the population size increases over time.

Figure 10 shows the results of bacterial growth rate (identified by their codes) in turbid OatF-F 7.3. For each strain, there are two dilutions (identified by -2 and -3). It can be observed that strain B -2 has the highest bacterial growth rate with its both dilutions in OatF-F in this trial with the rate of 1.723 (±0.16). Strain B is followed by strain C -2 with the growth rate of 1.509 (±0.29) and 10^{-3} dilution of B (B-2) also exhibited a relatively high growth rate of 1.404 (±0.77) (Detailed information can be found in the Appendix 2).

Nevertheless, strains F and A exhibited the highest ODs, indicating that they achieved the highest bacterial densities. However, interestingly, despite their high bacterial densities, the growth rates of strains F and A, as calculated using R Studio, were relatively lower compared to other strains.

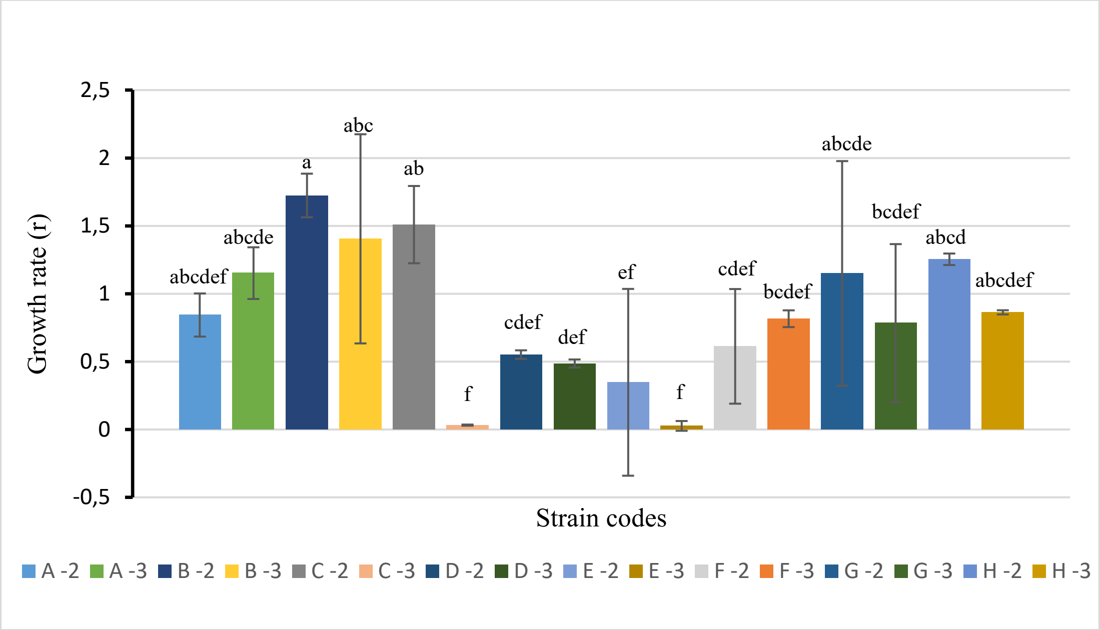

Figure 10. Growth rates (r) of selected strains in turbid OatF-F 7.3 (Results are average ±SD of replicates, n= 5)

A high growth rate could indicate that the bacteria are able to utilize the nutrients in the medium effectively and that the conditions are favorable for growth, but it does not guarantee that the strain is growing optimally in that specific medium. A bacterial strain showing a high OD in a bacterial growth curve may be indicative of a high growth rate, but it is not necessarily proportional to each other (Masoodi et al., 2021). There are several factors that can affect the relationship between optical density and growth rate such as competition with other indigenous bacteria or other environmental conditions such as pH (Sutton, 2006; Beal et al., 2020).

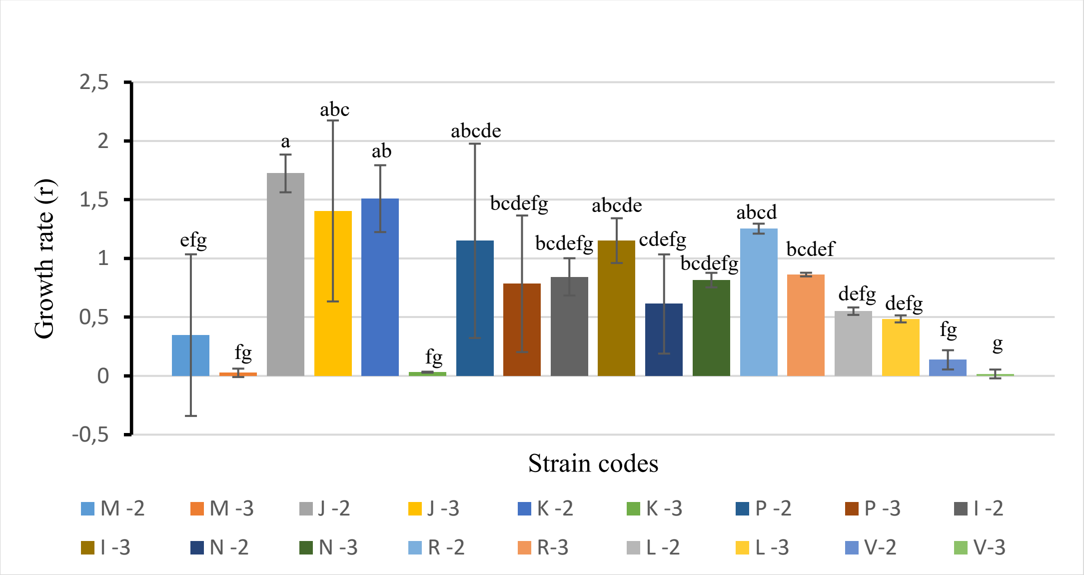

Figure 11. Growth rates (r) of selected strains in clear OatF-F 3.3 (Results are average ±SD of replicates, n=5)
Values with different superscript letters differ significantly.

It was observed that J -2 has the highest bacterial growth rate in OatF-F 3.3 (clear) in this trial with the rate of 1.724 (±0.16). Strain J is followed by strain K with the growth rate of 1.509 (±0.28) suggesting intermediate growth, and J -3 exhibited slightly lower growth rate of 1.404 (±0.77) compared to strain K. Strain R -2 has a growth rate of 1.253 (±0.04), indicating relatively slower growth compared to J -2, J -3, and K -2 (Figure 11).

In terms of bacterial density, the I strain displayed higher values compared to the other strains. However, the growth rate of the I strain was found to be average. On the other hand, the L strain exhibited lower growth rate compared to most of the other strains, despite showing high final optical densities.

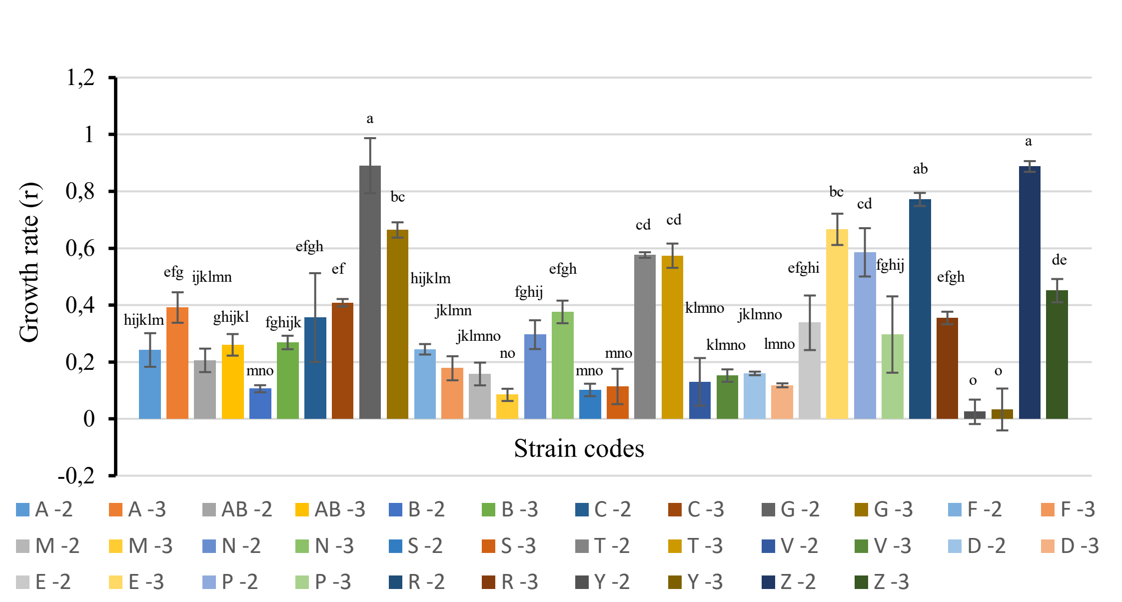

Figure 12. Growth rates (r) of selected strains in OatF-C (Results are average ±SD of replicates, n=5; H-2 strain is missing from the figure)
Values with different superscript letters differ significantly.

Strain H -2 has the highest growth rate of 22.273 (Appendix 2), (it has been excluded from the graph to enhance visibility and make it easier to observe the other bars) which is significantly higher than all other strains, indicating that it had the fastest bacterial growth rate among all the strains tested. Since it is remarkably higher than the other strains, so it's possible that the higher growth rate observed in the strain is due to experimental artifacts, such as errors in data collection or analysis, variability in experimental conditions, or other factors that may affect the growth curve. In addition, the strain with the higher growth rate may have acquired genetic mutations that promote faster growth, such as mutations that affect regulatory genes, metabolic pathways, or cell division processes. Strain G -2 has the second highest growth rate of 0.890 (±0.1), which is relatively high compared to other strains, suggesting relatively fast growth. Strain Z -2 has a growth rate of 0.887 (±0.02), which is similar to G -2, indicating fast growth (Figure 12).

Strain M, which showed the best growth performance according to the growth curves, was found to have a relatively lower growth rate (Mean_r=0.158 ±0.04 and 0.084±0.02). Strain A, which showed the second-best growth, also exhibited a similar trend of generally lower growth rate (Mean_r=0.242 ±0.06 and 0.392±0.05). Strain V, which also has been shown to have high OD in OatF-C, was determined to have a considerably lower growth rate with both dilution of 0.130±0.08 and 0.152±0.02, respectively (Appendix 2).

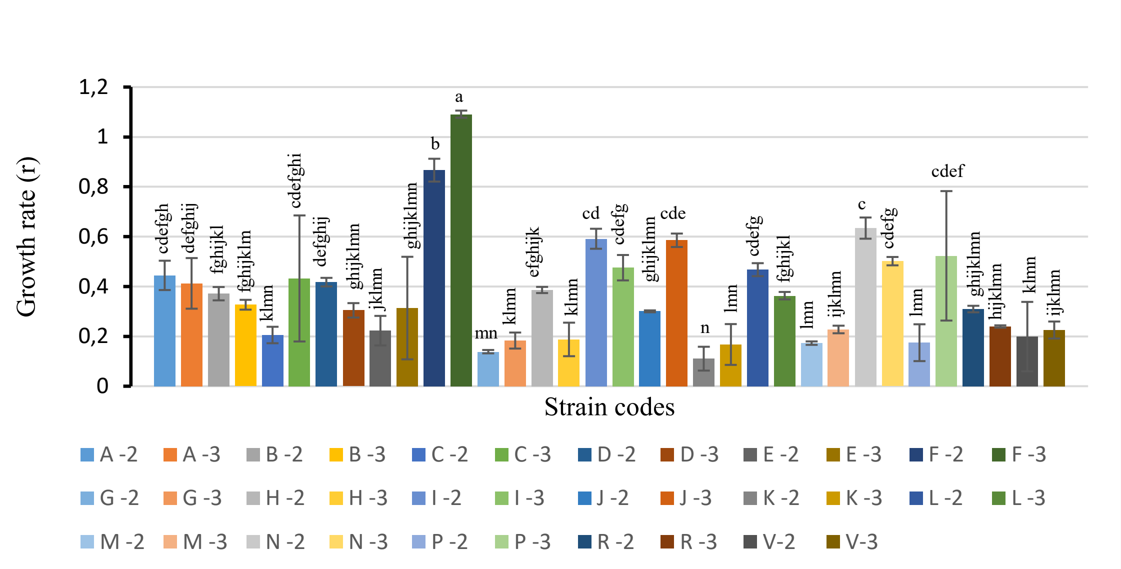

Figure 13. Growth rates (r) of selected strains in Faba-C (Results are average ±SD of replicates, n=5)
Values with different superscript letters differ significantly.

Strain F with its both dilutions presented the highest growth rate in Faba-C media with mean growth rates of 1.091 (±0.01) and 0.867 (±0.05), respectively (Figure 13). This indicates that this strain is able to grow at a faster rate than the others. Strain F was followed by strain N -2 with a mean growth rate of 0.634 (±0.04). These strains exhibit relatively higher growth rates compared to other strains in Faba-C. Strain F belongs to *Lc. lactis* subsp. *lactis* and N belongs to *Lb. plantarum* species.

according to the growth performance L strain exhibited the best growth. Following the L strain, the N strain displayed relatively higher growth rates compared to other strains. Nevertheless, it is important to note that a high OD measurement does not necessarily equate to a fast growth rate, and vice versa. For instance, strain A was identified as one of the strains exhibiting favorable growth and high optical density (OD), however, upon further examination of its growth rates, it was determined to be average compared to other strains.

The reasons for the higher growth rates of these strains could be attributed to various factors, such as genetic characteristics, metabolic capabilities, and adaptation to the specific growth conditions of the Faba-C media. These strains may have efficient utilization of nutrients present in Faba-C, resulting in higher growth rates compared to other strains in the study. When the enzymatic activities of these two strains were examined, it was found that Phophatase acid and Phosphoamidase were common among the strong enzymatic activities. These enzymes are responsible for the release of inorganic phosphate from organic phosphate compounds, which can then be used by microorganisms for energy metabolism and growth. In addition, the availability of free inorganic phosphate can also affect the pH of the medium and subsequently impact the activity of other enzymes involved in the fermentation process (Lidbury et al., 2022).

In their study, Herrera-Ponce et al. (2014) concluded that the oat-based medium they prepared had a significant impact on the growth kinetics of *Lb. rhamnosus*, *Lb. casei*, and *Lb. acidophilus* strains. Similarly, in this thesis, the effect of three cereal and legume-based media on the growth kinetics and patterns of strains was observed. Statistical analysis using R Studio showed that there was a significant difference among the media in terms of their impact on the growth kinetics of the strains ($p<0.05$).

3.3.1 Bacterial growth rate calculation on media with and without shaking overnight using R studio

The growth kinetics and growth curves of selected bacterial strains in overnight and non-incubated media were examined and their growth rates were calculated and compared in R Studio. Inocula were prepared in a similar way as in Bioscreen experiment as described above. Number of the cell were in the range of 6.2 log cfu/ml- 7.2 log cfu/ml for Faba-C and 5.7 log cfu/ml -7.4 log cfu/ml for OatF-C.

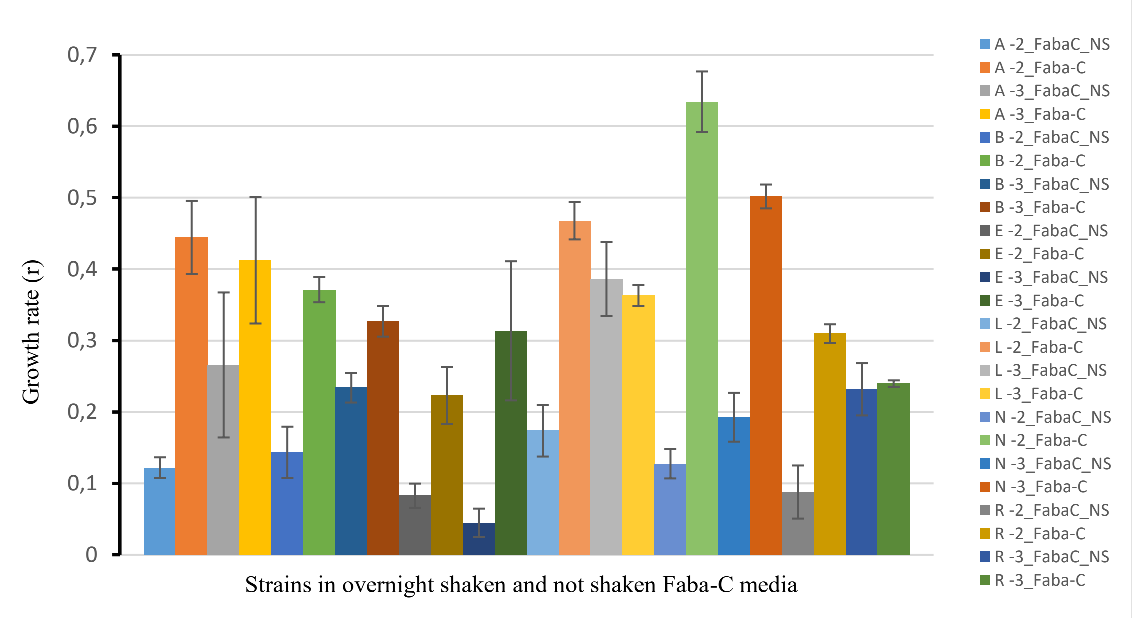

Figure 14. Growth rates (r) of selected strains in Faba-C with and without incubated overnight with shaking (Results are average ±SD of replicates, n=5)
*NS= Not incubated overnight with shaking

The strains with the highest mean growth rates (r) are N -2 (Mean_r = 2.709±0.13), B -2 (Mean_r = 2.644±0.14), L -2 (Mean_r = 2.605±0.17), V -2 (Mean_r = 2.344±0.12), and R -2 (Mean_r = 2.304±0.09) (Figure 14). In line with this, it was observed that strain N achieved a high level of bacterial density in Faba-C medium that was not incubated overnight with shaking. These strains exhibit relatively high growth rates compared to the other strains in the Faba-C, as indicated by their higher values of mean growth rate (r). This suggests that these strains have the potential for rapid growth and proliferation in the given media.

It could be observed that the bacterial growth rate is higher in the Faba-C medium incubated overnight without shaking, as compared to the medium that is incubated overnight with shaking.

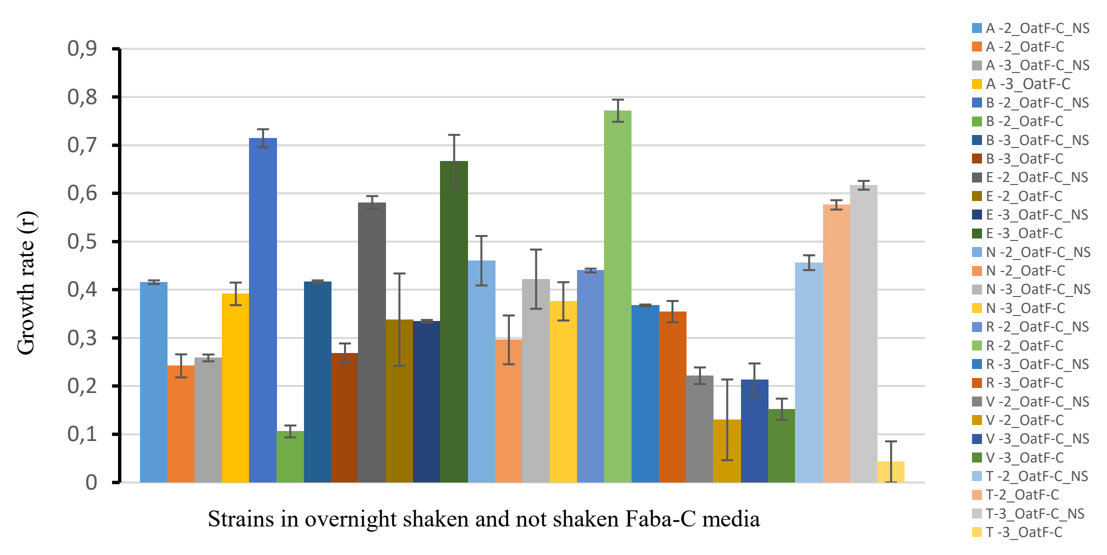

Figure 15. Growth rates (r) of selected strains in Oat-C with and without incubated overnight with shaking (Results are average ±SD of replicates, n=5)
*NS= Not incubated overnight with shaking

B -2 in not incubated overnight with shaking media exhibited the highest growth rate of 0.714 (±0.02), indicating the fastest growth among all the strains. While strain T -3 displayed the second-highest growth rate in OatF-C without incubated overnight with shaking (Mean_r=0.617±0.01). Moreover, L -2 has been found to demonstrate a relatively high growth rate of 0.635±0.01 and be considered as the third-highest growth rate observed among the strains (Figure 15). In OatF-C medium that was not shaken overnight, strains N and R demonstrated high bacterial growth. However, these strains exhibited an average bacterial growth rate.

Comparing the growth rates of the different strains in OatF-C medium with and without overnight incubation with shaking, it appears that for most of the strains, the growth rate is higher in the media without overnight incubation with shaking except for the strains A-3, E-3, R-2, and T-2.

The overnight shaking process could impact the physical properties of the medium, such as viscosity or particle size distribution, which could impact bacterial growth. Without shaking process, there may have been differences in the physical properties of the medium, which could have favored bacterial growth and metabolism (Du et al., 2014; Huang et al., 2018). In addition, in the absence of shaking, the nutrients in the medium may have been more stable, resulting in a more consistent and favorable environment for bacterial growth. Another reason could be that the shaking process could have increased oxygen availability in the medium, which may not be favorable for the growth of LAB, as they are facultative anaerobes. Without shaking, the oxygen availability in the medium could be lower, which may have provided a more favorable environment for LAB growth (Leroy & De Vuyst, 2004). Moreover, overnight incubation with shaking can lead to increased enzymatic activities of indigenous bacteria due to enhanced metabolism. This can potentially result in the breakdown of certain compounds in the medium, leading to changes in the chemical composition and nutrient availability.

3.4 Biolector-based analysis of bacterial biomass and pH profiles in cereal and legume-based media

3.4.1 Bacterial biomass profiling in cereal and legume-based media

The Biolector is a specialized instrument used for monitoring microbial growth and fermentation processes in real-time. It combines microscale bioreactor technology with high-throughput capabilities, allowing for simultaneous monitoring of multiple samples under controlled conditions.

The aim of performing this experiment was to evaluate the growth and fermentation characteristics of different LAB strains in cereal and legume-based media. The Biolector allows to measure and monitor the biomass change (i.e., growth) and pH variation of the bacterial cultures in these media in real-time, providing valuable insights into the growth dynamics and metabolic activity of the strains in different conditions.

The Biolector used to monitor the pH variation in the bacterial cultures over time, which can provide insights into the fermentation profiles of the strains in the prepared media. pH changes can indicate the production of organic acids or other metabolites during fermentation, which can influence the flavor, aroma, and nutritional properties of the final fermented product (Pejcz et al., 2021). It also enables to efficiently screen and evaluate a large number of LAB strains in different media, providing a comprehensive dataset for analysis and interpretation.

Russo et al. (2016) specified initial inoculation levels of 8.9 log cfu/g for the production of functional food using oat flour. In a study conducted by Angelov et al. (2005) to obtain an oat-based fermented drink using *Lb. plantarum* and *Lb. paracasei* strains, initial inoculum levels of 7-8 log cfu/ml were used. Similar initial inoculum levels were used by Rizello et al. (2010a), Curiel et al. (2015), Coda et al. (2015), Montemurro et al. (2019), and Rizello et al. (2019) to that of Angelov et al. (2005). In this study, initial inoculation levels were determined to be 7.11-8.51 log cfu/ml for all media from overnight grown cells.

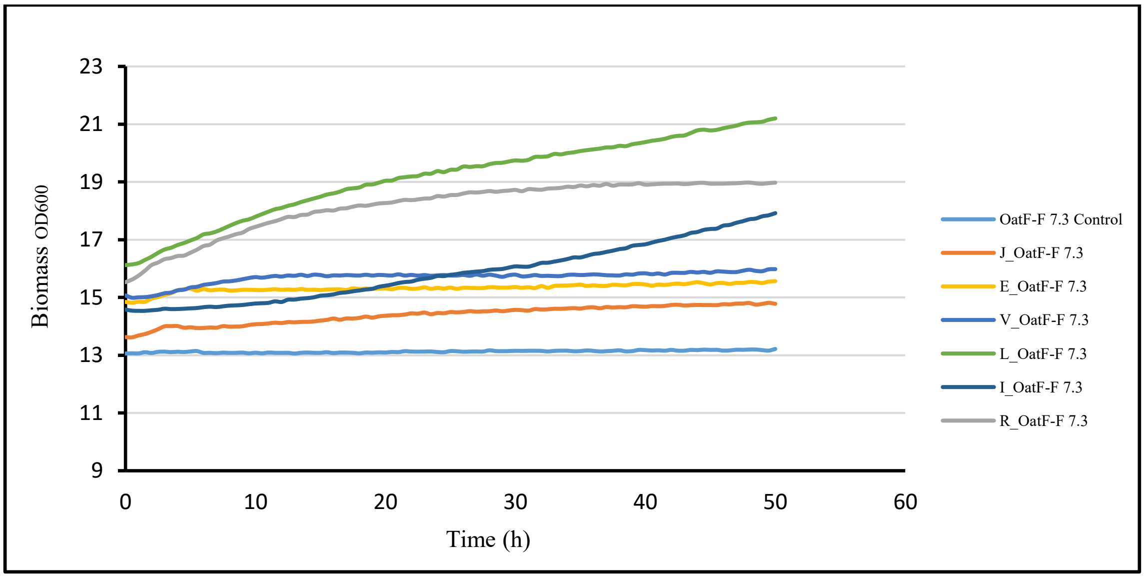

Figure 16. Biomass change from Biolector cultivation with selected strains in OatF-F 7.3

The growth of L strain in OatF-F 7.3 medium was found to be superior compared to other strains, showing better grow and reaching higher ODs. The final OD for L strain was observed to be 21.2 (±0.47). Following L strain, R strain showed an increase in OD over time, from 15.53 OD (±0.04) to 18.97 OD (±0.18). Moreover, similarly strain I exhibited a good biomass increment reaching 17.92 OD (±1.11). In contrast, other strains did not exhibit a noticeable increase in OD during the fermentation period, demonstrating overall stable biomass (Figure 16).

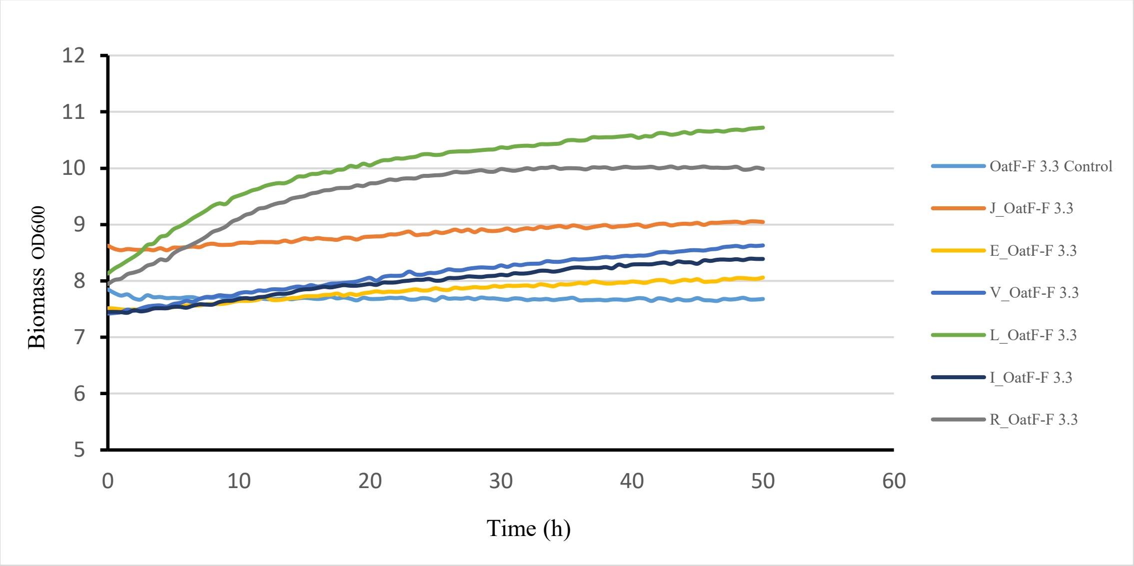

Figure 17. Biomass change from Biolector cultivation with selected strains in OatF-F 3.3

The OatF-F 7.3 and OatF-F 3.3 media were prepared from the same raw material (oat flour), but the turbidity of OatF-F 7.3 was found to be higher than that of OatF-F 3.3. Since both media were prepared from the same raw material, it was expected that the same strains would exhibit similar growth in both media. Consistent with this expectation, L and R strains showed the highest increase in biomass, similar to OatF-F 7.3. Final OD of strain L was found to be 10.72 (±0.37) and for the strain R 9.99 (±0.20) (Figure 17). Nevertheless, the growth of the I strain in OatF-F 3.3 was found to be unremarkable in comparison to its growth in OatF-F 7.3. Based on the findings from the Bioscreen experiment, it was observed that strain L also demonstrated high bacterial densities during bacterial growth.

It seems that strain B already starts from high ODs at the beginning, thus giving a misleading result (Figure 18). The A strain on the other hand was found to have remarkable biomass increment compared to other strains, showing better grow, and reaching higher ODs. The final OD for A strain was observed to be 9.66 (±0.20). Following A strain, The F strain showed a slow and slight increase in OD over time from 8.03 OD (±0.20) to 8.86 OD (±0.04). In contrast, other strains did not exhibit a noticeable increase in OD during the fermentation period, demonstrating overall stable biomass.

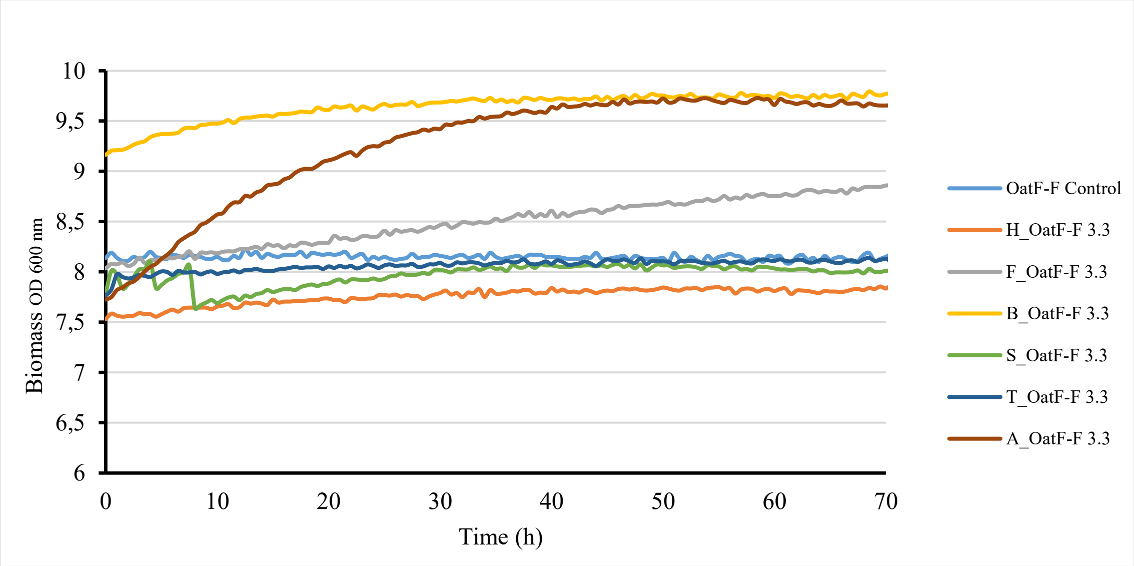

Figure 18. Biomass change from Biolector cultivation with selected strains in OatF-F 3.3

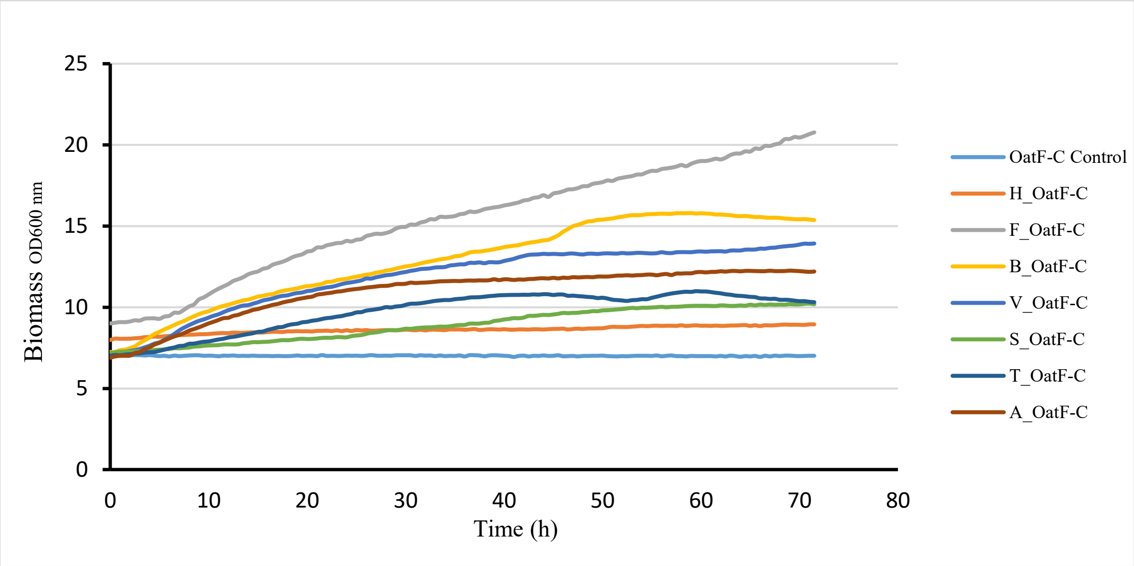

Figure 19. Biomass change from Biolector cultivation with selected strains in OatF-C

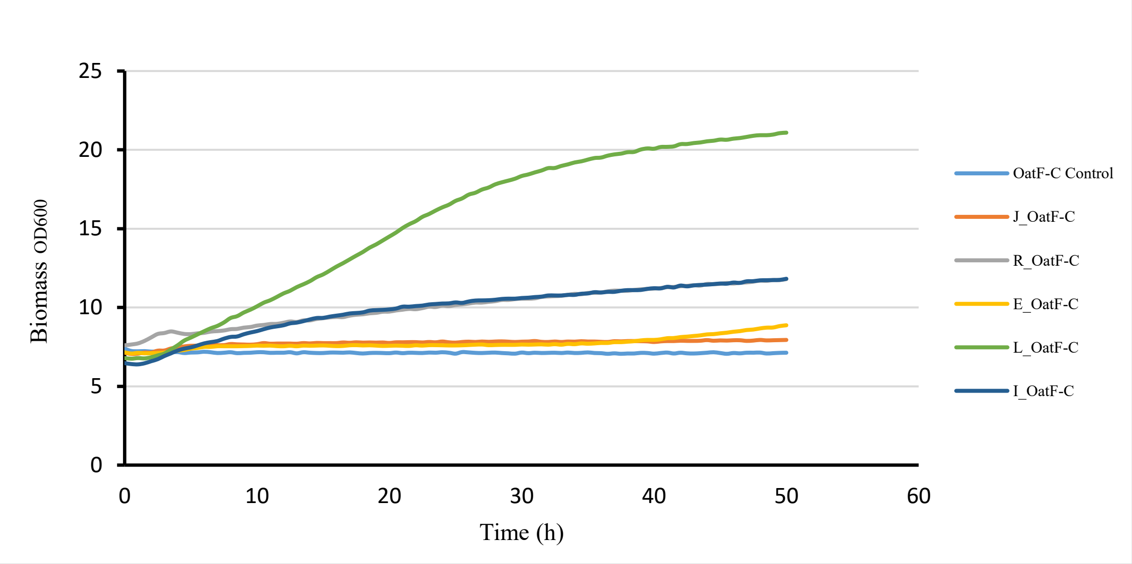

Figure 20. Biomass change from Biolector cultivation with selected strains in OatF-C

In the OatF-C medium, the most substantial increase in biomass was observed in the F strain. It exhibited a significantly higher increase in biomass compared to other strains, reaching a final OD of 21.77 (±1.24). It also showed the ability to reach higher OD values than other strains in the OatF-F medium. The strain L similar to OatF-F presents high biomass values in OatF-C medium reaching 21.1 (±0.001). Followingly the strain B exhibited good increase in biomass with an initial OD of 7.24 (±0.05) increasing to 15.37 (±1.09). The final OD of strain V were comparable and proximate to those of strain B with 13.92 (±0.76) (Figure 19-20).

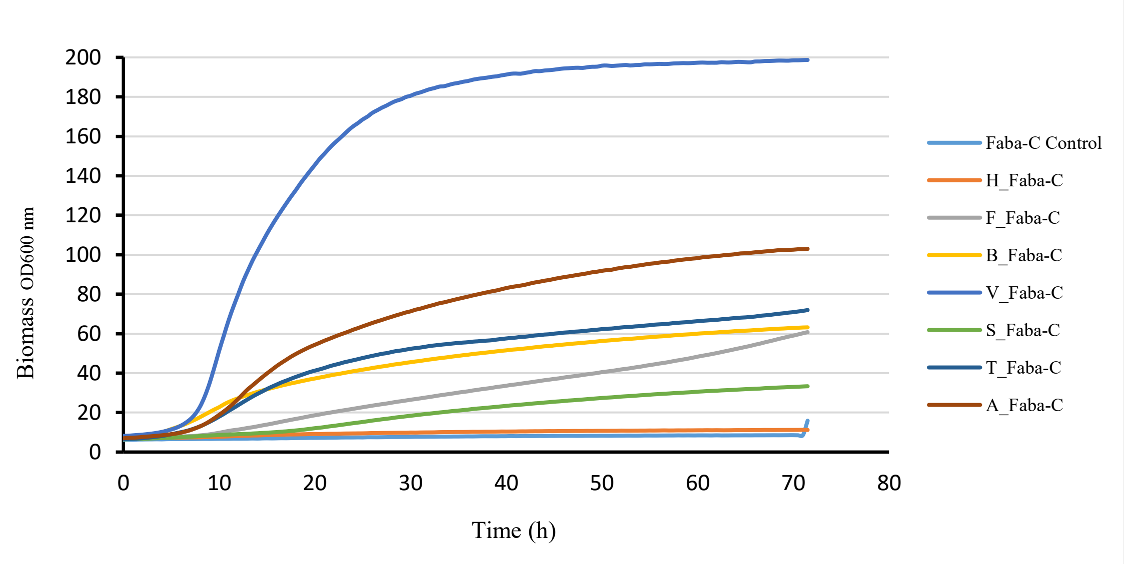

Figure 21. Biomass change from Biolector cultivation with selected strains in Faba-C

Biolector cultivation included the addition of Faba-C. From the results of Bioscreen analysis, which had shown that many strains exhibited a significantly high cell density when grown in Faba-C. The purpose of this addition was to gain further understanding about the growth behavior of different microbial strains under varying environmental conditions.

The V strain demonstrated the most substantial growth in biomass in the Faba-C medium, with a significantly higher increase in comparison to other strains, resulting in a final OD of 198.69 (±3.08). Despite the medium's low turbidity, the readings of OD have been found high (Figure 21). On the other hand, the V strain exhibited substantial bacterial growth when cultured in the Faba-C medium. The A strain exhibited the second-best growth, although significantly lower than the V strain, starting from an initial OD of 6.96 (±0.78) and reaching 102.95 (±4.43) at the end of the experiment. Standard deviations between the replicates were observed high. This can be due to the differences in the conditions in the wells even though the same media is used. There could be some changes in the conditions such as pH, nutrient availability or oxygen levels. On the other hand, this could also be due to external factors such as an error made while preparing the cells.

Upon analyzing the cell counts following the fermentation process, Herrera-Ponce et al. (2014) reported that the highest bacterial count in simple oat media was obtained after 24 hours of fermentation, with 9.3±0.01 log cfu/ml for *Lb. casei*. Similar values were obtained by Rizello et al. (2010a), where 9.3±0.3 log cfu/ml - 9.9±0.5 log cfu/ml were noted for *L. plantarum* and *L. rossiae*, respectively. Mårtesson et al. (2001) obtained viable counts higher than 7 log cfu/ml after fermentation. Counts exceeding 9 log cfu/ml were obtained after 24 hours of fermentation in the study conducted by Curiel et al. (2015), while the same counts were observed by Coda et al. (2015) after 48 hours of fermentation. In this study,the cell counts did not show significant changes after fermentation in OatF-F 7.3 medium, ranging from 6.1 (strain J) to 6.7 (strain I). In contrast, in OatF-F 3.3 medium, bacterial counts slightly increased after fermentation, ranging from 6.30 (strain B) to 8.3 (strain E). Cell viability obtained after fermentation in OatF-C medium ranged from 6.5 (strain H) to 8.3 (strain T). Based on these results, similar cell viability ranges were observed in both OatF-F and OatF-C media, despite being obtained with different strains.

In this thesis, initial inoculation levels in Faba-C medium were monitored from 7.1 log cfu/ml (strain E) to 8.5 log cfu/ml (strain B). After cultivation, cell viability counts ranged from 6.7 log cfu/ml (strain H) to 8.4 log cfu/ml (strain S). LAB counts were reported to increase to 5.2±0.1 log cfu/g after fermentation by Verni et al., 2017. In a study on the fermentation of black beans, similar and higher final counts to this study were obtained with 7 log cfu/g (Granito and Alvarez, 2006). A 4-log increase in lentil fermentation was also reported in another study (Montemurro et al., 2019). Doblado et al. (2003) reported that final counts of 6-8 log cfu/ml were obtained and the number of *L. plantarum* strains decreased at the end of fermentation. This situation was also observed in this thesis study. Some strains were found to decrease in number at the end of the fermentation period. This could be attributed to the extended fermentation period, as in practical applications, fermentation is commonly carried out overnight.

The post-fermentation cell count results showed that strain E had the highest plate count of 8.3 log cfu/ml in OatF-F medium. However, strains L, R, and B had high bacterial biomass in the same medium. The T strain had the highest count of 8.3 log cfu/ml in OatF-C, while strains F and L had the highest biomass value. In Faba-C medium, the S strain had the highest count of 8.4 log cfu/ml, but V and A were the strains with the highest biomass.

3.4.2 pH profiling in cereal and legume-based media

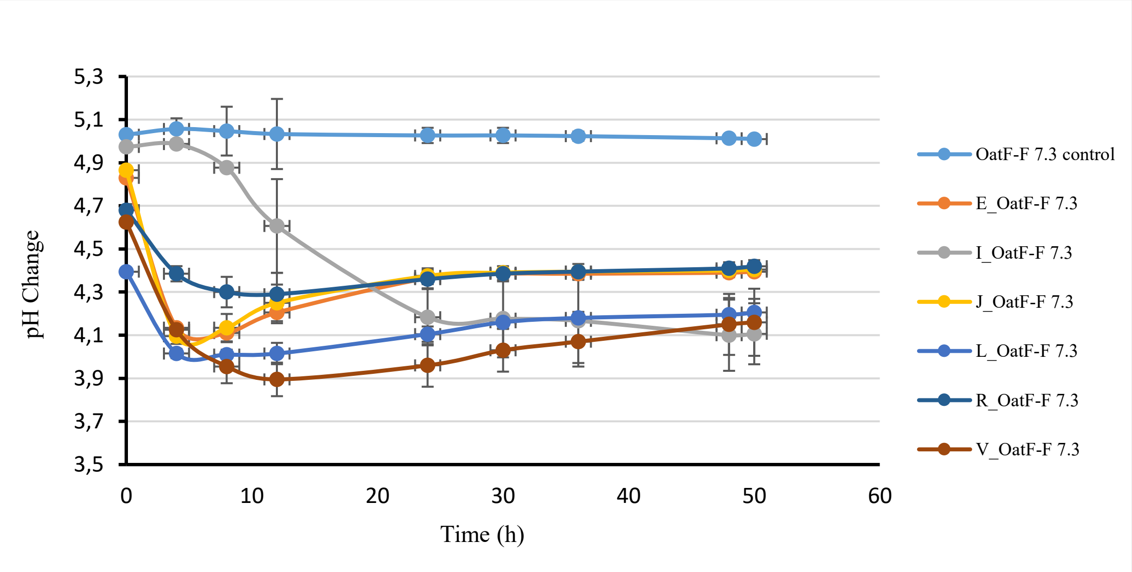

Figure 22. pH Change in OatF-F 7.3 with selected strains at time points (Results are average ±SD of replicates, n=2)

The Biolector cultivation in the OatF-F 7.3 medium revealed that strain I was the most effective in reducing pH in the initial 10 hours with 0.87 units to a final pH 4.11 (±0.141). Strain V also exhibited good pH drop similarly in the first 10 hours of the fermentation to a final pH of 4.16 (±0.15). Strain J, starting from a higher pH level of 4.86 (±0.01), attained final pH level to 4.40 (±0.01). Furthermore, strain E displayed a rapid pH decrease in the initial 10 hours and achieved final pH levels comparable to strain J (Figure

22). In the OatF-F 7.3 medium, strain L displayed superior biomass increase and bacterial growth compared to other strains. However, when it comes to pH reduction, strain I and V were the most effective, with strain J coming in second.

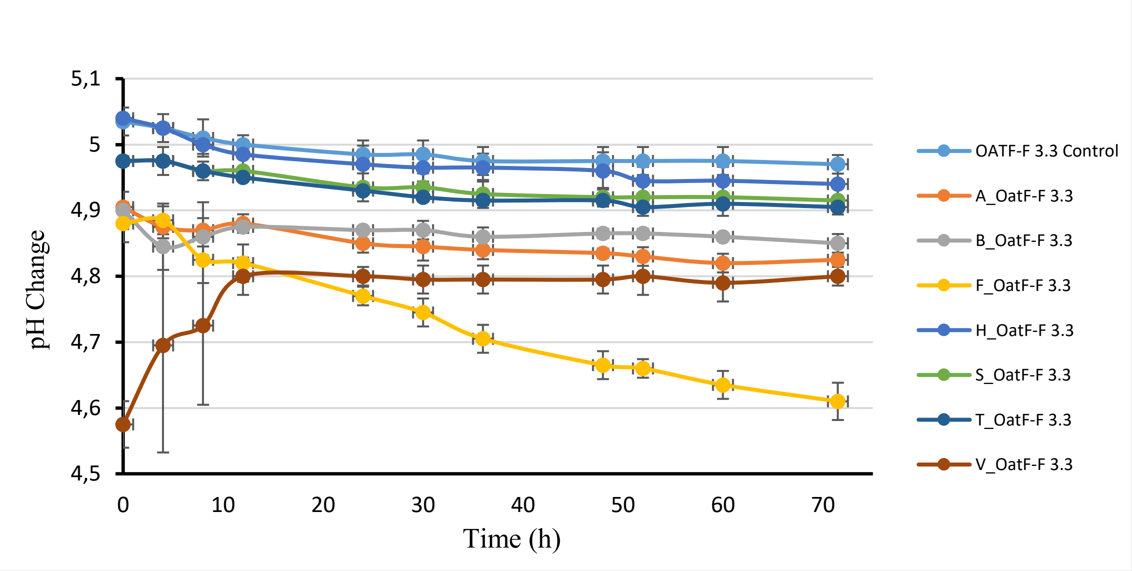

Figure 23. pH Change in OatF-F 3.3 with selected strains at time points (Results are average ±SD of replicates, n=2)

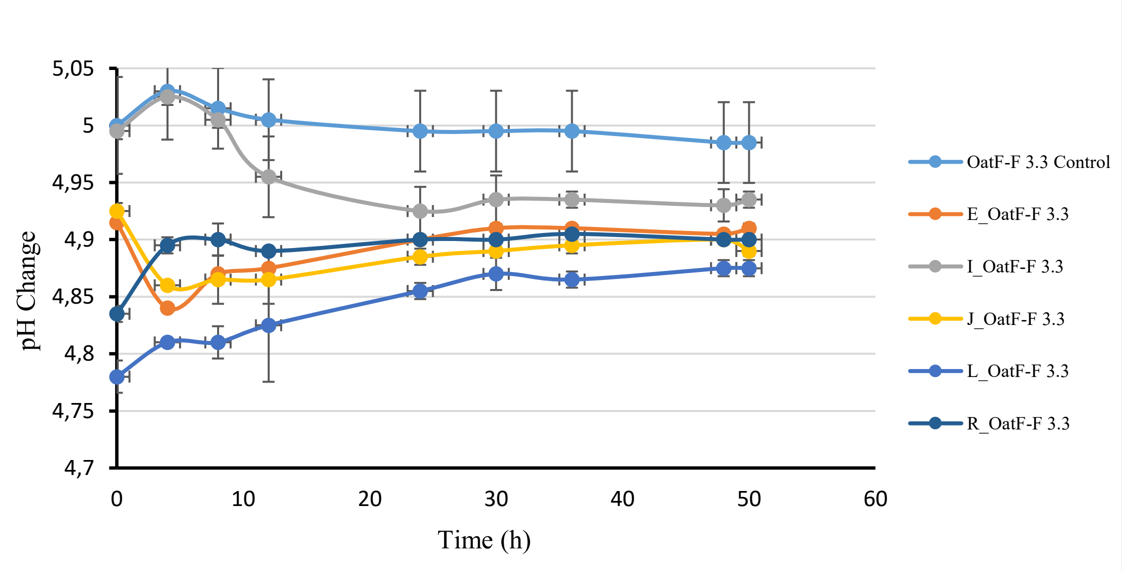

Figure 24. pH Change in OatF-F 3.3 with selected strains at time points (Results are average ±SD of replicates, n=2)

It was observed that utilized strains did not exhibit an effective pH reduction in OatF-F 3.3 in general. It was observed that the initial pH levels of the inoculated strains were between 5.04 (±0.00) and 4.57 (±0.03). Interestingly, some strains have been shown to increase pH during fermentation such as in the case of strain V (Figure 23) and L (Figure 24). Strain F provided the most effective and significant pH reduction lowering the pH values to 4.61 (±0.03). In contrast to the observations in Faba-C and OatF-F media, strain A, which was anticipated to have the highest biomass value and best bacterial growth in OatF-F, did not prove to be effective in reducing pH (with only a reduction of 0.08 units). Despite exhibiting the highest biomass increase and ODs in OatF-F 3.3 medium, L and R strains were unable to achieve a significant reduction in pH values. The results indicate that the strain exhibiting the best bacterial growth or highest biomass value did not necessarily lead to the most effective pH reduction.

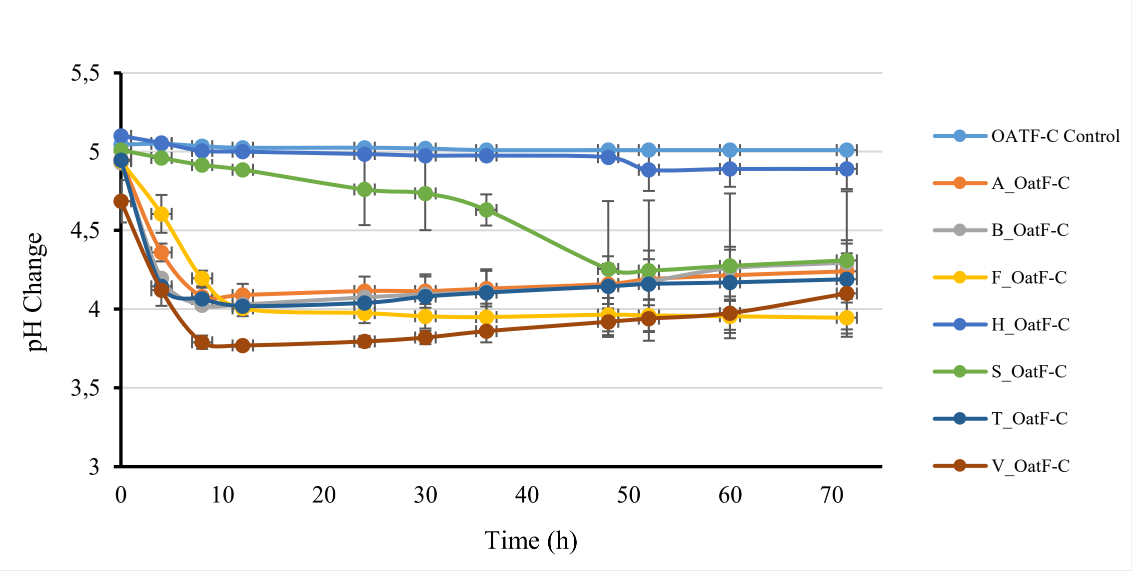

Figure 25. pH Change in OatF-C with selected strains at time points (Results are average ±SD of replicates, n=2)

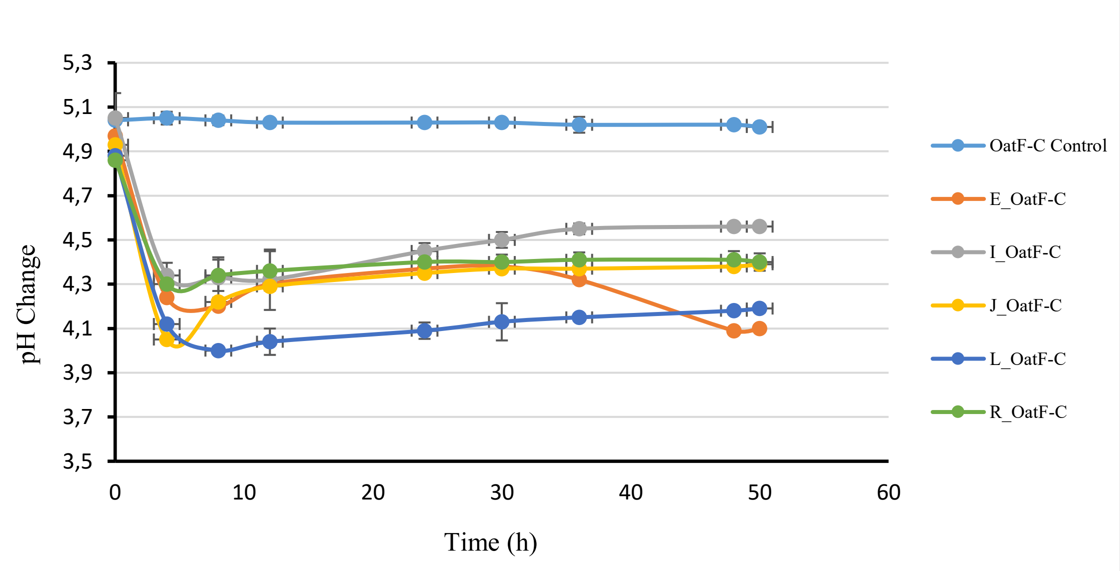

Figure 26. pH Change in OatF-C with selected strains at time points (Results are average ±SD of replicates, n=2)

All the strains used in OatF-C medium demonstrated a reduction in pH during fermentation except for the strain H, which was also the case in Faba-C medium. Typically, the strains exhibited the most effective pH reductions within the first 10 hours of fermentation. Strain F exhibited the lowest pH values and the highest pH decrease (0.995 units) from an initial pH of 4.94 (±0.01) to a final pH of 3.94 (±0.12) (Figure 25). The E strain resulted in a final pH of 4.1 (±0.01) with a reduction of 0.87 units (Figure 26). Similarly, strain V demonstrated good pH reductions (Figure 25) with a rapid decrease in the first 10 hours of fermentation but caused a slight increase in pH after 60th hour, ultimately providing a final pH of 4.10 (±0.25). Strain T closely followed strain V and provided a pH of 4.19 (±0.23). Consistent with the pH results strain F also presented the best bacterial growth with high OD values in OatF-C medium. This observation implies that a higher bacterial biomass could lead to a decrease in pH values.

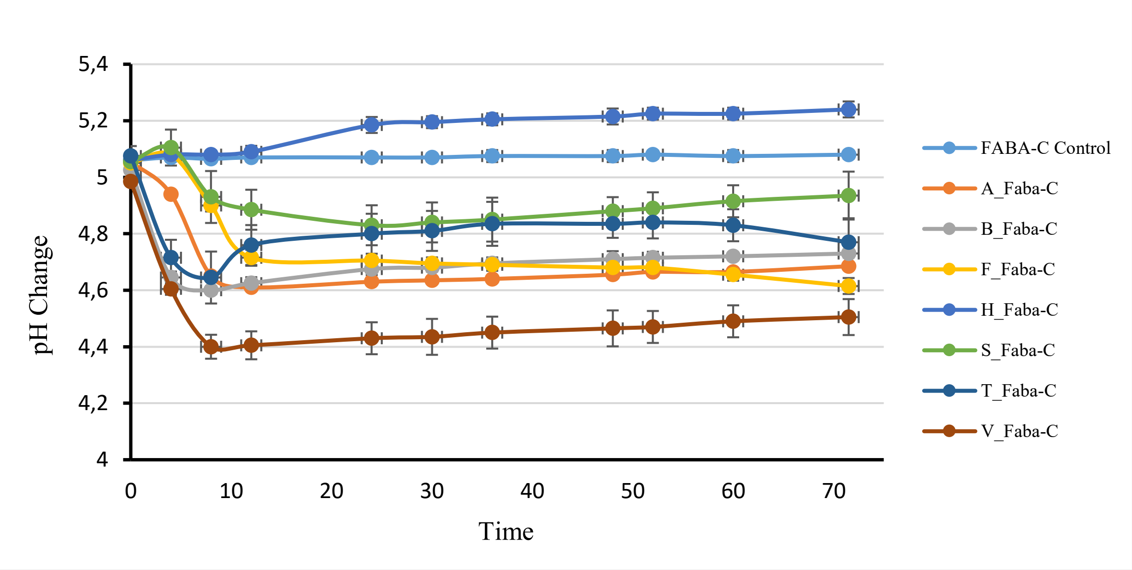

Figure 27. pH Change in Faba-C with selected strains at time points (Results are average ±SD of replicates, n=2)

It can be stated that all the strains used in Faba-C medium showed a slight decrease in pH during the fermentation process, except for strain H. There has been no drastic decrease observed in pH in Faba-C medium The strains demonstrated the most effective pH reduction during the initial 10 hours of fermentation. Among the strains, the lowest final pH of 4.5 (±0.06) was observed with strain V (Figure 27). Strain F also exhibited good pH reduction from 5.05 (±0.03) to 4.6 (±0.03). Consistent with the pH results, strain V showed the best bacterial growth and the highest biomass increase in Faba-C medium. This can be explained by the remarkably high OD of this V strain, which distinguishes it from other strains in Faba-C.

In a study conducted on bean fermentation, *Lb. helveticus*, *Lb. paracasei*, *Lb. plantarum*, *Lb. rhamnosus*, *Lb. acidophilus*, *Lb. casei* and *Lb. delbrueckii* subsp. *lactis* were the strains that provided a significant decrease in pH and were found to reduce it from the initial pH of 6.5-6.3 to the range of 4.7-3.7 (Ziarno et al, 2019). Similar low pH values of 4.31 have been reported in the fermentation of black beans using lactobacilli. In a study in which legume flours were fermented for 24 hours, final pH values between 4.0-4.4 were obtained (Curiel et al., 2015). In a study investigating the fermentation of faba protein rich fraction, the final pH value after 48 hours was reported as 4.6 (Coda et al., 2015). In this thesis, the initial pH values for Faba-C were in the range of 4.98-5.07. In the Biolector cultivation, after 72 hours of fermentation, the pH was found to be in the range of 4.50-5.24, close to the result of Coda et al. (2015). In a similar study, the final pH range was determined as 4.91-5.78 after 24 hours of fermentation in faba sourdough (Verni et al., 2017). It can be seen that the pH values obtained are close and similar to each other. Slightly higher pH values were obtained with the strains employed in this thesis. This result suggests that prolonged fermentation may lead to higher pH reductions.

Verni et al., 2017 found that the strains providing the least pH reduction were *P. pentosaceus* I76, I214, F77; *W. cibaria* F110; *Lb. sakei* F1410 and *Lc. lactis* F55. In this study, *Levilactobacillus brevis* and *Lacticaseibacillus paracasei* were found to be the least effective pH reducing strains in Faba-C. *P. pentosaceus* and *Lc. lactis subsp. lactis* were determined as the strains that caused the highest pH reduction. Verni et al (2017) similarly reported that *P. pentosaceus* F15 was the most effective strain and that other strains such as *W. koreensis* I06, I19, I148; *W. cibaria* F16, *Enterococcus* spp. F09; *P. pentosaceus* F15 and *Lb. sakei* F71 were amongst the strains that reduced pH. In the study of Rizello et al (2019), similar to this thesis, *P. pentosaceus* was the bacterium that caused the highest pH decrease in faba flour fermentation and decreased the pH from 6.19 to 4.48.

After 16 hours of fermentation, the final pH of oat products was found to be approximately 3.9 (Russo et al., 2016). In this thesis, the final pH values of OatF-F and OatF-C after 72 hours of fermentation were 4.11 (±0.14) and 3.94 (±0.12) after cultivation, respectively.

Angelov et al. (2005) obtained pH values below 4.5 with *Lb. paracasei* in oat-based drink after 8 hours of fermentation. Gupta et al., 2010 obtained pH values above 4.0 after 8 hours of fermentation. In a related study, higher acidification was reported in oat-based products after 16 hours of fermentation (Mårtensson et al., 2001). In a study where 24 h fermentation was applied to legume flours, final pH values between 4.0-4.4 were achieved (Curiel et al., 2015). In the light of the literature and the result obtained, it can be interpreted that prolonging the fermentation time in oat-based medium for a certain period of time has a positive effect on acidification. In the same study, it was reported that *Leu. mesenteroides* strain provided one of the highest pH decreases in non-dairy based medium (Mårtensson et al., 2001). In this thesis, the strain with the highest pH decrease was strain I (*W. confusa*) for OatF-F (Figure 22), strain F (*Lc. lactis* subsp. *lactis*) for OatF-C (Figure 25) and strain V (*P. pentosaceus*) for Faba-C (Figure 27).

In order to ensure microbiological safety and extend the shelf life of the product, it is crucial for the pH to rapidly decrease below 4.6. The findings of this study, conducted with the Biolector system, revealed that the most significant decrease in pH occurred during the initial 10 hours of fermentation. This early pH drop is of great importance in achieving the desired microbiological safety and stability of the product.

3.5 Scale-up fermentation

To gain a better understanding of the growth characteristics of certain strains, the Bioscreen experiment was increased in volume to 5 mL. In this experiment, both shaken and not shaken media were utilized and compared to each other. The four selected strains, namely A, L, N from the *Lactiplantibacillus plantarum* species, and R from the *Lacticaseibacillus paracasei* species, exhibited the most favorable growth performance during the Bioscreen experiment. These strains were consequently chosen for further scale-up fermentation. Samples were collected at specific time points, including 0, 4, 8, and 24 hours of overnight growth, and subjected to enumeration and pH measurements. This allowed for a comprehensive assessment of the strains' growth patterns and behavior under different conditions.

Combining OD measurements with plate counting can help validate and verify the accuracy of OD measurements. By performing colony forming unit assay in parallel, which directly measures viable cell counts, it can provide a reference point to confirm the accuracy of OD measurements and ensure the reliability of the data obtained (Beal et al., 2020). Colony forming unit can provide direct evidence of microbial growth by quantifying viable cells, whereas OD measurements provide an estimate of biomass based on light scattering. By combining both methods, it can be possible to confirm the growth dynamics of the microbial population and obtain a more robust understanding of their growth patterns over time.

Colony forming assay and pH measurements can provide additional information beyond OD measurements. Colony forming provides direct information on viable cell counts, which can be important for assessing cell viability. pH measurements can provide insights into changes in the environmental conditions during fermentation, which can affect microbial growth and metabolism.

Russo et al., 2016 reported the initial inoculation level as 8.9 log cfu/g for the functional food they aimed to produce using oat flour. Angelov et al., 2005 used initial inoculum levels of 7-8 log cfu/ml in their study to produce oat-based fermented drink with *Lb. plantarum* and *Lb. paracasei* strains. In this thesis, the initial inoculation levels were determined as 7.43 (±0.21) – 8.36 (±0.35) for this fermentation.

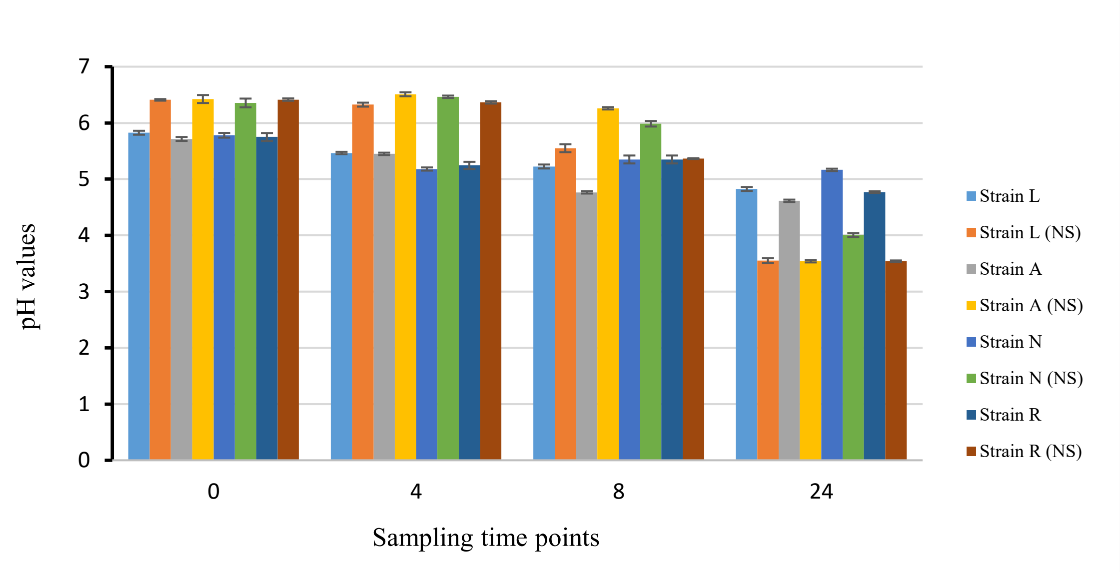

Figure 28. pH variations in OatF-F 3.3 with and without overnight shaking (NS) with selected strains at time points (Results are average ±SD of replicates, n=2)

There was a statistically significant difference in pH values obtained with the strains inoculated in the OatF-F media that was incubated overnight with shaking compared to the OatF-F media that was not incubated overnight with shaking ($p<0.05$).

During the first 8 hours of fermentation in the OatF-F medium, higher pH values were observed in the medium that was not incubated overnight with shaking. However, after overnight fermentation, the pH values in the medium not incubated overnight with shaking showed a drastic decrease and were significantly lower than those in the medium incubated overnight with shaking. The lowest pH value was monitored with the pH value of 3.5 (±0.02) in the OatF-F without incubated shaking overnight at the end of the fermentation (Figure 28). Overall, at the end of the fermentation the pH values ranged between 5.1 (±0.02) - 3.5 (±0.01).

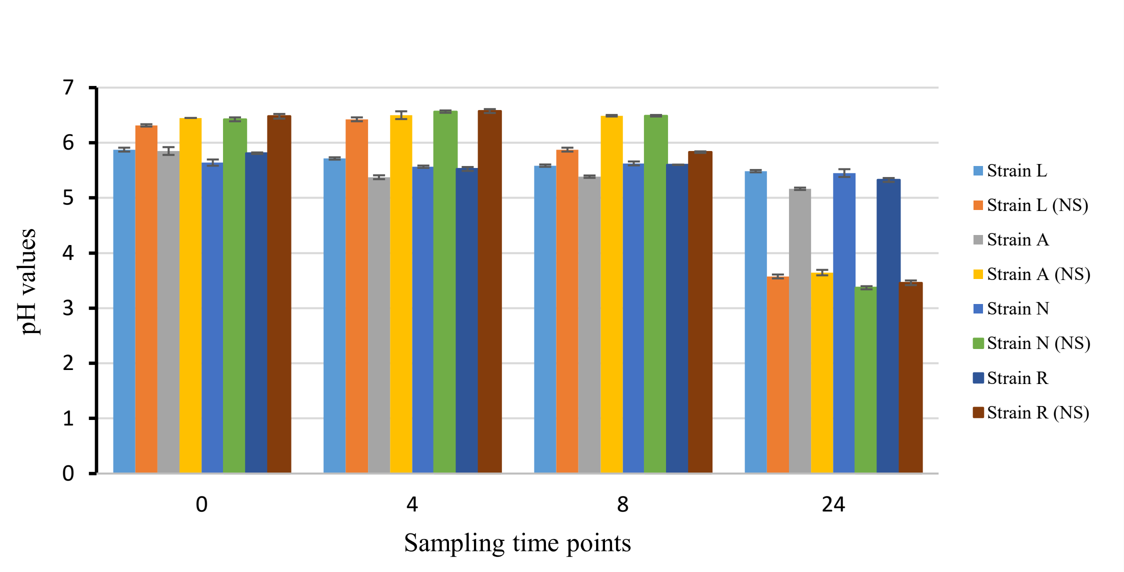

Figure 29. pH variations in OatF-C with and without overnight shaking (NS) with selected strains at time points (Results are average ±SD of replicates, n=2)

The pH values obtained from strains inoculated in the OatF-C media incubated overnight with shaking were significantly different from those obtained from the same media that was not incubated overnight with shaking ($p<0.05$). In OatF-C media, a similar trend to OatF-F was observed where the lowest pH value of 3.4 (±0.03) was obtained in the media that was not incubated overnight with strain N. Overall, lower pH values were obtained in OatF-C media without incubated overnight with shaking compared to other media, with pH values ranging from 3.64 (±0.05) to 3.4 (±0.03). In general, the final pH values in OatF-C both with and without shaking overnight was found ranging from 5.5 (±0.02) to 3.4 (±0.03) at the end of the fermentation (Figure 29).

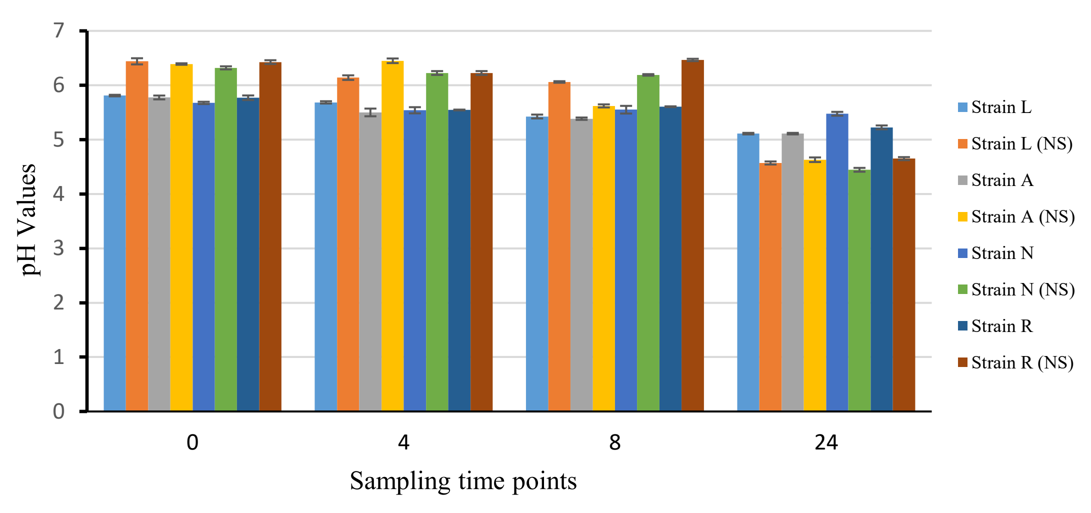

Figure 30. pH variations in Faba-C with and without overnight shaking (NS) with selected strains at time points (Results are average ±SD of replicates)

The pH values of strains grown in OatF-C media under shaking overnight were significantly different from those grown in the same media without shaking overnight ($p<0.05$). A similar trend was observed in Faba-C, but the decrease in pH was less and slower in Faba-C medium. The lowest pH obtained was 4.4 (±0.03) observed in Faba-C medium obtained with strain N and not incubated overnight with shaking (Figure 30). In general, the final pH values in Faba-C for both with and without shaking overnight was found ranging from 5.5 (±0.02) to 4.4 (±0.03) at the end of the fermentation.

Table 5. pH changes in OatF-F with and without overnight shaking (NS) with selected strains

Time	Strain L (±SD) n=2	Strain L (NS) (±SD) n=2	Strain A (±SD) n=2	Strain A (NS) (±SD) n=2	Strain N(±SD) n=2	Strain N (NS) (±SD) n=2	Strain R (±SD) n=2	Strain R (NS) (±SD) n=2
0	5.8±0.03	6.4±0.01	5.7±0.03	6.4±0.07	5.8±0.04	6.3±0.08	5.7±0.07	6.4±0.02
4	5.5±0.02	6.3±0.03	5.4±0.02	6.5±0.03	5.1±0.03	6.5±0.02	5.2±0.06	6.4±0.02
8	5.2±0.03	5.5±0.07	4.8±0.02	6.3±0.02	5.3±0.07	6.0±0.05	5.3±0.07	5.4±0.01
24	4.8±0.03	3.5±0.04	4.6±0.02	3.5±0.02	5.1±0.02	4.0±0.03	4.8±0.01	3.5±0.01

Table 6. pH changes in OatF-C with and without overnight shaking (NS) with selected strains

Time	Strain L (±SD) n=2	Strain L (NS) (±SD) n=2	Strain A (±SD) n=2	Strain A (NS) (±SD) n=2	Strain N(±SD) n=2	Strain N (NS) (±SD) n=2	Strain R (±SD) n=2	Strain R (NS) (±SD) n=2
0	5.9±0.03	6.3±0.02	5.8±0.07	6.5±0.00	5.6±0.06	6.4±0.03	5.8±0.01	6.5±0.04
4	5.7±0.02	6.4±0.03	5.4±0.03	6.5±0.07	5.6±0.02	6.6±0.02	5.5±0.03	6.6±0.03
8	5.6±0.02	5.9±0.03	5.4±0.02	6.5±0.01	5.6±0.03	6.5±0.01	5.6±0.00	5.8±0.01
24	5.5±0.02	3.6±0.03	5.2±0.02	3.6±0.05	5.4±0.07	3.4±0.03	5.3±0.03	3.5±0.04

Table 7. pH changes in Faba-C with and without overnight shaking (NS) with selected strains

Time	Strain L (±SD) n=2	Strain L (NS) (±SD) n=2	Strain A (±SD) n=2	Strain A (NS) (±SD) n=2	Strain N (±SD) n=2	Strain N (NS) (±SD) n=2	Strain R (±SD) n=2	Strain R (NS) (±SD) n=2
0	5.8±0.01	6.4±0.06	5.8±0.03	6.4±0.01	5.7±0.02	6.3±0.03	5.8±0.04	6.4±0.03
4	5.7±0.02	6.1±0.04	5.5±0.07	6.4±0.04	5.5±0.06	6.2±0.03	5.5±0.01	6.2±0.03
8	5.4±0.03	6.1±0.01	5.4±0.02	5.6±0.03	5.5±0.07	6.2±0.01	5.6±0.01	6.5±0.02
24	5.1±0.01	4.6±0.03	5.1±0.01	4.6±0.04	5.5±0.03	4.4±0.03	5.2±0.03	4.6±0.03

Mårtensson et al., 2001 reported that 6 of the 9 strains used in their study for fermenting non-dairy media achieved a final pH of 4.5 or less after 24 hours of fermentation. In the scale-up fermentation trial, a 24-hour fermentation process was carried out. In one study, the pH range of faba sourdough after 24 hours of fermentation was 4.91-5.78 (Verni et al., 2017). It can be seen that the pH values obtained are close and similar to each other. After 24 hours of fermentation, the lowest pH values were 4.6 (±0.02), 5.2 (±0.02) and 5.1 (±0.01) in OatF-F, OatF-C and Faba-C media incubated overnight with shaking, respectively.

However, lower pH values could be obtained in the scale-up experiment with the medium that was not incubated overnight with shaking. In this experiment, the lowest pH values obtained for OatF-F, OatF-C and Faba-C were 3.5 (±0.02), 3.4 (±0.03) and 4.4 (±0.03), respectively. The lower final pH values after fermentation may be due to several factors. In the scale-up experiment with media that were not shaken overnight, the initial pH of the media was found to be higher, between 6.3 and 6.5, although it was set to 5.9 during media preparation. This initial pH might be more favorable for some strains. Moreover, shaking promotes aeration and increases oxygen availability in the medium. LAB can ferment sugars both in the presence and absence of oxygen, but they tend to produce more lactic acid in the absence of oxygen. If the medium was not shaken, reduced oxygen availability could have created anaerobic conditions that favored lactic acid production and resulted in a higher decrease in pH.

In one study, the *Leu. mesenteroides* strain achieved one of the highest pH reductions in non-dairy based medium (Mårtensson et al., 2001). Among the strains employed in the scale-up experiment, strain A was found to be the most effective in reducing pH in OatF-F and OatF-C (in the medium incubated overnight with shaking), and strains A and L in

Faba-C. Both strain A and L were found to be belonging to the species *Lactiplantibacillus plantarum.*

Table 8. Plate count analysis results of selected strains in the media incubated overnight with shaking

Strain	Media	Initial count*	4 h count*	8 h count*	24 h count*
L	OatF-F	8.2±0.02	5.4±0.07	5.5±0.67	6.5±0.03
	OatF-C	8.1±0.03	5.6±0.07	6.1±0.00	6.2±0.13
	Faba-C	8.1±0.12	5.5±0.09	6.3±0.07	6.2±0.13
A	OatF-F	7.9±0.08	5.8±0.09	5.7±0.12	6.2±0.05
	OatF-C	7.8±0.34	4.8±0.14	4.9±0.08	6.3±0.02
	Faba-C	7.4±0.21	4.5±0.28	5.4±0.04	5.6±0.07
N	OatF-F	7.7±0.17	4.5±0.28	4.9±0.08	6.4±0.02
	OatF-C	7.7±0.00	4.8±0.21	5.9±0.08	6.3±0.01
	Faba-C	7.8±0.00	4.8±0.80	5.3±0.75	6.1±0.04
R	OatF-F	8.2±0.00	5.5±0.02	5.4±0.04	5.7±0.12
	OatF-C	8.4±0.05	5.5±0.01	5.5±0.04	5.6±0.00
	Faba-C	8.2±0.02	5.4±0.05	5.5±0.02	5.6±0.07

*log cfu/ml

Table 9. Plate count analysis results of selected strains in the media without incubated overnight with shaking

Strain	Media	Initial count*	4 h count*	8 h count*	24 h count*
L_NS**	OatF-F	7.3±0.06	7.8±0.05	8.4±0.57	8.2±0.11
	OatF-C	7.4±0.06	8.3±0.75	8.7±0.01	8.4±0.00
	Faba-C	7.4±0.08	7.9±0.07	9.0±0.11	10.4±0.14
A_NS**	OatF-F	6.7±0.00	7.3±0.02	8.2±0.02	10.0±0.00
	OatF-C	6.6±0.07	7.2±0.04	8.2±0.02	10.1±0.10
	Faba-C	6.6±0.07	6.0±0.00	8.2±0.02	10.3±0.02
N_NS**	OatF-F	6.4±0.08	6.9±0.12	8.0±0.09	10.2±0.02
	OatF-C	6.4±0.13	6.6±0.07	8.0±0.12	10.1±0.06
	Faba-C	6.4±0.13	6.8±0.10	7.9±0.34	10.3±0.05
R_NS**	OatF-F	8.0±0.09	8.8±0.00	8.9±0.04	10.0±0.00
	OatF-C	8.0±0.06	8.3±0.00	8.5±0.00	9.4±0.04
	Faba-C	8.0±0.03	8.4±0.03	8.6±0.07	10.0±0.11

*log cfu/ml
**In the medium without incubated overnight with shaking

During fermentation with selected strains in the medium that was incubated overnight with shaking, the inoculation levels varied between 7.4 (±0.21) and 8.4 (±0.35). For OatF-F, the inoculation levels ranged from 7.7 (±0.17) to 8.2 (±0.00), for OatF-C they ranged from 7.7 (±0.00) to 8.4 (±0.35), and for Faba-C medium they varied from 7.4 (±0.21) to 8.2 (±0.02).

During fermentation in the medium incubated overnight with shaking, a reduction in bacterial population was noted, particularly within the first 4 hours of fermentation. Although after 8 hours the bacterial population started to increase, it did not recover to the initial levels. Evaluation of pH changes in parallel with bacterial counts revealed that the pH data were in agreement with the bacterial counts. As the bacterial population did not increase, the decline in pH was not substantial.

The highest decrease in pH was obtained with strain A in OatF-F medium, and the final pH level was 4.6 (±0.02), which decreased from 5.7 (±0.03). Similarly, in OatF-C medium, the highest decrease was observed with strain A, which decreased the initial pH value of 5.8 (±0.07) to a final pH level of 5.2 (±0.02). In Faba-C medium, the highest decrease was achieved with strains L and A, both of which caused the pH to reach the final value of 5.1 from the initial value of 5.8.

In the medium that was not incubated overnight with shaking, inoculation levels ranged from 6.4 (±0.13) log cfu/ml to 8.04 (±0.06) log cfu/ml, depending on the type of medium. The OatF-F medium had inoculation levels ranging from 6.4 (±0.08) to 8.0 (±0.09) log cfu/ml, OatF-C medium had inoculation levels ranging from 6.4 ± 0.13 to 8.0 (±0.06) log cfu/ml, and Faba-C medium had inoculation levels ranging from 6.6 (±0.07) to 8.0 (±0.03) log cfu/ml. All media showed an increase in bacterial counts during fermentation compared to the initial inoculation levels. After fermentation, the lowest bacterial count was observed with strain L in OatF-F medium at 8.2 (±0.11) log cfu/ml, while the highest count was observed with strain L in Faba-C medium at 10.4 (±0.14) log cfu/ml. Bacterial counts increased after the 8th hour of fermentation. The highest bacterial counts were observed in the fermentation process when using strain L in the Faba-C medium and strain N in the OatF-C medium. In the case of OatF-F medium, the highest bacterial count was found with strain L in the media subjected to overnight shaking, while strain N exhibited the highest count in the non-shaking media. When pH changes during fermentation were considered, a significant decrease in pH was observed in parallel with the increase in bacterial counts. The lowest pH of 3.4 (±0.03) was achieved with strain L in OatF-C medium. The highest decrease in pH was observed in OatF-F medium with a decrease of 2.9 units with strains A, L, and R. In OatF-C medium, the highest decrease in pH was 3 units with strains N and R, but the lowest final pH value of 3.4 (±0.03) was achieved with strain N. In Faba-C medium, the highest decrease of 1.9 units was also observed with strain N, and the final pH value achieved was 4.4 (±0.03).

Generally, it has been determined that the selected strains in the media that were not incubated overnight with shaking showed better growth and an increase in counts during fermentation. Consistent with this, it has been determined that pH decreases were more noticeable in these media. Lower final pH counts were observed after fermentation.

3.6 Characterization of Sugar Compositions in Fermented Samples Post Biolector Cultivation

Rhamnose was not detected in any of the samples at a concentration above the limit of detection (n.a.). This means that the concentration of rhamnose in the sample is below the level at which it can be reliably detected and quantified using the methods used in the experiment.

In OatF-F 3.3 medium, raffinose was not detected in the control sample or in the samples after fermentation using the selected strains. However, in the OatF-F 3.3 medium, an increase in raffinose concentration was observed following fermentation using only J strain (*Lc. lactis* subsp. *lactis*), with a detected level of 0.25 g/L. The OatF-F 7.3 medium, which was prepared from the same raw material but observed to be more turbid, had a detected raffinose concentration of 0.96 g/L in the control sample. Reductions in raffinose concentration of 61.99%, 67.88%, and 90.74% were observed for L (*Lactiplantibacillus plantarum*), J (*Lc. lactis subsp. lactis*), and R (*Lacticaseibacillus paracasei*) strains, respectively. The highest decrease in raffinose concentration was obtained in the OatF-F 7.3 medium using the R strain (*Lacticaseibacillus paracasei*) (final concentration of 0.09 g/L). In the OatF-C medium, the raffinose concentration in the control sample was relatively low at 0.74 g/L, while complete degradation was achieved with the J (*Lc. lactis subsp. lactis*), L (*Lactiplantibacillus plantarum*), F (*Lc. lactis subsp. lactis*), S (*Levilactobacillus brevis*), T (*Leu. mesenteroides* subsp. *mesenteroides*), and V (*P. pentosaceus*) strains. A reduction of 92.24% was achieved with the R strain. The utilization of raffinose by the employed strains was observed to be more effective in the OatF-C medium. Rizzello et al. (2010) reported a 45% decrease in raffinose concentration in wheat germ sourdough fermented with *Lb. plantarum* compared to raw wheat germ. In a study examining the fermentation of wheat, barley, and quinoa, complete hydrolysis of raffinose was achieved with a lactic acid bacterial consortium (Montemurro et al., 2019). According to Teixeira et al. (2012), raffinose, an α-galactoside of sucrose, can be found in legumes at varying concentrations. In this study, interestingly, no raffinose was detected in the control Faba-C medium or in any sample following fermentation using the selected strains. It is possible that the raffinose concentration in the Faba-C medium was below the detection limit of the equipment or that the preparation of the Faba-C medium with overnight incubation and shaking may have caused raffinose degradation during the incubation period due to endogenous enzymes or bacteria in the faba bean protein concentrate. However, in a study where 27 LAB were employed for faba bean fermentation, a reduction in raffinose concentration from an initial concentration of 0.85 g/kg to 0.50 g/kg was reported in faba bean dough with the use of *Leu. mesenteroides* strain, which resulted in the highest reduction (Verni et al., 2017). In a similar study examining soybean and faba bean fermentation using *Leu. mesenteroides*, it was found that raffinose fell below the detection limit (Xu et al., 2017a). In another study by the same researchers, only faba bean flour fermentation was performed using *Leuconostoc* sp. and *Weisella* sp., and complete degradation of raffinose was reported (Xu et al., 2017b). Similarly, Rizzello et al. (2019) found total hydrolysis of raffinose after fermentation of faba bean with *Weisella*, *Pediococcus*, and *Leuconostoc* sp. Lower decreases in raffinose concentration were obtained by Adeyemo and Onilude (2014). A 28% decrease in raffinose concentration was achieved after traditional fermentation of soybean with *Lb. plantarum*. In a study employing yogurt starter cultures for white bean seed fermentation, the raffinose concentration was found to be less than other studies with a 17% decrease (Ziarno et al., 2019). In a study examining the effect of fermentation of different types of beans on FODMAPs, the highest reductions in raffinose content were found in chickpea and pea flours with 64% and 57.2% reductions, respectively, after fermentation with *Lb. plantarum* and *Lb. brevis* (Curiel et al., 2015). Fermentation of traditional African legumes with *Lb. plantarum*, *Lb. fermentum*, and *Pediococcus acidilactici* achieved reductions of up to 79% in raffinose concentration (Adewumi and Odunfa, 2009). In another study examining soybean fermentation, a raffinose concentration of 0.23 g/L was found after fermentation (Battissini et al., 2018). In this thesis, raffinose was detected at a concentration of 0.25 g/L in oat-based medium OatF-F 3.3 with strain J. Conversely, in a study examining the fermentation of Australian legumes, Kaczmarska et al. (2017) found a 275% increase in raffinose content in lupin.

In this thesis, sucrose was found at a very low concentration (0.05 g/L) in the OatF-F 7.3 medium, and complete degradation was achieved after fermentation with the L (*Lactiplantibacillus plantarum*) strain. Lower reductions were obtained with the J strain (*Lc. lactis subsp. lactis*), resulting in a 24.46% decrease after fermentation. On the contrary, the R strain (*Lacticaseibacillus paracasei*) showed a 268% increase in sucrose concentration after fermentation. Similarly, sucrose concentration was determined to be 0.03 g/L in the OatF-F 3.3 medium, and a significant increase in sucrose concentration was observed with the R strain (final concentration of 0.88 g/L), while a 91% increase was observed with the J strain. A slight increase of 2% was observed with the A strain (*Lactiplantibacillus plantarum*). Similar results were also reported by Adewumi and Odunfa (2009), where a significant increase in sucrose content was observed after fermentation with *Lb. plantarum* at 24, 48, and 72 hours. This study also observed reductions in sucrose concentration in the OatF-F 3.3 medium, where complete hydrolysis was achieved with the L strain, and a 53.44% reduction was observed with the B strain (*Lactiplantibacillus plantarum*). In wheat germ fermentation, lower reductions of 35.97% were reported (Rizzello et al., 2010). The highest sucrose concentration was found in the OatF-C medium, with a concentration of 2.29 g/L, and more effective reductions were achieved with the strains used for fermentation compared to other media. Complete degradation was achieved with the A (*Lactiplantibacillus plantarum*) and R strains (*Lacticaseibacillus paracasei*), while near-complete reductions were achieved with the T (*Leu. mesenteroides* subsp. *mesenteroides*), J (*Lc. lactis subsp. lactis*), and L strains (*Lactiplantibacillus plantarum*) at rates of 99.33%, 99.12%, and 99.08%, respectively. A lower reduction rate of 59.81% was observed with the H strain (*Levilactobacillus brevis*). According to Blandino et al. (2003), LAB utilize sucrose as an energy source during the fermentation process, resulting in a reduction in sucrose concentration.

In this thesis, it was determined that the sucrose concentration in the control sample of Faba-C medium was 0.80 g/L. Complete degradation was achieved with strain A (*Lactiplantibacillus plantarum*) at the sucrose concentration. Results close to complete degradation were obtained with strains T (*Leu. mesenteroides* subsp. *mesenteroides*) and B (*Lactiplantibacillus paraplantarum*) at 98.38% and 98.30%, respectively. However, an increase of 43.21% was observed in sucrose content in the Faba-C medium with strain S (Levilactobacillus brevis). This increase in sucrose can be explained by the enzymatic hydrolysis of oligosaccharides such as raffinose and sucrose (Verni et al., 2017). In another study, it was suggested that the breakdown of stachyose and raffinose was due to the α-galactosidase secreted by LAB (Adewumi and Odunfa, 2009). Lower reduction rates than those in this thesis study were reported by Rizzello et al. (2019), who used *P. pentosaceus* to ferment fava bean dough, resulting in a 44% decrease. After the fermentation of soy flour, a 54% reduction in sucrose concentration was reported (Kaczmarska et al., 2017). Similarly, lupin fermentation resulted in a decrease of 14% or less (Kaczmarska et al., 2017). Higher reductions were reported by Granito and Alvarez (2006), who found an 86.34% reduction in sucrose concentration during the fermentation of black beans by LAB. A similar result was obtained with the fermentation of pigeon pea flour, resulting in a 91% reduction in sucrose (Torres et al., 2006). Complete degradation has also been achieved in other studies. Xu et al. (2017a) reported that sucrose was undetectable in the doughs after the fermentation of soybeans and fava beans with *Leu. mesenteroides*. In another study by the same researcher, similar results were obtained, and it was reported that sucrose was utilized to different extents during fermentation. In fermented fava bean doughs, sucrose was not detected after fermentation (Xu et al., 2017b). An increase of 32% was detected in sucrose concentration after the fermentation of raw soybeans with *Lb. plantarum* (Adeyemo and Odunfa, 2014), similar to the 43.21% increase in sucrose content observed in this thesis study.

In the OatF-F 7.3 medium, glucose was detected at a concentration of 0.92 g/L. Following fermentation with strain L (*Lactiplantibacillus plantarum*), 64.40% glucose degradation was achieved, followed by strain J (*Lc. lactis subsp. lactis*) with a 58.41% decrease. Strain R (*Lacticaseibacillus paracasei*) resulted in lower degradation, with a decrease of 16.43%. In the control OatF-F 3.3 medium, a lower glucose concentration was detected at 0.05 g/L. Complete degradation of glucose was achieved with strain L, while dramatic increases were observed after fermentation with strains R and J (*Lc. lactis subsp. lactis*) (7065% and 814%, respectively). Similar increases of 750% in glucose concentration were reported during natural fermentation of beans (Granito et al., 2002). Following fermentation with *Lb. casei*, the glucose concentration in wheat sourdough was found to be 0.20 g/100g dm, whereas no glucose was detected in unfermented sourdough. A lower amount of glucose was also detected after fermentation with *Lb. plantarum*, at 0.05 g/100g dm (Pejcz et al., 2021). A 61% increase in glucose concentration was reported after fermentation of raw soybeans with *Lb. plantarum*, although at a lower value. In another study, glucose levels for lupin and soy were reported to increase from 0.7 and 0.4 mg/g to 15 mg/g and 13 mg/g, respectively, after fermentation (Kaczmarska et al., 2017). The increase in glucose levels could be attributed to damaged starch and the activity of amylose and amiloglucosidase (Gänzle, 2014).

In OatF-C medium, glucose levels were determined to be 0.86 g/L, which is close to the value obtained for the control of OatF-F 7.3 medium. Although complete degradation was not achieved with the selected strains, degradation levels close to complete degradation were obtained. The highest degradation was achieved with the S strain (*Levilactobacillus* *brevis*), with 94.85%. This was followed by the V strain (*P. pentosaceus*) with 93.82% degradation. Additionally, the R (*Lacticaseibacillus paracasei*) and J strains (*Lc. lactis subsp. lactis*) provided hydrolysis at rates of 93.43% and 92.70%, respectively. Overall, it appears that the strains were more effective in hydrolyzing glucose in the OatF-C medium and achieving reductions in concentration. In a study by Rizzello et al. (2019), glucose was not detected after fermentation. In a study on wheat germ fermented with Lb. plantarum, it was reported that glucose concentration decreased by 51% after fermentation (Rizzello et al., 2010).

In Faba-C medium, glucose was detected at a concentration of 1.49 g/L under control conditions. Generally, an increase in glucose levels was observed in Faba-C medium after fermentation with many strains. However, complete degradation of glucose was achieved with A strain (*Lactiplantibacillus plantarum*), and near-complete degradation was achieved with V strain (*P. pentosaceus*) with a 95.13% reduction. On the other hand, T (*Leu. mesenteroides* subsp. *mesenteroides*) and S strains (*Levilactobacillus brevis*) resulted in an increase in glucose concentration of 60.92% and 43.97%, respectively, after fermentation in Faba-C medium. Xu et al. (2017a) detected varying concentrations of glucose after fermentation of faba bean doughs. The highest glucose concentration was 1.19% in faba bean dough supplemented with 15% sucrose. Granito and Alvarez (2006) reported a significant increase in glucose concentration by 51.43% in black bean fermentation with LAB. This increase in glucose concentration could be due to the release of glucose and fructose from sucrose, consumption of other sugars by LAB in the media, and production of lactate, which increases the pH of the media and leads to the hydrolysis of sucrose into glucose and fructose. In contrast to these results, complete hydrolysis or reduction was achieved in other studies after fermentation. Xu et al. (2017b) reported that glucose was not detected after fermentation of faba bean dough. Similarly, Verni et al. (2017) reported that the LAB they used in their study had the ability to utilize glucose in faba bean.

4 Conclusion

The objective of this thesis was to screen available food fermentation LAB for optimum fermentation potentials and health promoting effects. The aim was to evaluate the fermentation performance, growth kinetics, acidification, anti-nutritional factors and FODMAPs as individual strains and consortium using a high throughput microreactor.

The screening of LAB strains in OatF-F, OatF-C, and Faba-C media using Bioscreen® provided valuable information regarding their growth patterns and enzymatic activities. The results revealed that specific strains exhibited superior growth and enzymatic capabilities, highlighting their suitability for different media compositions. In OatF-F medium, the F-coded strain belonging to *Lc. lactis subsp. lactis* species showed the most robust bacterial growth, indicating its potential as a favorable strain for this medium. *Lactiplantibacillus plantarum* strains (coded as A, L, and N) also exhibited strong growth, making them suitable candidates for OatF-F medium. Similarly, in OatF-C and Faba-C media, *Lactiplantibacillus plantarum* exhibited the highest bacterial growth compared to other strains.

The experiment comparing incubation with shaking overnight and without shaking overnight provided valuable insights into the impact of the preparation step on nutrient release and growth kinetics of bacterial strains. In OatF-C medium, strain N consistently exhibited the highest bacterial growth, regardless of whether the medium was incubated overnight with shaking or not. In Faba-C medium without overnight shaking, the selected bacterial strains demonstrated significant growth, with many of them reaching final OD values exceeding 2.4 units.

The utilization of R Studio programming for the calculation of bacterial growth rates has provided valuable quantitative insights into the dynamics of bacterial growth and proliferation. The growth rate serves as a crucial parameter in microbiology research, indicating the speed at which bacterial populations multiply over time. It is important to note that as a result from this study high growth rates do not necessarily guarantee optimal growth in a specific medium.

The shaking process during overnight incubation affected the properties of the medium and potentially influenced bacterial growth rates. The increased oxygen availability from shaking may be less favorable for LAB, which prefer low-oxygen environments. In contrast, the absence of shaking and lower oxygen availability seemed to create a more conducive environment for the growth of LAB. In addition, overnight incubation with shaking could stimulate increased enzymatic activities of indigenous bacteria due to enhanced metabolism. This could lead to the breakdown of certain compounds in the medium, altering the chemical composition and nutrient availability. The knowledge gained from this experiment can be valuable for future research for designing strategies to enhance bacterial growth in various applications.

The use of the Biolector instrument has offered valuable knowledge regarding the growth and fermentation behavior of LAB strains in media derived from cereals and legumes. In the OatF-C medium, the F strain (*Lc. lactis subsp. lactis*) exhibited the most substantial increase in biomass, while the V strain (*P. pentosaceus*) demonstrated the most significant growth in the Faba-C medium. These findings highlight the strain-specific responses to different media compositions. Regarding cell viability counts after fermentation, variations were observed across different studies and among strains and media. Some strains exhibited decreases in numbers at the end of the fermentation period, which is consistent with previous findings.

pH profiling experiments demonstrate that bacterial growth and biomass production do not necessarily correlate with the strains' ability to effectively reduce pH. *Lc. lactis subsp. lactis* exhibited the highest pH reduction in OatF-C. While in the Faba-C medium, the most effective pH reduction occurred during the initial 10 hours of fermentation with P. pentosaceus which also displayed the best bacterial growth and highest biomass increase. The findings underscore the need to consider strain characteristics and specific capabilities when designing fermentation processes aimed at pH reduction.

The scale-up fermentation experiment conducted in this study provided valuable observations into the growth characteristics and behavior of selected strains in various conditions. By increasing the volume of the Bioscreen experiment and comparing shaken and not shaken media, a comprehensive assessment of the strains' growth patterns was achieved. The results revealed significant differences in pH values between media incubated overnight with shaking and those not incubated overnight. Similar trends were observed across the OatF-F, OatF-C, and Faba-C media. The lower final pH values observed in the scale-up experiment in the media without overnight shaking. The most effective strains in reducing pH across all media were determined to be strains A and L, both of which were identified as members of the species *Lactiplantibacillus plantarum*. Furthermore, the highest bacterial counts were observed in the fermentation process when using strain L and N. Strain L along with the strain A and N, all belonging to

Lactiplantibacillus plantarum, demonstrated effective pH reduction and bacterial growth capabilities, indicating its potential suitability for scaling up fermentation processes and product development. However, additional experiments and research are required to assess the growth behavior in larger-scale fermentation and evaluate the sensory properties of the samples.

The findings from this thesis study indicate that the selected strains of LAB have the capability to alter the levels of raffinose, sucrose, and glucose in diverse media. The fermentation process showed varying effects on these sugars depending on the strain and the medium used. The OatF-C medium proved to be the most effective in raffinose degradation, with complete degradation achieved by several strains. Sucrose concentration was found to be relatively low in the OatF-F 7.3 and OatF-F 3.3 media, and reductions in sucrose concentration were achieved with most strains. Glucose concentrations varied in the different media. Reductions in glucose concentration were observed with most strains in the OatF-F 7.3 and OatF-F 3.3 media. The OatF-C medium resulted in more effective reductions in glucose concentration, with near-complete degradation achieved by several strains. The increase in glucose concentration observed with some strains in the Faba-C medium.

Overall, the findings from this thesis study provide a foundation for future research endeavors in the field of microbial fermentation. By investigating the underlying mechanisms, optimizing cultivation conditions, and exploring specific outcomes such as pH reduction and sugar metabolism, researchers can advance the knowledge of microbial behavior and contribute to the development of optimized fermentation strategies that enable the creation of innovative and high-quality plant-based food products.

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APPENDIX 1

Appendices

Medium compositions

Table 1. Growth media and solutions used in the study.

Medium	Typical formula	g/L	Manufacturer
de Man, Rogosa and Sharp Medium (MRS) MRS Agar	Peptone `Lab-Lemco' powder Yeast extract Glucose Sorbitan mono-oleate Dipotassium hydrogen phosphate Sodium acetate 3H2O Triammonium citrate Magnesium sulphate 7H2O Manganese sulphate 4H2O Agar	10.0 8.0 4.0 20.0 1 ml 2.0 5.0 2.0 0.2 0.05 10	Oxoid Ltd. CM 361/CM 359
de Man, Rogosa and Sharp Medium (MRS) MRS Broth pH 6.2 ± 0.2 @ 25°C	Peptone `Lab-Lemco' powder Yeast extract Glucose Sorbitan mono-oleate Dipotassium hydrogen phosphate Sodium acetate 3H2O Triammonium citrate Magnesium sulphate 7H2O Manganese sulphate 4H2O	10.0 8.0 4.0 20.0 1 (ml) 2.0 5.0 2.0 0.2 0.05	Oxoid Ltd. CM 361/CM 359
Legume and Cereal - based media pH 5.6-5.9 at at 25°C	Oat flour, oat protein concentrate or faba protein concentrate	100	-
Peptone saline diluent (PS) pH 7.0 +/- 0.2 at 25°C	Peptic digest of animal tissue Sodium chloride	1.0 8.0	Neogen

Growth kinetics of selected strains in cereal-legume based media

Table 1. Growth kinetics of selected strains in OatF-F 7.3 (turbid) calculated with R Studio

Strain code	Mean_r*	K*	t_mid**
A -2	0.843±0.16	0.131±0.01	3.292±0.22
A -3	1.151±0.19	0.134±0.01	4.026±0.19
B -2	1.723±0.16	0.136±0.02	2.820±0.16
B -3	1.404±0.77	0.091±0.005	-8.313±28.14
C -2	1.509±0.28	0.048±0.003	2.418±0.07
C -3	0.032±0.004	0.029±0.001	-4.598±8.07
D -2	0.551±0.03	0.272±0.01	5.641±0.15
D -3	0.486±0.03	0.255±0.01	9.613±0.22
E -2	0.347±0.69	0.037±0.06	-4.030±11.98
E -3	0.026±0.04	0.017±0.02	-1.385±19.01
F -2	0.612±0.42	0.195±0.12	3.819±2.41
F -3	0.816±0.06	0.155±0.01	7.713±0.16
G -2	1.150±0.83	0.083±0.03	3.363±3.89
G -3	0.784±0.58	0.048±0.03	2.566±1.93
H -2	1.253±0.04	0.181±0.03	4.061±0.09
H -3	0.863±0.01	0.149±0.003	8.679±0.05

Results are average ±SD of replicates, n=5

*K represents the carrying capacity of the bacterial population

**t_mid represents the time at which the bacterial population reaches its midpoint in terms of growth.

Table 2. Growth kinetics of selected strains in OatF-F 3.3 (clear) calculated with R Studio

Strain code	Mean_r	K*	t_mid**
M -2	0.347±0.69	0.037±0.01	-4.030±11.98
M -3	0.026±0.04	0.017±0.01	-13.853±19.01
J -2	1.724±0.16	0.136±0.02	2.820±0.16
J -3	1.404±0.77	0.092±0.005	-8.313±28.14
K -2	1.509±0.28	0.048±0.003	2.418±0.07
K -3	0.032±0.004	0.029±0.001	-45.978±8.07
P -2	1.150±0.83	0.083±0.01	3.363±3.89
P -3	0.784±0.58	0.048±0.01	2.566±0.22
I -2	0.843±0.16	0.131±0.06	3.292±0.22
I -3	1.151±0.19	0.134±0.02	4.026±0.19
N -2	0.612±0.42	0.195±0.12	3.819±2.41
N -3	0.816±0.06	0.155±0.01	7.713±0.16
R -2	1.253±0.04	0.181±0.03	4.061±0.09
R-3	0.863±0.01	0.149±0.03	8.679±0.05
L -2	0.551±0.03	0.272±0.03	5.641±0.09
L -3	0.486±0.03	0.255±0.003	9.613±0.22
V -2	0.137±0.08	0.037±0.01	-15.732±7.02
V -3	0.017±0.04	0.010±0.02	-0.717±1.30

Results are average ±SD of replicates, n=5
*K represents the carrying capacity of the bacterial population
**t_mid represents the time at which the bacterial population reaches its midpoint in terms of growth.

Table 3. Growth kinetics of selected strains in OatF-C calculated with R Studio

Strain	Mean_r	K*	t_mid**
A -2	0.242±0.06	0.355±0.02	13.085±1.05
A -3	0.392±0.05	0.260±0.02	14.502±0.65
AB -2	0.206±0.04	0.103±0.01	7.685±0.63
AB -3	0.260±0.04	0.091±0.01	9.779±0.91
B -2	0.106±0.01	0.248±0.01	17.248±1.03
B -3	0.269±0.02	0.248±0.02	14.358±0.24
C -2	0.356±0.16	0.146±0.14	12.648±17.03
C -3	0.408±0.013	0.051±0.002	7.263±0.22
G -2	0.890±0.1	0.015±0.001	7.016±0.22
G -3	0.664±0.03	0.023±0.001	12.391±0.1
H -2	22.273±13.45	0.009±0.001	-0.197±0.24
H -3	0.547±0.74	0.008±0.001	3.541±8.77
F -2	0.245±0.02	0.202±0.003	9.699±0.26
F -3	0.178±0.04	0.164±0.01	15.566±1.45
M -2	0.158±0.04	0.134±0.02	15.954±2.36
M -3	0.084±0.02	0.048±0.01	29.540±5.35
N -2	0.296±0.05	0.463±0.04	12.636±1.15
N -3	0.376±0.04	0.435±0.01	17.352±0.57
S -2	0.102±0.02	0.108±0.09	24.064±4.83
S -3	0.114±0.06	0.099±0.03	28.519±7.82
T -2	0.576±0.01	0.268±0.004	10.096±0.03
T -3	0.574±0.04	0.230±0.01	12.182±0.12
V -2	0.130±0.08	0.306±0.04	13.414±5.97
V -3	0.152±0.02	0.244±0.01	14.863±2.18
D -2	0.160±0.01	0.151±0.002	9.249±0.23
D -3	0.118±0.01	0.128±0.01	16.527±0.77
E -2	0.338±0.1	0.267±0.02	19.307±0.96
E -3	0.666±0.05	0.311±0.01	19.361±0.78
P -2	0.586±0.08	0.169±0.01	17.418±0.25
P -3	0.297±0.13	0.233±0.04	22.674±4.15
R -2	0.772±0.02	0.207±0.002	4.782±0.04
R -3	0.355±0.02	0.270±0.01	11.631±0.28

Table 3. Growth kinetics of selected strains in OatF-C calculated with R Studio (continue)

Strain	Mean_r	K*	t_mid**
Y -2	0.025±0.04	0.025±0.05	2.685±28.94
Y -3	0.033±0.07	0.016±0.03	8.111±18.14
Z -2	0.887±0.02	0.203±0.01	7.630±0.09
Z -3	0.451±0.04	0.260±0.03	11.326±0.42

Results are average ±SD of replicates, n=5
*K represents the carrying capacity of the bacterial population
**t_mid represents the time at which the bacterial population reaches its midpoint in terms of growth.

Table 4. Growth kinetics of selected strains in Faba-C calculated with R Studio

Strain	Mean_r	K	t_mid
A -2	0.445±0.06	0.580±0.05	25.787±0.26
A -3	0.413±0.10	0.468±0.09	31.712±1.17
B -2	0.371±0.03	0.497±0.02	25.829±0.55
B -3	0.327±0.02	0.460±0.02	30.712±0.73
C -2	0.205±0.03	0.302±0.08	18.191±1.5
C -3	0.432±0.25	0.072±0.08	12.621±5.96
D -2	0.418±0.02	0.202±0.01	13.139±0.09
D -3	0.304±0.03	0.208±0.02	18.999±0.52
E -2	0.223±0.06	0.213±0.04	57.929±1.26
E -3	0.314±0.21	0.138±0.1	40.616±23.00
F -2	0.867±0.05	0.323±0.01	11.058±0.08
F -3	1.091±0.01	0.269±0.01	12.804±0.04
G -2	0.139±0.01	0.273±0.01	44.441±1.07
G -3	0.183±0.03	0.132±0.01	43.604±3.35
H -2	0.386±0.01	0.113±0.003	16.242±0.03
H -3	0.188±0.07	0.129±0.19	32.467±22.93
I -2	0.592±0.04	0.217±0.003	7.904±0.07
I -3	0.475±0.05	0.199±0.01	9.443±0.08
J -2	0.300±0.004	0.346±0.01	21.222±0.19
J -3	0.585±0.03	0.167±0.01	22.494±0.22
K -2	0.111±0.05	0.268±0.12	18.868±13.95
K -3	0.167±0.08	0.064±0.01	13.090±2.72
L -2	0.468±0.03	0.737±0.02	9.502±0.25
L -3	0.363±0.01	0.585±0.02	14.644±0.12
M -2	0.173±0.01	0.134±0.01	14.810±0.74
M -3	0.228±0.01	0.036±0.001	23.639±0.51
N -2	0.634±0.04	0.708±0.02	14.140±0.24
N -3	0.502±0.02	0.622±0.01	19.192±0.22

Table 4. Growth kinetics of selected strains in Faba-C calculated with R Studio (continue)

Strain	Mean_r	K*	t_mid**
P -2	0.175±0.07	0.274±0.06	27.161±4.47
P -3	0.523±0.26	0.215±0.07	24.143±2.83
R -2	0.310±0.01	0.618±0.02	9.581±0.15
R -3	0.240±0.005	0.506±0.01	18.124±0.17
V -2	0.199±0.14	0.226±0.02	9.908±4.07
V -3	0.225±0.03	0.135±0.01	16.479±2.65

Results are average ±SD of replicates, n=5
*K represents the carrying capacity of the bacterial population
**t_mid represents the time at which the bacterial population reaches its midpoint in terms of growth.

Table 5. Growth kinetics of selected strains in Faba-C without incubated overnight with shaking (NS*) calculated with R Studio

Strain	Mean_r	K**	t_mid***
A -2	1.977±0.12	1.788±0.01	8.676±0.07
A -3	1.798±0.27	1.761±0.10	13.025±0.04
B -2	2.644±0.14	1.809±0.04	5.616±0.03
B -3	2.473±0.23	1.863±0.02	9.616±0.04
E -2	1.976±0.08	1.856±0.02	12.127±0.04
E -3	1.956±0.04	1.786±0.02	13.961±0.07
L -2	2.605±0.17	1.812±0.04	4.869±0.04
L -3	2.582±0.39	1.897±0.05	8.770±0.08
N -2	2.709±0.13	1.860±0.02	9.595±0.06
N -3	1.819±0.19	1.905±0.03	13.198±0.05
R -2	2.304±0.09	1.744±0.04	5.345±0.01
R -3	1.916±0.23	1.741±0.04	10.667±0.07
V -2	2.344±0.12	1.878±0.03	5.043±0.02
V -3	2.298±0.06	1.909±0.02	8.632±0.09

*NS= Not incubated overnight with shaking

**K represents the carrying capacity of the bacterial population

***t_mid represents the time at which the bacterial population reaches its midpoint in terms of growth

Table 6. Growth kinetics of selected strains in OatF-C without incubated overnight with shaking (NS*) calculated with R Studio

Strain	Mean_r	K**	t_mid***
A -2	0.416±0.004	0.370±0.002	8.545±0.06
A -3	0.259±0.01	0.340±0.01	14.608±0.19
B -2	0.714±0.02	0.255±0.004	4.686±0.07
B -3	0.416±0.003	0.261±0.004	9.203±0.05
E -2	0.581±0.01	0.349±0.01	7.351±0.09
E -3	0.335±0.003	0.281±0.005	10.027±0.04
L -2	0.635±0.01	0.350±0.005	4.830±0.03
L -3	0.363±0.01	0.373±0.01	9.346±0.08
N -2	0.460±0.05	0.397±0.005	9.829±0.2
N -3	0.422±0.06	0.329±0.01	13.181±0.25
R -2	0.440±0.004	0.347±0.004	5.911±0.06
R -3	0.367±0.002	0.342±0.005	11.250±0.03
T -2	0.456±0.01	0.345±0.005	8.150±0.08
T -3	0.617±0.01	0.285±0.005	8.775±0.03
V -2	0.222±0.01	0.306±0.01	6.931±0.16
V -3	0.214±0.03	0.319±0.02	11.743±0.75

*NS= Not incubated overnight with shaking

**K represents the carrying capacity of the bacterial population

***t_mid represents the time at which the bacterial population reaches its midpoint in terms of growth

Bacterial counts

Table 1. Bacterial counts of Bioscreen experiment in OatF-F 7.3

Strain code	Strain name	Bacterial counts*
A	*Lactiplantibacillus plantarum*	6.7
B	*Lactiplantibacillus paraplantarum*	7.5
C	*Lacticaseilactobacillus paracasei* subsp. *paracasei*	7.2
D	*Pediococcus pentosaceus*	7.3
E	*Lactococcus* sp.	7.3
F	*Lactococcus lactis subsp. lactis*	6.0
G	*Levilactobacillus brevis*	6.3
H	*Levilactobacillus brevis*	6.0

*log cfu/ml, overnight growth

Table 2. Bacterial counts of Bioscreen experiment in OatF-F 3.3

Strain code	Strain name	Bacterial counts*
I	*Weissella confusa*	7.0
J	*Lactococcus lactis* subsp. *lactis*	7.2
K	*Lacticaseilactobacillus paracasei* subsp. *paracasei*	6.5
L	*Lactiplantibacillus plantarum*	7.5
M	*Pediococcus parvulus*	6.0
N	*Lactiplantibacillus plantarum*	7.4
P	*Leuconostoc* sp.	7.3
R	*Lacticaseibacillus paracasei*	7.2

*log cfu/ml, overnight growth

Table 3. Bacterial counts of Bioscreen experiment in OatF-C

Strain code	Strain name	Bacterial counts*
A	*Lactiplantibacillus plantarum*	6.00
B	*Lactiplantibacillus paraplantarum*	7.06
C	*Lacticaseilactobacillus paracasei* subsp. *paracasei*	6.88
D	*Pediococcus pentosaceus*	7.04
E	*Lactococcus* sp.	7.08
F	*Lactococcus lactis* subsp. *lactis*	6.48
G	*Levilactobacillus brevis*	6.30
H	*Levilactobacillus brevis*	6.18
M	*Pediococcus parvulus*	7.10
N	*Lactiplantibacillus plantarum*	6.18
P	*Leuconostoc* sp.	6.70
R	*Lacticaseibacillus paracasei*	7.00
S	*Levilactobacillus brevis*	6.00
T	*Leuconostoc mesenteroides* subsp. *mesenteroides*	6.30
V	*Pediococcus pentosaceus*	7.22
Y	*Liquorilactobacillus nagelii*	6.60
Z	*Weissella confusa*	6.48
AB	*Lacticaseilactobacillus paracasei* subsp. *paracasei*	6.85

*log cfu/ml, overnight growth

Table 4. Bacterial counts of Bioscreen experiment in Faba-C

Strain code	Strain name	Bacterial counts*
A	*Lactiplantibacillus plantarum*	7.43
B	*Lactiplantibacillus paraplantarum*	7.75
C	*Lacticaseilactobacillus paracasei* subsp. *paracasei*	6.00
D	*Pediococcus pentosaceus*	7.45
E	*Lactococcus* sp.	7.08
F	*Lactococcus lactis subsp. lactis*	6.60
G	*Levilactobacillus brevis*	7.28
H	*Levilactobacillus brevis*	6.48
I	*Weissella confusa*	7.23
J	*Lactococcus lactis subsp. lactis*	7.53
K	*Lacticaseilactobacillus paracasei* subsp. *paracasei*	7.45
L	*Lactiplantibacillus plantarum*	7.32
M	*Pediococcus parvulus*	6.85
N	*Lactiplantibacillus plantarum*	7.51
P	*Leuconostoc* sp.	7.66
R	*Lacticaseibacillus paracasei*	7.60
V	*Pediococcus pentosaceus*	7.78

*log cfu/ml, overnight growth

APPENDIX 4 (1/2)

Bacterial counts in Biolector

Table 1. Bacterial counts in OatF-F 7.3 in Biolector experiment

Strain code	Strain Name	Initial inocula*	Bacterial counts*
J	Lactococcus *lactis* subsp. *lactis*	8.31	6.11
R	*Lacticaseibacillus paracasei*	8.11	6.30
L	*Lactiplantibacillus plantarum*	8.23	6.30
I	*Weissella confusa*	7.88	6.70
E	Lactococcus sp.	8.10	6.65
V	*Pediococcus pentosaceus*	8.13	6.30

*log cfu/ml

Table 2. Bacterial counts in OatF-F 3.3 in Biolector experiment

Strain code	Strain Name	Initial inocula*	Bacterial counts*
J	Lactococcus *lactis* subsp. *lactis*	8.31	6.48
R	*Lacticaseibacillus paracasei*	8.11	7.88
L	*Lactiplantibacillus plantarum*	8.23	7.88
I	*Weissella confusa*	7.88	8.31
E	Lactococcus sp.	8.10	8.35
H	Levilactobacillus brevis	7.04	6.81
F	*Lactococcus lactis subsp. lactis*	7.45	6.78
B	*Lactiplantibacillus paraplantarum*	8.51	6.30
V	*Pediococcus pentosaceus*	7.95	6.60
S	*Levilactobacillus brevis*	7.11	7.00
T	Leuconostoc mesenteroides subsp. *mesenteroides*	7.78	6.95
A	*Lactiplantibacillus plantarum*	7.76	6.60

*log cfu/ml

Table 3. Bacterial counts in OatF-C in Biolector experiment

Strain code	Strain Name	Initial inocula*	Bacterial counts*
J	Lactococcus *lactis* subsp. *lactis*	8.31	7.00
R	*Lacticaseibacillus paracasei*	8.11	7.90
L	*Lactiplantibacillus plantarum*	8.23	8.02
I	*Weissella confusa*	7.88	8.11
E	Lactococcus sp.	8.10	7.98
V	*Pediococcus pentosaceus*	8.13	8.15
H	Levilactobacillus brevis	7.04	6.48
F	Lactococcus lactis subsp. lactis	7.45	7.70
B	Lactiplantibacillus paraplantarum	8.51	6.88
V	Pediococcus pentosaceus	7.95	8.11
S	Levilactobacillus brevis	7.11	8.31
T	Leuconostoc mesenteroides subsp. *mesenteroides*	7.78	8.35
A	Lactiplantibacillus plantarum	7.76	8.15

*log cfu/ml

Table 4. Bacterial counts in Faba-C in Biolector experiment

Strain code	Strain Name	Initial inocula*	Bacterial counts*
H	Levilactobacillus brevis	7.04	6.70
F	Lactococcus lactis subsp. lactis	7.45	7.93
B	Lactiplantibacillus paraplantarum	8.51	7.90
V	Pediococcus pentosaceus	7.95	8.02
S	Levilactobacillus brevis	7.11	8.36
T	Leuconostoc mesenteroides subsp. *mesenteroides*	7.78	7.98
A	Lactiplantibacillus plantarum	7.76	8.15

*log cfu/ml