Sveriges lantbruksuniversitet
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Department of Molecular Sciences

Probiotics
– how does it affect our health?

Probiotika
– hur påverkar det vår hälsa?

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Independent project in Food Science – Bachelor project • 15 credits
Agricultural Programme – Food Science
Molecular Sciences, 2018:19
Uppsala, 2018

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Credits: 15 credits
Level: First cycle, G2E
Course title: Independent project in Food Science – bachelor project
Course code: EX0669
Programme/education: Agricultural programme – Food Science

Place of publication: Uppsala
Year of publication: 2018
Title of series: Molecular Sciences
Part number: 2018:19
Online publication: https://stud.epsilon.slu.se

Keywords: probiotics, microbiota, intestinal microbiota, probiotic mechanism of action, irritable bowel syndrome, IBS, microbial ecology, gut microbiota

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Abstract

The human body consists of about 10 times as many bacteria as our own body cells. Only the gastrointestinal tract can hold up to 400 different bacterial species, where the majority of the bacteria are concentrated to the large intestines. We are born sterile without any microbiota, but thereafter colonizing and development of the microbiota occur over time. Probiotics are good bacteria that are supplied via food or as dietary supplements. The most common of probiotic bacteria belongs to the genera *Lactobacillus* or *Bifidobacterium*. They are classified as safe bacteria which makes them very popular to use in probiotic products. Both genera are Gram positive, rod-shaped and can ferment carbohydrates to short-chain fatty acids that lower the pH and make it unfavorable for other bacteria. Some guidelines for probiotics are that it must contain live bacteria and be resistant to gastric acid and bile salts.

In this literature study focus has been on how probiotics affect the gastrointestinal tract which in turn can affect the health. To narrow down the work four different probiotic strains that are common on the Swedish market have been selected for further studies: *Lactobacillus reuteri* Protectis DSM 17938, *Lactobacillus rhamnosus* GG (LGG), *Lactobacillus plantarum* 299v and *Bifidobacterium animalis* BB-12.

The general mechanism of action for probiotic bacteria is that they temporarily can persist in the intestine and be able to produce antimicrobial substances that can inhibit pathogenic bacteria. Probiotic bacteria should also be able to compete with other bacteria about nutrition and bind to epithelial cells to strengthen the immune system.

Studies have shown that *Lactobacillus rhamnosus* GG reduces the risk of suffering from antibiotic-associated diarrhea both in children and adults and that *Lactobacillus reuteri* DSM 17938 reduces the time of crying with 30-70 minutes per day in infants with colic.

Analyzing the effect of probiotics is a challenge as the effect can vary widely from individual to individual. Also, most studies vary greatly in performance, duration and dose given to patients. There are also more studies on mono strained probiotics than on multi strained. Due to the fact that multi strained probiotics makes it more difficult to evaluate which strain has had the best effect the studies are harder to compare.

Since probiotics are classified as safe and harmless bacteria it can always be a good start to treat gut dysbiosis with probiotics.

Keywords: intestinal microbiota, probiotics, probiotic mechanism of action, gut microbiota, irritable bowel syndrome, IBS, microbial ecology

Sammanfattning

En människokropp består av ungefär 10 gånger så många bakterier som egna kroppsceller. Bara mag- och tarmkanalen rymmer upp till 400 olika bakteriearter, där bakterierna är mest koncentrerade i tjocktarmen. Vi föds sterila utan någon mikrobiota varefter vi snabbt koloniseras och mikrobiotan utvecklas med tiden. Probiotika är goda bakterier som tillförs via livsmedel eller som kosttillskott. De vanligaste släktena av probiotiska bakterier är *Lactobacillus* och *Bifidobacterium*. De är klassade som säkra bakterier vilket gör att de är väldigt populära att använda i probiotiska produkter. Båda släktena är Gram-positiva, stavformade och kan fermentera kolhydrater till kortkedjiga fettsyror som sänker pH och gör det ogynnsamt för andra bakterier. Några riktlinjer för probiotiska produkter är att de måste innehålla levande bakterier som är resistenta mot magsaft och gallsalt.

I denna litteraturstudie har fokus legat på hur probiotika påverkar mag- och tarmkanalen som i sin tur kan påverka hälsan. För att smalna av arbetet har fyra olika probiotiska stammar, som är vanligt återkommande på den svenska marknaden, valts ut för att studeras mer ingående: *Lactobacillus reuteri* Protectis DSM 17938, *Lactobacillus rhamnosus GG* (LGG), *Lactobacillus plantarum* 299v och *Bifidobacterium animalis* BB-12.

Den generella verkningsmekanismen för probiotiska bakterier är att de under en tid stannar i tarmen och då producerra antimikrobiella substanser som kan inhibera patogena bakterier. Probiotiska bakterier ska även kunna konkurrera med andra bakterier om näring och om att binda till epitelceller för att stärka immunförsvaret.

Studier har visat att *Lactobacillus rhamnosus* GG minskar risken för att drabbas av antibiotikaassocierad diarré både hos barn och vuxna, och att *Lactobacillus reuteri* DSM 17938 sänker gråttiden hos spädbarn med kolik med 30-70 minuter per dag.

Att analysera effekten av probiotika är en utmaning då effekten kan skifta mycket från individ till individ, samtidigt som de flesta studier varierar i utförande, varaktighet och dos som patienterna får. Det finns också fler studier på probiotika med en stam än på produkter med flera. På grund av att en produkt med flera stammar gör det svårare för att utvärdera vilken av stammarna som har gett bäst effekt blir studierna svårare att jämföra.

Eftersom probiotika klassificeras som säkra och ofarliga bakterier kan det alltid vara en bra början att behandla tarmdysbios med probiotika.

Nyckelord: intestinal microbiota, probiotics, probiotic mechanism of action, gut microbiota, irritable bowel syndrome, IBS, microbial ecology

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Abbreviations and explanations

AAD	antibiotic-associated diarrhea
BB-12	strain of *Bifidobacterium animalis spp. lactis*
CFU	colony forming units
GI	gastrointestinal
GRAS	Generally Recognized As Safe
IBS	irritable bowel syndrome
ID	infectious diarrhea
IgA	immunoglobulin A
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
LGG	*Lactobacillus rhamnosus GG*
NGS	Next Generation Sequencing
RCT	randomized controlled clinical trial
SCFA	short-chain fatty acid
VSL#3	combined probiotic: *L. casei, L. plantarum, L. acidophilus, L. delbrueckii subsp. bulgaricus, B. longum, B. breve, B. infantis, Streptococcus salivarius subsp. thermophilus*

1 Introduction

1.1 What is probiotics?

Probiotics is a hot topic today with many commercials on television marketing new products that can provide a well-functioning intestine, which in turn, can lead to an overall healthier lifestyle and wellbeing. The human body consists of almost ten times more bacteria than the body's own cells and most of the bacteria are concentrated to the intestines and more specifically the colon (Gerritsen *et al.*, 2011). It has been established that the gastrointestinal (GI) tract holds about 400 different species of bacteria (Gerritsen et al., 2011). During birth the newly born comes from a sterile environment and immediately a bacterial colonization starts to build up (Araya *et al.*, 2006). Some bacteria are harmful while others favour us. These friendly bacteria that are presented in the colon and promotes a functional eco-system and maintains the homeostasis within the body are called microbiota. Unlike the existing microbiota that are established in the gut, favorable bacteria that comes from food or supplements which contain friendly bacteria, are called probiotics (Roos, 2018).

Probiotics are believed to be newly discovered but there is evidence going all the way back to the Old Testament that people consumed fermented milk (Fuller, 1992). Through the years fermented milk has been a way of preventing food spoilage and in the beginning of the 20th century Elie Metchnikoff, professor at the Pasteur Institute in Paris, determined that the microflora of the gut influenced the wellbeing of humans and that people who drank fermented milk lived longer. Going back in the history when the diet consisted of natural food from a natural environment there were no need of probiotics. But over time both the lifestyle and the environment have changed, and several causes can contribute to a dysbiosis in the gut. Some effects can be connected to inferior diet with artificial and processed food, antibiotics that expels the favourable bacteria or a more excessive cleanliness. Nowadays

probiotics instead has become a supplement for repairing deficiencies in the human body (Fuller, 1992)

There are a lot of species and strains that can be used as probiotics, and the far most used in Sweden are strains of *Lactobacillus* (Fuller, 1992) and *Bifidobacterium* but certain strains of *Lactococcus*, *Streptococcus, Pediococcus* and *Leuconostoc* can also be used. Strains of *Lactobacillus* and *Bifidobacterium* are stated as safe and are the far most used ones. They are Generally Recognized As Safe (GRAS) which means that there is no doubt that they are not harmful. According to the FAO/ WHO probiotics are "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". There are some guidelines for probiotics (Araya *et al.*, 2006):

- the product must contain living microorganisms
- there must be an adequate dose of probiotics pointed out on the product for achieving an effect
- the probiotic bacteria must survive not only in the passage through the gastrointestinal but also survive and proliferate in the gut which means the bacteria needs to be resistant towards gastric juices and bile.

Probiotic bacteria can ferment carbohydrates that contribute to a low pH in foods. They are mostly found in fermented foods, but nowadays it can be found in juices and other drinks, if added. The fermented foods can be everything from yoghurt and cheese to sauerkraut and kimchi. In the gut the bacteria of the microbiota and some probiotic bacteria can digest dietary fibers that cannot be digested in the small intestines. Fibers that contribute to a more beneficial microbiota are called prebiotics. The probiotic bacteria can also compete and inhibit the action of other pathogenic bacteria by competing for nutrients or compete for binding to the mucosal surface of the intestines. They can also stimulate enzymatic reactions and synthesize vitamins (Fuller, 1992).

1.2 Products containing common probiotic strains in Swedish food- and drugstores

Most Swedish supermarkets have a large stock of products that contain probiotics. Also, Swedish drug stores offers a lot of different supplements that can contribute to a healthier microflora. The recommended dose of intake per day for probiotics is 10^9-10^{10} colony forming units (CFU) to get a positive effect of the health (Hemaiswarya *et al.*, 2013), but even lower doses can show positive effects.

Lactobacillus is the most popular used probiotic and there are a lot of strains that are used in different products. *Lactobacillus* can ferment carbohydrates to lactic acid, acetic acid and ethanol while *Bifidobacterium* can ferment carbohydrates and the end products are acetic acid and lactic acid (O'Callaghan & van Sinderen, 2016). Both *Lactobacillus* and *Bifidobacterium* are Gram positive bacteria. *Lactobacillus* are oxygen tolerant, rod-shaped and non-spore-forming bacteria. They are mostly found naturally in animal feed, ensilage and milk products and are added to food products like sour cream, cheese and fermented vegetables to give it the characterizing taste and a longer sustainability. *Bifidobacterium* naturally occurs in the intestines of mammals, as well as the oral cavity of humans and in sewages (O'Callaghan & van Sinderen, 2016). They are rod-shaped and sensitive to oxygen.

Many companies have patent on different strains and have even given them names that only that company uses. The Trademark for a probiotic strain extends over 20 years, and then that strain is allowed to be used by other companies if renamed (Roos, 2018). There are many products on the market and four species that are repeatedly used on the Swedish markets are:

1.2.1 Lactobacillus reuteri

L. reuteri was discovered by the German microbiologist Gerhard Reuter (Britton, 2017). A lot of different strains of *L. reuteri* are found and in Sweden and the strain *Lactobacillus reuteri* Protectis DSM 17938 is strongly associated with the company BioGaia that has patent on the strain and that makes supplements in shapes of tablets, liquids or powder form. *L. reuteri* can be one of the first bacteria that the newly born baby meets through the birth and breastfeeding. The bacteria can naturally live in humans but often in relatively low numbers. *L. reuteri* can produce a metabolic compound, reuterin, that can inhibit pathogenic bacteria (Karimi, 2017).

1.2.2 Lactobacillus rhamnosus

Lactobacillus rhamnosus GG (LGG) was discovered 1985 by Sherwood Gorbach and Barry Goldin (Segers & Lebeer, 2014). On the Swedish market the *L. rhamnosus* can be found in Bifiform from the drug stores where it is sold as lactic acid pills. The strain can produce antimicrobial compounds, bacteriocins, that can inhibit anaerobic bacteria such as *Clostridium*, *Staphylococcus* etc (Gorbach *et al.*, 2017). The bacteria can lower the pH in the intestines and inhibit pathogenic organisms (Hemaiswarya *et al.*, 2013).

1.2.3 *Lactobacillus plantarum*

L. plantarum can ferment a lot of different carbohydrates and have a high tolerance towards bile and acids (Molin, 2015). *L. plantarum* increases the amount of acetic acid and propionic acid that makes the environment unfavourable for pathogenic bacteria (Ducrotte *et al.*, 2012). Probi mage is a product in Swedish drug stores that contains *Lactobacillus plantarum* 299v as a single strain (*Probi Mage 80 kapslar*, 2018). Also the trademark Proviva that provides fruit drinks in supermarkets contain *Lactobacillus plantarum* 299v as a single strand (ProViva, 2013).

1.2.4 *Bifidobacterium animalis spp. lactis*

B. animalis spp. lactis can be found in the colon of mammals and is resistant to oxidative stress and acidity (Quigley, 2017). The company Chr. Hansen A/S have had a patent on the strain *Bifidobacterium animalis* DSM 15954 which they named BB-12. The strain can be found in products of Bifiform and in certain types of yogurts sold in the supermarkets.

1.3 Isolation of bacteria

Lactobacillus reuteri was first isolated 1990 from a peruvian woman's breastmilk (BioGaia). The bacteria are common in the microbiota of humans and is isolated from breastmilk, vagina, oral cavity, stomach, small intestine, colon and feces. It can survive acids and bile salts that naturally occurs in the intestinal tract.

Lactobacillus rhamnosus was first isolated from healthy human feces by Sherwood Gorbach and Barry Goldin in 1985 (Gorbach *et al.*, 2017). Due to its characteristics as resistance to bile and acid, good growth and adhesion capacity to the epithelial cells it is preferable as probiotics (Segers & Lebeer, 2014).

Lactobacillus plantarum have been isolated from healthy human intestinal flora (Molin, 2015). The bacteria was isolated and studied in a science project in Lund 1986 to come up with a nutrition supplement for patients with tube feeding (ProViva, 2013).

Bifidobacterium animalis spp. lactis was discovered by the French paediatrician Henry Tissier that determined that there were a higher number of *Bifidobacterium* in healthy children compared to children with diarrhea (Araya *et al.*, 2006). The first isolation was taken from milk cultures, which makes milk products a very good

environment for the probiotic bacteria to grow in (Quigley, 2017). *B. animalis spp. lactis* can be divided into two subspecies; *B. animalis* and *B. lactis*. *B. animalis* is resistant to oxidative stress and acidity.

1.4 Probiotic bacterial mechanism of action

There are a lot of mechanisms that the probiotic bacteria can work through, and it differs a lot from one species or strain to another. Favorable for probiotic bacteria is to colonize the gut, produce antimicrobial substances, compete with other bacteria for binding to epithelial cells, compete for nutrients and modulate the immune system (Araya *et al.*, 2006). One general problem that can cause a lot of disorders and diseases in the human intestines often starts with a leaky gut (Roos, 2018). In the same way as nutrients can pass the epithelial cells from the intestines to the blood, the bacteria can pass it the same way and cause diseases.

The gastrointestinal tract is complex and starts with a layer of mucus that provides the intestines with a protective barrier (Karimi, 2017). The mucus layer is the first immune barrier the bacteria faces. The attachment to the mucus often comprises binding protein that the probiotic bacteria secretes. Furthermore, the probiotics can produce antimicrobial compounds like bacteriocins, hydrogen peroxide, nitric oxide, lactic acid, acetic acid and butyric acid that impairs the environment for other bacteria (Hemaiswarya *et al.*, 2013).

If a pathogen penetrates the mucus it faces the epithelial cells that are the major defense of the immune system in the gut (Karimi, 2017). These epithelial cells are bound together with junctions. The tight junction which is the first barrier of the epithelial junctions control the regulation of micro- and macro molecules between the epithelial cells. The two other junctions are adhesion junction and desmosome. They regulate the communication and adhesion between the cells. There is evidence that pathogens can enfeeble the tight junctions between the epithelial cells while probiotics can amplify the mucosal layer, repair the junctions and the binding proteins by extrude metabolites that activate genes.

2 Purpose and question at issue

The aim of this study was to get an understanding how probiotics works and to compare the most used probiotic strains in Sweden to each other to get a bigger knowledge in what effect they have in the body. To narrow down the area the greatest importance has been to study the probiotic effect on mostly healthy people or people suffering from minor gut diseases or disorders like irritable bowel syndrome (IBS), antibiotic-associated diarrhea (AAD) and abdominal pain.

The questions requested was:

- Which probiotic bacteria are the most common used in Sweden and where have they been isolated from?
- What have earlier studies shown? What positive and non-positive effects have the probiotic bacteria shown?
- What characteristics do the bacteria have in the intestines?
- Are there any differences between the mechanism of action according to the probiotic bacteria?

3 Method

To get a background about what probiotic bacteria are, where they come from and what they do the book *Probiotics - the scientific basis* written by R. Fuller was used. Even some articles about microflora, gastrointestinal tracts and probiotics were read to get a greater knowledge about the human body and how the bacterial flora of the gut affects the human health. For further studies a literature survey was performed were most of the articles were found by searching in the database *Web of Science*. Both clinical studies and META-analysis were studied. Clinical studies were used to see what certain studies have shown and the META-analysis were read and evaluated to compare a large stock of clinical trials to get a trustworthy outcome and a more accurate result. The relevance of the articles was assessed by their models so that the clinical studies compared were randomized, double-blinded and placebo-controlled. Great importance was given to the year of publicity and most of the material was published between 2013 and 2017.

4 Results

4.1 Efficiency of probiotics

The efficiency of probiotics can vary. Four different META-analysis has been audited and summarized below.

One META-analysis compiles the probiotic effect of *Lactobacillus rhamnosus* GG compared to a placebo effect in children and adults suffering from antibiotic-associated diarrhea (AAD) (Szajewska & Kolodziej, 2015). The analysis compared 12 different randomized controlled clinical trials (RCTs) and 1499 patients participated. The daily intake of probiotic varied between $4*10^8$ to $12*10^{10}$ colony forming units (CFU) per day. The probiotics was compared with placebo or with no additional treatment. In total this META-analysis showed that *L. rhamnosus* GG reduced the risk of getting the AAD from 22.4% to 12.3%. for all participants When the results were divided between adults and children the results differed significantly between the groups. The group of children that had an intake of probiotics reduced the risk of getting AAD from 23% to 9.6% while in the group of adults it was reduced from 22.2% to 13.7%. The results showed that the AAD significantly was reduced in children but not in adults.

Another META-analysis evaluated the effect of *Lactobacillus reuteri* DSM 17938 and other treatments on infants suffering from colic (Gutierrez-Castrellon *et al.*, 2017). The META-analysis consisted of 32 randomized controlled clinical trials (RCTs) with 2242 patients randomized into 9 different groups that compared different treating techniques: massage, acupuncture, drugs, herbal, dietetics, reassurance, different manipulations and a placebo.

Table 1. *Shows different treatments that was given to infants with colic.*

Diet	Hydrolyzed formulas, isolated soy protein formulas, prebiotic added infant formulas
Lactobacillus reuteri DSM 17938	*Lactobacillus reuteri* DSM 17938 (10^8 CFU/ day)
Drugs	Dicyclomine, cimetropium or simethicone
Herbal	Fennel seed oil, *Menta piperita, Melissa officinalis, Matricariae recutita* or *Foeniculum vulgare*
Acupuncture	Local or systematic acupuncture
Manipulative	Car-rides interventions, decrease of stimulating actions, chiropractic techniques
Massage	Spinal massage or any kind of massage
Reassurance	Familiar caregivers support, counseling therapies
Control	Placebo

Randomized clinical trials (RCTs) used were published between 1960 and 2015. The results in *table 2* shows that the *L. reuteri* was far most effective compared to the other methods. For 95% of the infants treated with *L. reuteri* the reduction of crying decreased with 30-70 minutes. On the second place came dietetics interventions.

Table 2. *Comparisons between different treatment for colic in infants. Weighted mean differences shows the reduction of crying in minutes, $Cl_{95\%}$ shows a 95% confidence interval, P value shows the probability and the I^2 is an imaginary number.*

Intervention	**Weighted mean differences (min)**	**$Cl_{95\%}$, min**	**P value**	**Heterogeneity I^2 (%), P value**
L. reuteri DSM 17938	-51.3	-30.5 to -72.2	0.0001	42, 0.08
Dietic	-44.3	-18.7 to -56.1	0.0001	83, 0.001
Manipulative	37.4	-21.5 to -67.0	0.001	78, 0.001
Massage	-40.0	-2.0 to -78.0	0.04	0, 0.83
Acupuncture	-11.2	2.0 to -23.0	0.08	0, 0,40
Herbal	-61.2	0.8 to -122.0	0.05	98, 0.001
Drugs	-30.0	-20.8 to -39.0	0.001	63, 0.01
Reassurance	-52-6	56.0 to -161.4	0.34	99, 0.001

The third META-analysis was a literature study that analyzed how probiotics affect different kinds of irritable bowel syndrome (IBS). The different syndromes compared was IBS with predominantly diarrhea (IBS-D), constipation (IBS-C), a mix of diarrhea and constipation (IBS-M) or unspecified (IBS-U) (Principi *et al.*, 2018). More than 800 articles were compiled, and they were published between January 2000 and June 2017. The participants needed to come up with at least two criteria to participate in these studies: pain associated with defecation, difference in feces frequency and/ or changes in the texture of the feces.

What was established was that when mucosal and/ or fecal microbiota was analyzed patients from the healthy control groups they had a larger diversity of the microbiota than patients suffering from IBS (Principi *et al.*, 2018). It also differed from children and adults since children do not have a full established microbiota. What was determined of the microbiota was that the beneficial bacteria such as *Lactobacillus spp.* and *Bifidobacterium spp.* did not play such a big role as it was supposed. The amount of these bacteria was reduced, increased or unchanged while the number of possible detrimental bacteria like *Clostridium spp.* was found in a higher concentration in patients suffering from IBS. The conclusions were that probiotic was significantly effective against IBS for a big part of the participants and strains *Bifidobacterium breve, Bifidobacterium longum* and *Lactobacillus acidophilus* showed the best results when treating IBS while *Bifidobacterium animalis, Bifidobacterium infantis, Lactobacillus casei, Lactobacillus plantarum* and *Lactobacillus bulgaricus*

had no effect. According to abdominal distension probiotics that improved were *Bifidobacterium breve, Bifidobacterium infantis, Lactobacillus casei* and *Lactobacillus plantarum*. For flatulence all the probiotics showed improvements. The data showed positive effects for the use of probiotics, but it needs more research for dose and duration to treat different types of IBS.

The fourth META-analysis compiled the effect of probiotic efficacy for a number of different GI diseases (Ritchie & Romanuk, 2012).

Table 3. *Shows the diseases that was analyzed in this META-analysis.*

Diseases analyzed
Pouchitis
Infectious diarrhea (ID)
IBS
Helicobacter pylori
Clostridium difficile disease
AAD
Traveler's diarrhea
Necrotizing Enterocolitis

The clinical trials used stretched between 1970 and 2011 and they were collected from Pubmed, Medline, Google Scholar, Embase, Biological Abstracts and Science Direct. The probiotics used are showed below.

Table 4. *Shows the probiotics used to treat different diseases in this META-analysis. The probiotics in bold are the two of the four most used strains in Sweden.*

Probiotics used
VSL#3
LGG
S. boulardii
B. infantis
L. acidophilus
L. casei
C. butyricum
E. faecum
L. plantarum
B. lactis
L. acidophilus + B. infantis

In total of eight diseases only Pouchitis, AAD, ID, IBS, *Helicobacter pylori* and *Clostridium difficile* were improved when treated with probiotics. Pouchitis was affected by all probiotic strains while Traveller's diarrhea and Necrotizing Enterocolitis did not show any significant effect of the probiotics. The probiotics that showed most effect according to all the GI diseases were VSL#3, *E. faecium*, *C. butyricum*, *L. acidophilus* combined with *B. infantis*, *B. lactis*, LGG, *L. casei* and *S. boulardii*. VSL#3 and *C. butyricum* showed best effect of all probiotics. It was also established that *L. acidophilus* as a single strain was more effective than in a mixed strain, and that the age of the participants or the dose that was given did not show any significant efficacy. On the other hand, the length of the treatment showed more efficacy for a longer treatment (9-240 weeks) than a shorter (3-4 weeks).

4.1.1 Efficacy of mechanism of action

As earlier mentioned in the introduction there are several possible mechanisms of action for the probiotic bacteria. One is to upregulate or downregulate immune molecules that affect the immune system. One hypothesis of a mechanism is the immunomodulation by probiotics (Hemaiswarya *et al.*, 2013). The probiotic bacteria bind to the mucus where it meets either the epithelial cells, the Microfold cells (M cells) or the Dendritic cells. The interaction stimulates both the epithelial cells to release Interleukin 6 (IL-6) that is a pro-inflammatory cytokine, and it stimulates the macrophages and Dendritic cells to produce TFN-α and IFN-γ.

Probiotic bacteria can also stimulate Mast cells to produce cytokine IL-4, and together with the cytokine IL-6 that the epithelial cells secrete and TGF-β they can change the production of IgM to IgA (Hemaiswarya *et al.*, 2013). It has also been shown that an increase of the antibodies IgM and IgG and a decrease of the inflammatory antibody IgE.

4.1.2 Multi strained probiotics

A multi strained probiotic supplement can either contain several strains of the same species or strains from different probiotic species (Timmerman *et al.*, 2004). As the gut contains more than 400 different species it has been hypothesized that a multi strained probiotic has a bigger chance to colonize the gut and produce a greater variety of microbial substances like bacteriocins that can work in synergism and inhibit pathogenic bacteria in a more effective way than a single strained probiotic. In one study both single strained probiotics, multi strained probiotics and multispecies showed positive effects on diseases but the multispecies showed best results. The disadvantages of multi strains or multispecies are that the probiotic bacteria can inhibit each other, and it can be hard to analyze the result to see which strain or species that promoted the best effect.

4.1.3 Single strained probiotics

A single strain probiotic only contain probiotic from one strain of a species (Timmerman *et al.*, 2004). The advantage of a single strained probiotic is that no other species or strains can interrupt or inhibit the mode of action. It makes it easier to evaluate the effect of a single strained probiotics as there is only one strain that can affect the outcome. The mono strain does not either have to compete with other probiotic strains about the nutrients. The disadvantage of using a single strain

probiotic supplement is that if the probiotics does not give any effects, there will not be any other strains that can complete the effect.

4.2 Survival in guts

There are several different methods to determine the gastrointestinal microbiota in humans. Old molecular techniques as quantitative polymerase chain reaction (qPCR), temperature or denaturing gradient gel electrophoresis, terminal-restriction fragment length polymorphism (T-RFLP) and fluorescent in situ hybridization (FISH) have been used to see the diversity of bacteria in the intestines (Gerritsen *et al.*, 2011). Nowadays a faster way of sequencing a human's whole genome is to use Next Generation Sequencing (NGS) which only takes one single day (Behjati & Tarpey, 2013). Another method of determine the microbiota of a person is to study the feces as the colon and feces contains more bacteria per gram comparison to the content of bacteria in the stomach or in the small intestines. It is also a god way because there is no need of surgical procedures like biopsies. From the fecal samples the biomass of microbial cells can be determined.

The probiotic bacteria must survive gastric acidity and bile salts (Bezkorovainy, 2001). Depending on the strength and length of exposure to acidity and bile salts probiotic bacteria can survive differently. Many probiotics work their way through the intestines where they may multiply and bind to the mucus but still do not colonize, therefore a continuous intake of probiotic supplement is favorable.

4.3 Favorable food for the probiotic bacteria

As mentioned earlier some foods are improved to favor the probiotic bacteria. Especially indigestible sources as dietary fibers and resistant starch can not be digested in the gut or in the small intestines. Later in the colon some of the probiotic bacteria can digest it and produce short chain fatty acids (SCFA). The SCFA lowers the pH and can inhibit proteolytic bacteria. The probiotic bacteria can even produce vitamins as K and B12 that the body is incapable to produce itself (Gerritsen *et al.*, 2011).

Foods that stimulate the positive bacteria in the intestines are called prebiotics. Prebiotics may also stimulate the growth of probiotic bacteria, and together they are mentioned synbiotics. The criteria for prebiotics is that they need physical properties that can withstand the digestive process (Wang, 2009). They need to be resistant to bile salt and acids, can be fermented by certain gut bacteria in the colon and/ or to

stimulate the growth of probiotic bacteria (Gibson *et al.*, 2004). The downside of prebiotics nowadays is that a large part of the population gets flatulence and stomachache because of the decrease of friendly bacteria in the gut due to their diets.

Studies have been performed to see what dietary fibers that promotes the probiotic bacteria best. Still the studies needs improvements and more research but it showed that inulin showed best effect towards probiotics (Gibson *et al.*, 2004).

Inulin is the far most researched dietary fiber and it belongs to the fructans of carbohydrates (Clase & Nertby Aurell, 2015). Inulin can be found in a large scale in Jerusalem artichokes. Pectin is another dietary fiber that can be found in the peel of different citruses and almost 100% of the pectin makes it way to the colon without being broken down. Also, beta glucans are common dietary fibers and the most popular way of intake comes from porridge as the oats contains lots of beta glucans.

4.4 Influence of external factors

Age, health, gender, ethnicity, geographic location, genetics and diet are all factors that can promote or inhibit the effect of probiotics (Marco & Tachon, 2013). There are evidences that the diet affects how much nutrients that are available for the probiotic bacteria and how well they can proliferate. Due to a modern diet a lot of people have a reduced number of certain members of the microbiota as they have not been given them enough nutrients to keep them alive.

For infants breast-feeding contra formula-feeding gives different intestinal microbiota (Gerritsen *et al.*, 2011). The breast-feed infants have a larger diversity of *Bifidobacterium spp*. while the formula-feed have a larger diversity of *Clostridia*, *Enterobacteriaceae*, *Bacteroides* and *Enterococcus*. Later during the first year when the baby starts to eat solid food the microbiota starts to develop into an adult acting microbiota.

A lot of elderly people suffer from decreased intestinal motility, as well as lost appetite which can lead to a decreased nutritional intake that affect the intestinal microbiota (Gerritsen *et al.*, 2011).

In a study the gut microbiota of European children from Italy was compared to the microbiota of children from Burkina Faso in Africa (De Filippo *et al.*, 2010). Though the diet in Burkina Faso consists in high fiber, lot of starch and plant

saccharides and less meat it showed that their microbiota was well established compared to the microbiota of European children.

5 Discussion

It can be a challenge to determine how effective probiotics are. The mechanism of action differs from bacteria to bacteria and it is not well established in which way each one works. Every single bacteria affects differently for each person depending on the composition of the microbiota, what you eat, how old you are and if you are healthy or not. On the other side another factor that makes it hard to analyze the way probiotics works is if the intake is combined with other probiotics that may inhibit or favor each other. It is tricky to see which of the probiotics that works best in a multiple probiotic supplement.

Some META-analysis determined that a bias was that studies that was supposed to be double-blinded ended up with the administrators revealing what supplement that was used, which can affect the results. Overall the bias with these kinds of studies is the ability to keep them double-blinded to exclude impact.

The difficulties in META-analysis is that there are fewer clinical studies comparing the effect of several probiotics treating different diseases. The existing ones are often completed by companies that wants to come up with a new product. The studies are also very different in the configuration. Daily dose of probiotics, the duration of the study and how often follow-ups exists differs from study to study.

There are much more clinical studies available on single strained probiotics than studies of multiple strained probiotics. That is because it is easier to evaluate the effect of a single stranded probiotics than a multi stranded. Overall there is not so many studies comparing different probiotic supplements when treating a disease which would have been interesting to see what strain that is most effective regarding to a specific disease.

There are a lot of evidence that probiotics contribute to health, and from all the clinical reports and META-analysis studied it showed that probiotic favor the

function of the intestines which in turn favors the immune system and mitigate most of the gut dysbiosis although with different effectivity. There need to be more studies of how probiotic bacteria effect the microbiota.

During the second half of the last century Western countries have develop antibiotics that impede pathogenic bacteria, but at the same time allergies, gut dysbiosis leading to IBS and some autoimmune disorders increased. Probably they go hand in hand as antibiotics can disrupt the microbiota. Therefore, probiotics can work as a supplement to keep the microbiota stable.

What can be said about the META-analysis studied is that most of the probiotics used showed a significant effect when treating diseases. There is evidence that probiotics show effect although it can be a little effect. As probiotic bacteria are divided not only in species but also in strains there are a wide range of probiotics available. To get the best effect out of a probiotic supplement one must keep in mind that different strains contribute with different effects when treating a disease. Also, the duration of treat, the dose and a continuous intake plays a big part in the outcome.

One must put great importance in what probiotic that is chosen for treating a specific disease.

6 Summary

Probiotics have been well established since the beginning of the 20th century but it is not until the last decade it has been a hot topic due to the trends of being healthy. The aim of this study was to get a greater knowledge about the most common probiotic strains used in Sweden and to get an understanding in how the mechanism of action works. Even if there are much more to discover there is evidence that probiotics contribute to a better health. Most of all clinical studies and META-analysis read in this report showed that probiotics was favorable when treating different diseases. When choosing a probiotic one should pay attention to which disease to be treated. There are still lots to discover and much more to research, but the future is bright, and probiotics is on the move.

7 References

Araya, M., Gopal, P., Lindgren, S.E., Lodi, R., Oliver, G., Saxelin, M.-L., Servin, A.L., Stanton, C., Gilliland, S.E., Morelli, L. & Reid, G. (2006). *Probiotics in food - Health and nutritional properties and guidelines for evaluation*.

Behjati, S. & Tarpey, P.S. (2013). What is next generation sequencing? *Archives of Disease in Childhood-Education and Practice Edition* 98(6), 236-238.

Bezkorovainy, A. (2001). Probiotics: determinants of survival and growth in the gut. *American Journal of Clinical Nutrition* 73(2), 399S-405S.

BioGaia *BioGaias probiotiska stammar*. [online] Available from: https://www.biogaia.se/var-forskning/lactobacillus-reuteri-stammar/. [Accessed 12/5].

Britton, R.A. (2017). *Lactobacillus reuteri*. In: H. Floch, M., *et al.* (Eds.) *The Microbiota in Gastrointestinal Pathophysiology*. pp. 89-97 Academic Press. ISBN 978-0-12-804024-9.

Clase, M. & Nertby Aurell, L. (2015). Fyra magiska fibrer. In. Food Pharmacy.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G. & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America* 107(33), 14691-14696.

Ducrotte, P., Sawant, P. & Jayanthi, V. (2012). Clinical trial: Lactobacillus plantarum 299v (DSM 9843) improves symptoms of irritable bowel syndrome. *World Journal of Gastroenterology* 18(30), 4012-4018.

Fuller, R. (1992). *Probiotics - The scientific basis*: Chapman & Hall. ISBN 0412408503.

Gerritsen, J., Smidt, H., Rijkers, G.T. & de Vos, W.M. (2011). Intestinal microbiota in human health and disease: the impact of probiotics. *Genes and Nutrition* 6(3), 209-240.

Gibson, G.R., Probert, H.M., Van Loo, J., Rastall, R.A. & Roberfroid, M.B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews* 17(2), 259-275.

Gorbach, S., Doron, S. & Magro, F. (2017). *Lactobacillus rhamnosus* GG. In: Floch, M.H., *et al.* (Eds.) *The Microbiota in Gastrointestinal Pathophysiology*. pp. 79-88 Academic Press. ISBN 978-0-12-804024-9.

Gutierrez-Castrellon, P., Indrio, F., Bolio-Galvis, A., Jimenez-Gutierrez, C., Jimenez-Escobar, I. & Lopez-Velazquez, G. (2017). Efficacy of Lactobacillus reuteri DSM 17938 for infantile colic: Systematic review with network meta-analysis. *Medicine* 96(51).

Hemaiswarya, S., Raja, R., Ravikumar, R. & Carvalho, I.S. (2013). Mechanism of Action of Probiotics. *Brazilian Archives of Biology and Technology* 56(1), 113-119.

Karimi, S. (2017). *Exploring Probiotics-Host Interactions.* Diss. Uppsala, Sweden:Swedish University of Agricultural Sciences (SLU).

Marco, M.L. & Tachon, S. (2013). Environmental factors influencing the efficacy of probiotic bacteria. *Current Opinion in Biotechnology* 24(2), 207-213.

Molin, G. (2015). *Lactobacillus plantarum* 299(2015-06-10)Available from: https://probi.com/sites/all/files/attachment_files/lp_299-15_2015-06-10_0.pdf. [Accessed 2018-04-24]
O'Callaghan, A. & van Sinderen, D. (2016). Bifidobacteria and Their Role as Members of the Human Gut Microbiota. *Frontiers in Microbiology* 7.
Principi, N., Cozzali, R., Farinelli, E., Brusaferro, A. & Esposito, S. (2018). Gut dysbiosis and irritable bowel syndrome: The potential role of probiotics. *Journal of Infection* 76(2), 111-120.
Probi Mage 80 kapslar. [online] Available from: https://www.apotea.se/probimage-80-kapslar?gclid=CjwKCAjw_47YBRBxEiwAYuKdw4yaO-cn9ihO1mamJphnB0Yn18jRcCL6dgdesEpdPlDk6-jkLPF1qRoCDTgQAvD_BwE. [Accessed 05/22].
ProViva *Mer information kring forskning och LP299V®*. [online] Available from: http://proviva-se.u5592509.fsdata.se/historien-om-proviva/magens-basta-van/mer-information-kring-forskning-och-lactobacillus-plantarum-299v/. [Accessed 16/5].
Quigley, E.M.M. (2017). *Bifidobacterium animalis spp. lactis*. In: Floch, M.H., *et al.* (Eds.) *The Microbiota in Gastrointestinal Pathophysiology*. pp. 127-130 Academic Press. ISBN 978-0-12-804024-9.
Ritchie, M.L. & Romanuk, T.N. (2012). A Meta-Analysis of Probiotic Efficacy for Gastrointestinal Diseases. *Plos One* 7(4).
Roos, S. (2018). In: Abrahamsson, J. (Ed.).
Segers, M.E. & Lebeer, S. (2014). Towards a better understanding of Lactobacillus rhamnosus GG - host interactions. *Microbial Cell Factories* 13.
Szajewska, H. & Kolodziej, M. (2015). Systematic review with meta-analysis: Lactobacillus rhamnosus GG in the prevention of antibiotic-associated diarrhoea in children and adults. *Alimentary Pharmacology & Therapeutics* 42(10), 1149-1157.
Timmerman, H.M., Koning, C.J.M., Mulder, L., Rombouts, F.M. & Beynen, A.C. (2004). Monostrain, multistrain and multispecies problotics - A comparison of functionality and efficacy. *International Journal of Food Microbiology* 96(3), 219-233.
Wang, Y.B. (2009). Prebiotics: Present and future in food science and technology. *Food Research International* 42(1), 8-12.