International Journal of Food Properties

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

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To cite this article: Hulya Demir (2020) Comparison of traditional and commercial kefir microorganism compositions and inhibitory effects on certain pathogens, International Journal of Food Properties, 23:1, 375-386, DOI: 10.1080/10942912.2020.1733599

To link to this article: https://doi.org/10.1080/10942912.2020.1733599

INTERNATIONAL JOURNAL OF FOOD PROPERTIES
2020, VOL. 23, NO. 1, 375–386
https://doi.org/10.1080/10942912.2020.1733599

Comparison of traditional and commercial kefir microorganism compositions and inhibitory effects on certain pathogens

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ABSTRACT
The aim of this study was to compare traditional and commercial kefir products and their microbiological compositions along with their inhibitory activities against various pathogens. Different media and inhibitory conditions were used for identification of microbiological groups. Each microbiological group was subjected to gram and fluorescence staining. Biochemical identification was performed according to gram staining. Vitek 2 Compact system was used for biochemical identification. Identification cards; Gram-negative (GN), Gram-positive (GP), Anaerobe and Corynebacterium (ANC), Neisseria-Haemophilus (NH), Yeas-mold (YM) were selected according to gram staining. Bruker MS (16 s rRNA) was used for microorganism identification. The inhibitory effect onsome pathogens were examined. A diverse spectrum of bacterial genera were identified in kefir samples including: *Lactobacillus* (six species), *Lactococcus* (one species), *Saccharomyces* (one species) *Streptococcus* (one species), *Bifidobacterium* (two species). While traditional kefir had a greater inhibitory effect on *Enterobacter cloacae* and *Escherichia coli* pathogens than commercial kefir, commercial kefir had a greater inhibitory effect on *Enterococcus faecalis* pathogen than traditional kefir. In general, traditional kefir had most effective inhibitory effect on pathogens.

ARTICLE HISTORY
Received 4 September 2019
Revised 30 January 2020
Accepted 15 February 2020

KEYWORDS
Probiotics; microbiological properties; microbial content; microbial growth inhibition mechanism

Introduction

According to archeological evidence, fermentation of food was an accidental discovery dating back to a few thousands of years. It only later occurred that most fermented foods had better storage lives and nutritional constituents; consequently, fermentation has become a popular food processing method.[1]

Due to their properties or changes occurring during fermentation, some microorganisms are beneficial for our health and for developing resistance to diseases, These live microorganisms that provide health benefit on the host are defined as probiotics. Increasing knowledge on the process of fermentation and on these probiotic species in general have paved the way for more research. As healthy nutrition and preventing the diseases are popular subjects, adding beneficial bacteria to our food sounds interesting.[2] One of these probiotics and fermented foods are kefir, fermented drink originating in the Caucasus Mountains. Kefir houses a vast variety of bioactive compounds, which are produced during fermentation, and a highly diverse microbiota. These microbes act either independently or synergistically to give kefir its various physiological, prophylactic and therapeutic properties. These properties provide many health benefits on human health. Lactose is a type of sugar found in high concentration in the milk and other dairy products, and it can only be absorbed in the intestines through hydrolysis.[3] Studies have shown that kefir loses its lactose content by 30% after fermentation, thus reducing complications of consumers with lactose intolerance.[4] The antibacterial properties of kefir have been associated with a number of factors. These include

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competition for available nutrients and the inherent action of organic acids, H_2O_2, acetaldehyde, CO_2 and bacteriocins produced during fermentation. The high lactic acid bacteria (LAB) content of kefir can reduce cholesterol levels up to 33% by inhibiting the absorption of exogenous cholesterol in the intestine. It is known that consuming probiotics regularly improves blood sugar levels, by positively modulating the composition of the intestinal microbiota and reducing intestinal permeability, oxidative stress and inflammation.[3] Even though the data is limited and controversial, there is evidence that probiotic bacteria or their fermented products have an important role in controlling blood pressure through their anti-hypertensive effects.[5]

Quirós et al.[6] have found that kefir can inhibit angiotensin-converting enzyme (ACE) activity through the action of bioactive peptides generated from casein during the milk fermentation process. In addition, Maeda et al.[7] have shown that kefir can exert its antihypertensive effect through the same mechanism mentioned above. Regularly consuming kefir positively modulates the composition of the intestinal microbiota and the immune system. Therefore, it is believed that this fermented milk may play an important role in carcinogenesis.

Hosono et al.[8] have shown that all bacterial strains isolated from kefir can bind to mutagens with rates above 98.5%. This could be eliminated even further through the feces to protect the colonocytes from damage. Kefir has been found to significantly reduce mutagenicity induced by methyl methane sulfonate, sodium azide and aflatoxin B1. Yogurt and milk, on the other hand, were found to reduce mutagenicity to a lesser degree, which may be explained by the presence of higher levels of conjugated linoleic acid isomers and butyric, palmitic, palmitoleic and oleic acids in kefir compared to milk and yogurt.

Traditional kefir has different characteristics in terms of its microbial populations, such as the ratios of key microorganisms, which affect the byproducts used in fermentation and the flavor of the food. Kefir is produced by fermentative activity of kefir grains, a natural starter culture, which contain a diverse range of inherent lactic acid bacteria, acetic acid bacteria and yeasts in a polysaccharide matrix of semi-hard granules. Microorganisms in the grains proliferate in milk and produce lactic acid and other flavor compounds, causing physicochemical changes with fermentation. One feature of kefir that differs from other fermented milk products is that kefir grains can be recovered after fermentation with a slight increase in grain biomass.[9]

Kefir is an excellent example of the co-occurrence of yeasts and bacteria. The lactic acid bacteria, lactococci, yeast and acetic acid bacteria contents of kefir grains differ due to variety of effects. Different reports have shown that kefir grains comprise of microbial content such as lactic acid bacteria, lactococci, yeast and acetic acid bacteria and the quality of the final product largely depends on the origin of the grains.[10] Both the traditional kefir and the commercially-available versions contain *Lactobacillus, Lactococcus*, and *Leuconostoc*. However, some commercial beverages labeled as "kefir" have significant differences as compared to traditional kefir in terms of their microbiology, such as lacking lactic acetic acid bacteria and a complex fungal community. Commercial kefir will often contain yeast, though the complexity of the population will be significantly lower. While traditional kefir contains *Saccharomyces cerevisiae, Pichia fermentans, Kazachastania unispora, a*nd *Kluyveromyces marxianus* and lactic in addition to many other smaller populations of yeast, the commercial variant only has *Saccharomyces cerevisiae*.[11,12] The characteristics of kefir are like those of other fermented dairy foods. In the research performed by Dillard and German commercial developments in nutraceuticals in terms of patents for anti-carcinogenic foods, anti-diabetogenic foods, cerebro-active foods, prebiotic oligosaccharides and *Bifidobacterium* probiotic foods, and foods for prevention of osteoporosis and rheumatoid arthritis have been investigated.[13]

To our knowledge, there is negligible study in the literature that compares the differences between various traditional kefir variants in terms of microbial composition or between commercial variants and traditional kefir made with grains. In this study, we aimed to compare traditional and commercial kefir products and their microbiological composition along with their inhibitory activity against various pathogens.

Materials and methods

Traditionally, kefir is made by adding kefir grains directly, where raw milk is boiled and cooled down to 20–25°C and inoculated with 2–10% (usually 5%) kefir grain. Following fermentation for 18–24 h at 20–25°C, grains are separated from milk by sieve filtering. It can then be dried at room temperature and kept at cold temperature for the next inoculation. Finally, kefir is ready for consumption after being stored at 4°C for a certain time.[14] Commercial kefir was obtained from market.

Microbiological analysis: tryptic lineage agar (TSA), sabouraud dextrose agar (SDA), potato dextrose agar (PDA), sabouraud dextrose agar with chloramphenicol medium (SDCA), tryphose sulfite cycloserine agar (TSCA), sulfide polymyxin sulfadizine agar (SPSA), parker agar (BPA), chromogenic coliform agar (CCA), tryptone even x-glucuronide agar (TBXA), violet red even agar (VRBA), MacConkeyA, blood agar, eosin methylene blue agar, standard methods agar (SMA), xylose lysine deoxycholate (XLDA), cetrimide fusidin cephaloridine agar (CFCA), mannitol egg yold polymyxin agar (MYPA), Palcam/OxfordA, bacillus acido terrestris agar (BATA), man, rogosa, sharpe agar (MRSA), yeast starch glucose agar (YRS), cooked meat medium (CMM), tryptone soy both (TSB), nutrient broth (NB), Muller-Kauffman tetrathionate novobiocin broth (MKTTn), Rappaport Vassiliadis soy peptone broth (RVSB), modified Rappaport Vassiliadis broth (MRVB), BrillantB, FB, LactoseB, phosphate buffered saline (PBS), Ringer's media and normal saline were used in the studies. Different incubation conditions were used according to the microorganism species. For example; *Lactobacillus* spp; 30° C, 40° C, 45°C and 24–72 h + 24 h, *Streptococci* group: 30° C, 37° C, 40° C, 45°C and 24–072 h + 48 h; mold-yeast: 22°C, 25°C, 30°C and 48–120 h + 48 h.

Identification methods: In all strains, gram staining was carried out to classify the starins according to their staining characteristics conferred by the walls of the bacteria; fast biochemical tests were carried out. Vitality was checked by fluorescence staining.

Identification was performed with Bruker MS (16 s rRNA). For identification and susceptibility by the VITEK 2 system (bioMérieux, Marcy d'Etoile, France), GN bacterial and identification test cards Anaerobe and Corynebacterium (ANC), Neisseria-Haemophilus (NH), Yeas-mold (YM) were used. Each decomposed microbiological group was subjected to gram and fluorescent staining.

Inoculant species with known numbers were used in order to determine the efficiency of traditional and commercial kefir on some pathogens. 2.6E +09 cfu/mL *Enterobacter cloacae*, 3.5E +08 cfu/gmL *Escherichia coli*, 6.2E+08 cfu/mL *Enterococcus faecalis* were added on 1 mL (1/10 ratio) of 1.3E+09 cfu/gmL commercial kefir, and 6.7E +09 cfu/gmL natural kefir, seperately in 10 ml conical centrifuge tubes. The samples were incubated at 37–40°C for 24 h. Man, Rogosa, and Sharpe (MRS) were placed in the agar and counted.

Results

The microbial contents of the kefir produced using traditional kefir grains and the kefir produced using commercial grains are shown in Table 1 and Table 2.

Bifidobacterium animalis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus krusei, Lactobacillus johnsonii, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactococcus lactis colony morphologies are shown below using GP 100X IM and Fluorescent Dyes 100X FL (Figures 1–11).

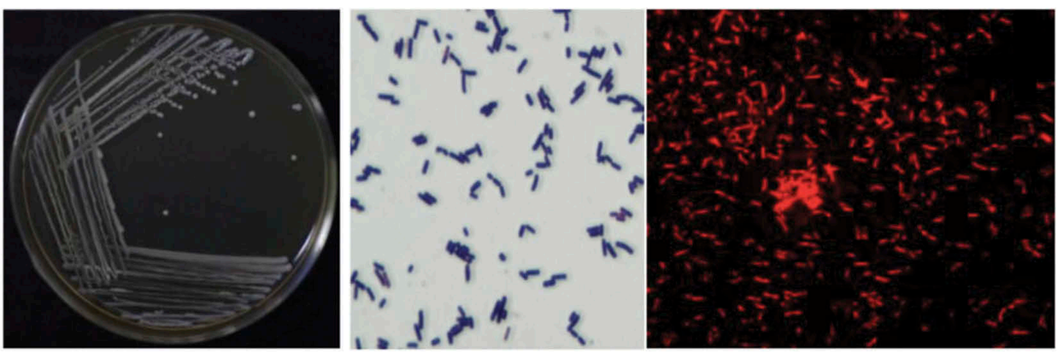
The inhibitory effect of traditional kefir on *Enterobacter cloacae* and *Escherichia coli* pathogens is greater than that of commercial kefir. Although inhibitory effect of commercial kefir on the *Enterococcus faecalis* pathogen seems to be higher than that of traditional kefir, in general, traditional kefir was found to be more effective in terms of inhibitory effects on pathogens. Inhibitory effect of natural kefir on pathogens was found to be as *Enterobacter cloacae* > *Escherichia coli*> *Enterococcus faecalis*. On the other hand, inhibitory effect of commercial kefir on pathogens was found to be as *Enterococcus faecalis*> *Escherichia coli*> *Enterobacter cloacae* (Table 3–5; Figure 12).

Table 1. Microbiological characteristics of traditional kefir.

Product	Microorganism	cfu/gmL
Traditional Kefir	*Lactobacillus acidophilus*	3.50E+09
	Lactobacillus bulgaricus	1.50E+08
	Saccharomyces spp	2.60E+09
	Lactobacillus plantarum	5.20E+07
	Streptococcus thermophilus	2.90E+08
	Lactobacillus reuteri	4.40E+06
	Lactococcus lactis	3.70E+07
	Lactobacillus johnsonii	5.10E+06
	Lactobacillus rhamnosus	3.80E+07
	Bifidobacterium longum	1.20E+07
	Bifidobacterium animalis	2.70E+07
	Total Non-Probiotic Microorganism	0.00E+00
	Total Probiotic Microorganism	6.70E+09

Table 2. Microbiological characteristics of commercial kefir.

Product	Microorganism	cfu/g-mL
Commercial Kefir	*Lactobacillus acidophilus*	2.00E+07
	Lactobacillus bulgaricus	1.50E+08
	Saccharomyces spp	5.80E+08
	Lactobacillus plantarum	1.80E+07
	Streptococcus thermophilus	4.50E+08
	Lactobacillus reuteri	7.10E+04
	Candida krusei	6.50E+07
	Lactococcus lactis	1.20E+06
	Total Non-Probiotic Microorganism	0.00E+00
	Total Probiotic Microorganism	1.30E+09

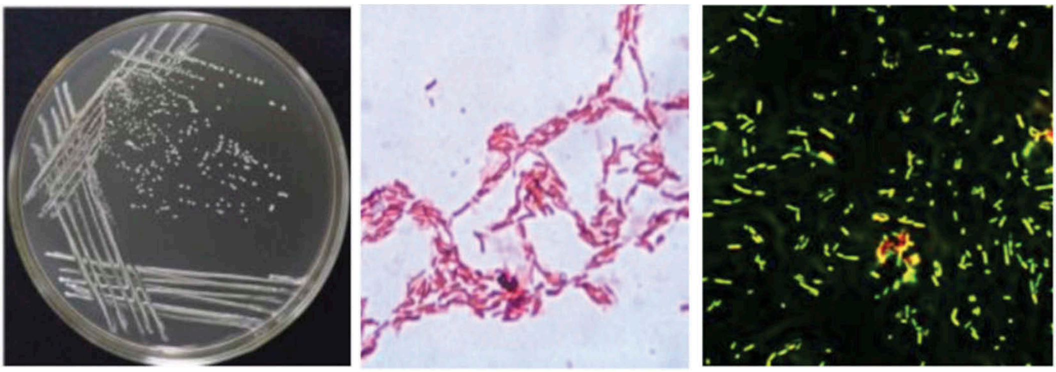


a- Colony morphology b-GP 100X IM c-Fluorescent dyes 100X FL

Figure 1. a) Colony formation of *Bifidobacterium animalis* on MRSA media (1.5 mm, smooth, shiny, white). b) Gram-positive staining of *B.animalis* (Under 100X light microscope). c) Fluorescent imaging of *B. animals* (Under 100X Fluorescent microscope).

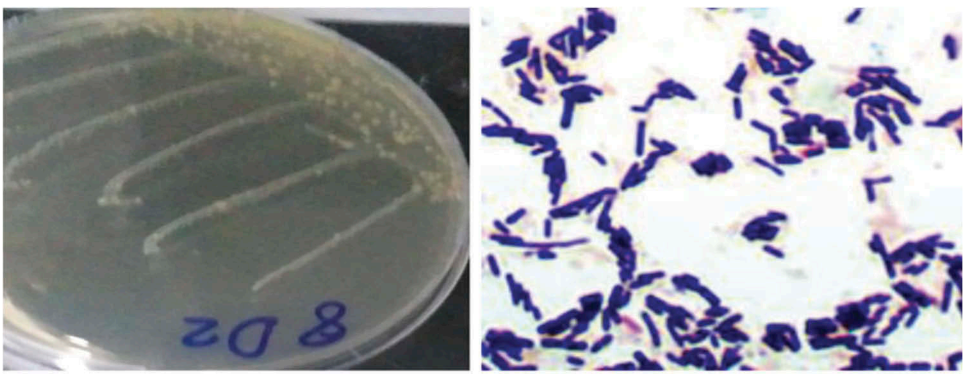
There was a statistically significant difference between the control and the commercial kefir groups in terms of mean number of microorganisms ($p < .05$). The mean number of microorganisms in the commercial kefir group was significantly lower than the mean number of microorganisms in the control group ((1.84E+06) vs. 1.19E+09).

There was also a statistically significant difference between the control and the traditional kefir groups in terms of mean number of microorganisms ($p < .05$). The mean number of microorganisms in the traditional kefir group was significantly lower than the mean number of microorganisms in the control group ((6.83E+05) vs. 1.19E+09).



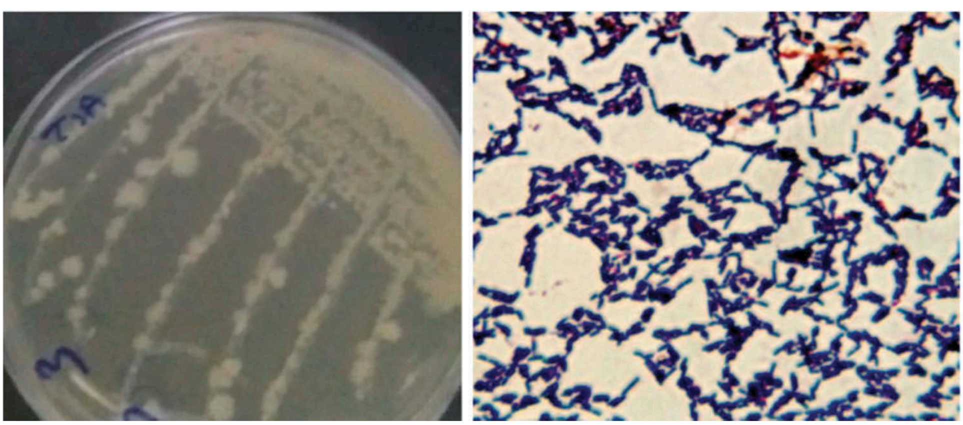
a-Colony morphology b-GP 100X IM c-Fluorescent dyes 100X FL

Figure 2. a) Colony formation of *Bifidobacterium longum* on TSA (1.5–3.0 mm, opaque/shiny, white). b) Gram-positive staining of *B. longum* (Under 100X light microscope). c) Fluorescent imaging of *B. longum* (Under 100X Fluorescent microscope).



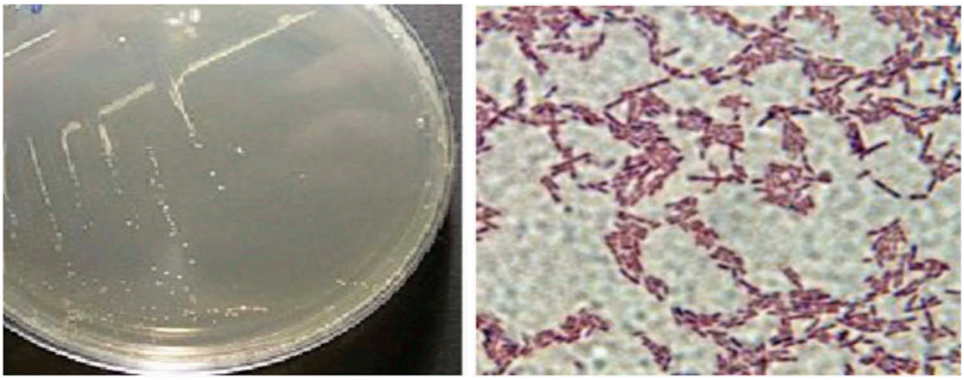
a-Colony morphology b-GP 100X IM

Figure 3. a) Colony formation of *Lactobacillus acidophilus* on TSA (1.5–2.5 mm, raised, shiny). b) Gram-positive staining of *L. acidophilus*(Under 100X light microscope).



a-Colony morphology b-GP100X IM

Figure 4. a) Colony formation of *Lactobacillus bulgaricus* on MRSA (2.0–3.5 mm, umbonate, raised). b) Gram-positive staining of *L. bulgaricus* (Under 100X light microscope).



a-Colony morphology b-GP100X IM

Figure 5. a) Colony formation of *Lactobacillus krusei* on skim milk (1.0–3.0 mm, small, shiny, white). b) Gram-positive statining of *L. Krusei* (Under 100X light microscope).

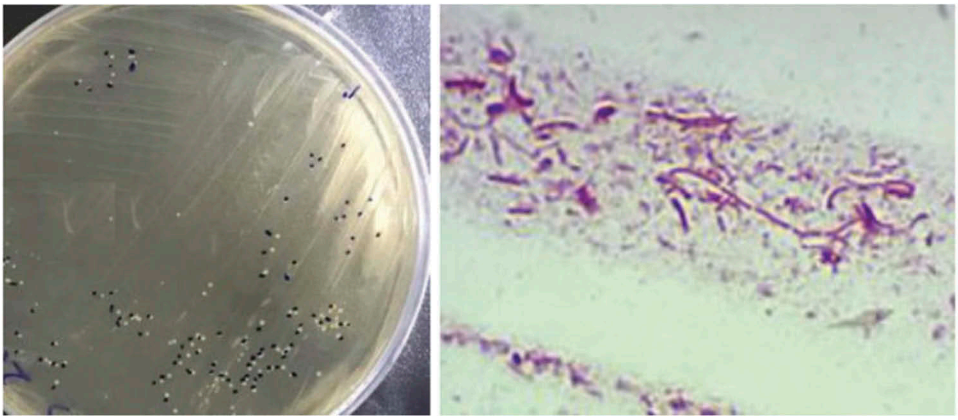
Colony morphology GP100X IM

Figure 6. a) *Colony formation of Lactobacillus johnsonii* on TSA (2.0–3.5 mm, shiny, white). b) Gram-positive staining of *L. johnsonii* (Under 100X light microscope).

Discussion

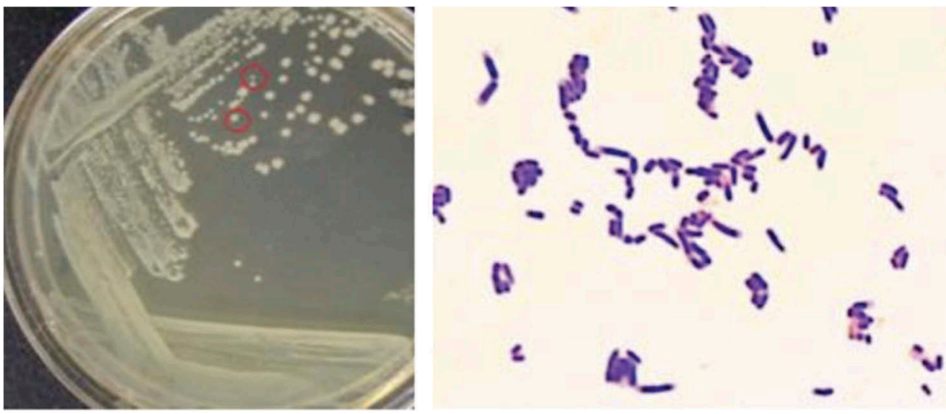
The microbial population in kefir has been reported to be an example of symbiotic community, which makes it difficult to identify and investigate the constituent microorganisms in it. Kefir has been reported to have a predominance of rod-shaped *Lactobacilli, Lactococci, Leuconostocs, Streptococcus* (LAB) in the outer layer in addition to yeasts (*Candida* sp., *Kluyveromyces* sp., *Saccharomyces* sp., *Torulopsis* sp., *Zygosaccharomyces* sp.) at the core, certain bacteria and yeasts in the intermediate region and a progressive change depending on the distance from the core.[15,16]

In this study, two different kefir microflora that contain many microorganisms, including: *Lactobacillus kefir, L. acidophilus, L. casei, L. helveticus, L. bulgaricus, L. reuteri, Bifidobacterium bifidum, Lb. brevis, Streptococcus thermophilus, Lactococcus lactis, Enterococcus durans* were analyzed. These microorganisms have such probiotic properties as alleviating lactose intolerance, treating certain types of diarrhea, strengthening the immune system, preventing cancer and lowering cholesterol levels.[17,18] It has been reported that probiotic lactobacilli and bifidobacteria break down



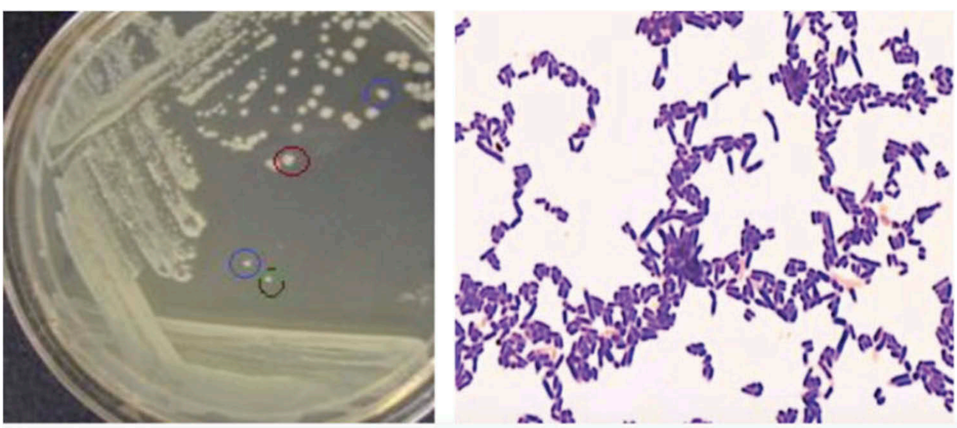
Colony morphology GP100X IM

Figure 7. a) Colony formation of *Lactobacillus plantarum* on TSA (1.5–2.5 mm, small, soft, shiny). b) Gram-positive staining of L. plantarum (Under 100X light microscope).



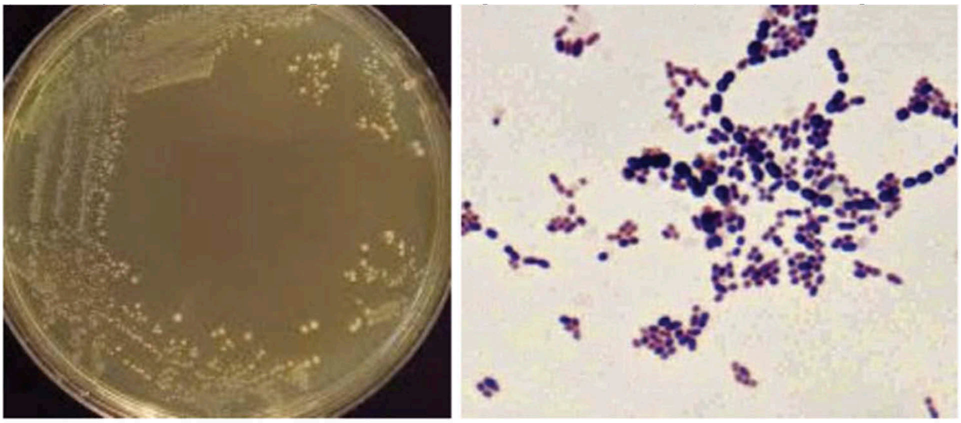
a-Colony morphology b-GP100X IM

Figure 8. a) Colony formation of *Lactobacillus reuteri* grow NA (flat,). b) Gram-positive staining of L.reuteri (Under 100X light microscope).



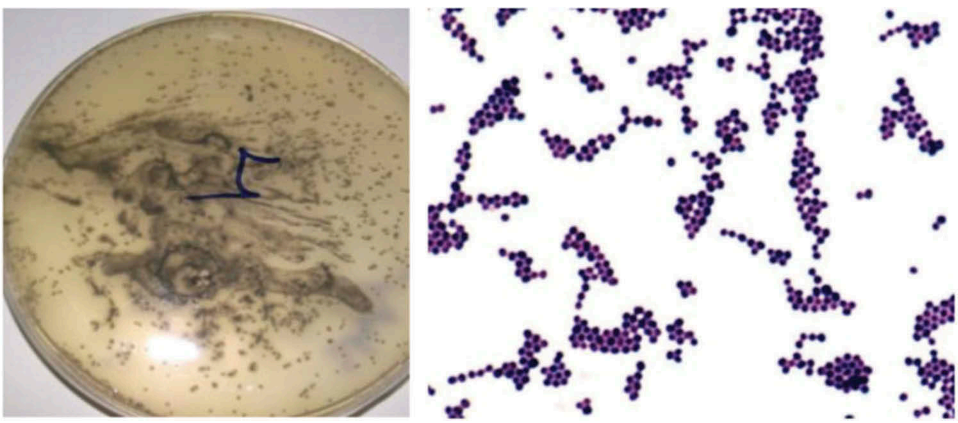
a-Colony morphology b-GP100X IM

Figure 9. a) Colony formation of *Lactobacillus rhamnosus* on Skim Milk Agar (flat, shiny, white). b) Gram-positive staining of *L. rhamnosus* (Under 100X light microscope).



a- Colony morphology b-GP100X IM

Figure 10. a) Colony formation of *Lactococcus lactis* on MRS Agar (1.5–3.0 mm soft, opaque/white). b) Gram-positive staining (Under 100X light microscope).



a-Colony morphology b-GP100X IM

Figure 11. a) Colony formation of *Staphylococcus spp*. on Baird Parker agar (1.5–2.0 mm, black). b) Gram-positive staining of *Staphylococcus spp*. (Under 100X light microscope).

Table 3. Inhibitory effect of commercial and traditional kefir on certain pathogens.

	Activity		
Microorganisms	Control inoculation count (cfu/gmL)	Count after commercial kefir application (cfu/gmL)	Count after traditional kefir application (cfu/gmL)
Enterobacter cloacae	2.60E+09	2.90E+06	1.20E+06
Escherichia coli	3.50E+08	2.40E+06	3.20E+05
Enterococcus faecalis	6.20E+08	2.10E+05	5.30E+05
Enterobacter cloacae	2.60E+09	2.90E+06	1.20E+06

bile salts into free acids, allowing faster removal of conjugated bile salts from the intestinal tract. Since free bile salts are excreted from the body, the synthesis of new bile acids from cholesterol can reduce the total cholesterol concentration in the body.[19]

Table 4. Mann-Whitney test results regarding the comparison of microorganism numbers according to control and commercial kefir groups.

	Groups	n	Mean	SD	Min	Max	Z	p
Microorganisms count	Control	3	1.19E+09	1.23E+09	3.50E+08	2.60E+09	−1.964	.050*
	Commercial Kefir	3	1.84E+06	1.43E+06	2.10E+05	2.90E+06		

*p<0.05 was accepted as statistically significance.

Table 5. Mann-Whitney test results regarding the comparison of microorganism numbers according to the control and the traditional kefir groups.

	Group	n	Mean	SD.	Min	Max	Z	p
Microorganisms count	Control	3	1.19E+09	1.23E+09	3.50E+08	2.60E+09	−1.964	.050*
	Traditional Kefir	3	6.83E+05	4.60E+05	3.20E+05	1.20E+06		

*p<0.05 was accepted as statistically significance.

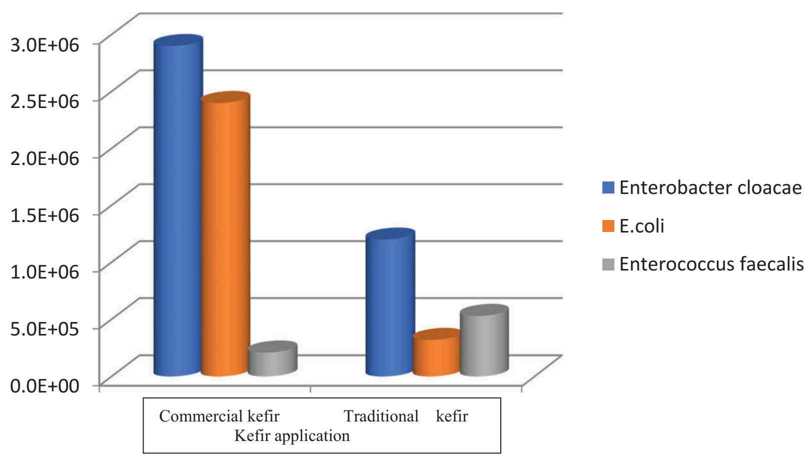


Figure 12. Inhibitory effect of commercial and traditional kefir on certain pathogens.

The fact that *Saccharomyces spp*. directly passes through the digestive tract, that its optimum temperature requirement is 37° C and that it inhibits the development of many pathogenic microorganisms indicate its probiotic specificity.[20] Witthuhn et al. have reported that LAB in kefir grains range from 6.40E+04 cfu/gmL to 8.50E+08 cfu/gmL and yeast levels range from 1.50E+05 to 3.70E+08 cfu/gmL. Yeast levels in various kefir products have been reported to range from 1E+03 cfu/gmL to 1E+06 cfu/gmL. The microbial content results of South African household kefir was consistent with our results.[21] Yeasts provide crucial growth nutrients including amino acids and vitamins, also they change pH, produce secret ethanol and CO_2 and they are known to have a key role in fermented dairy products. They are more enduring and show more diverse enzymatic profiles compared to lactic acid bacteria, although they make up only about 1% of the kefir microorganism.[22] Increasing body of evidence suggests that the yeast species from kefir can be used as probiotics.[23]

Besides the fact that the yeasts in grains provide a better environment for the growth of bacteria in kefir, the metabolites yeasts produce better flavor and mouthfeel. Previous research on that era shows that kefir has above 20 species of yeast, and the predominant ones among these can be defined as *Candida* and *Saccharomyces*.[22,24] However, research on the yeasts in kefir is less than bacteria research in the literature.

The traditional kefir used in this research was microbially very dissimilar to commercial kefir. Commercial kefir contains *Lactobacillus acidophilus, Lactobacillus bulgaricus, Saccharomyces spp., Lactobacillus plantarum, Streptococcus thermophilus, Lactobacillus reuteri, Lactococcus lactis*. In contrast, traditional kefir contains *Lactobacillus acidophilus, Lactobacillus bulgaricus, Saccharomyces spp., Lactobacillus plantarum, Streptococcus thermophilus, Lactobacillus reuteri, Lactococcus lactis, Lactobacillus johnsonii, Lactobacillus rhamnosus*, as well as *Bifidobacterium longum, Bifidobacterium animalis.*

The microbial population of kefir is important in terms of research on food quality control and bioactive products that can be produced from them. Kefir samples have diverse spectrum of bacterial genera: These include *Lactobacillus* (six species), *Lactococcus* (1), *Saccharomyces* (1), *Streptococcus* (1) and *Bifidobacterium* (2). The bacterial species identified in the current study are given in Tables 1 and 2. We found that the number of *lactobacilli* (10^9cfu/g) was higher than *lactococci* (10^7cfu/g) in kefir grains and this result is in accordance with the findings of previous studies.[25,26] Lactic acid bacteria are the major acid producers in kefir fermentation. In fermentation, the bacteria release certain compounds that can effect the health either positively or negatively. Some examples of bacteria that cause positive effects include *Lactobacillus, Streptococcus*, and *Bifidobacteria* of lactic acid bacteria, which are the most significant acid producers during the process. Many previous studies have shown that bifidobacteria can prevent or help treating colorectal cancer.[27] Another property of probiotics is that they can improve the intestinal microbial balance, where they protect the microbiota from exogenous pathogens.[27] Bifidobacteria are anaerobic microorganisms, usually growing in 10% CO_2 environments. However, some species can tolerate oxygen. Bifidobacteria differ from lactobacilli by the carbohydrates they use in their metabolic activities. Bifidobacteria use the fructose-6 phosphate pathway in hexose metabolism, while the lactobacilli use the glucose-6 phosphate pathway. Important products formed at the end of this pathway are acetic acid and L (+) lactic acid. They do not form high acids O_2 from glucose, and their catalase, nitrate reduction, indole formation and gelatin hydrolysis are negative.[28]

L. plantarum often is the dominant *Lactobacillus* species in traditional fermented prevalent species included *L. fermentum, L. brevis, L. casei, E. facecalis, E. faecium*. Except for *E. faecium* and *E. faecalis*, they were also dominant species in local fermented food. Contrary to our expectations, there were no bifidobacteria in commercial kefir and these results are in agreement with the results of Hall et al.[29]

Even though many studies have been conducted through the years, the microbiological composition of kefir grains is still not fully known. The composition of kefir microflora depends heavily on the origin of the grains, the local culture conditions and the processes of storage and elaboration.[30] The findings of our study revealed that kefir has stronger inhibitory activities against *Enterobacter cloacae, Escherichia coli* and *Enterococcus faecalis* strains than the control group. The precise microbial growth inhibition mechanism is not known, as it may be due to the antagonistic action of different microorganisms in kefir. These microorganisms can prevent the accession, establishment, replication and pathogenic action of certain pathogenic microorganisms.[31] Recent studies on humans have shown that probiotics can alter the gut microbiota, thus improving metabolic functions.[32] These include organic acids (lactic and acetic acids), carbon dioxide, hydrogen peroxide, ethanol, diacetyl and peptides (bacteriocins) that can reduce food pathogens, prevent bacterial damage in storage and food intake and help in treating and preventing gastro-intestinal and vaginal infections. Lactic and volatile fatty acids penetrate the microbial cell membrane and lead to the acidification of the cytoplasm and the inhibition of enzyme activity. This may be the explanation for the inhibitory activity against *Escherichia coli*, and *Salmonella* sp. The acids dissociate at higher intracellular pH levels and produce hydrogen ions. These then interfere with crucial metabolic functions including oxidative phosphorylation, which may explain the inhibitory activity against aerobic species.[33] It is though that the antibacterial activity against different pathogens may be due to organic acids and specific antibodies that are produced by acetic acid bacteria, yeasts[34] and undissociated lactic and acetic acids produced in fermentation[30] or hydrogen peroxide produced by LAB.[35] In the study of Santos et al., the antagonistic property of isolated strains of lactobacilli against *E. coli* (43/58 strains), *L. monocytogenes* (28/58 strains), *Salmonella typhimurium* (10/58 strains), *S. enteritidis* (22/58 strains), *Shigella flexneri* (36/58 strains) and *Y. entero*colitica (47/58 strains) have been emphasized.[36]

Conclusion

In conclusion, probiotic microorganisms can result in many positive effects on health, including positively modulating the intestinal microbiota and controlling diseases associated with dysbiosis (e.g., obesity). The positive effects of probiotics are owed to the intrinsic properties of the strains and the quantity of viable cells ingested every day. Kefir is composed of various species of lactic acid bacteria, acetic acid bacteria and yeasts and has approved effects on obesity.[28] Probiotic bacteria produce inhibitory antimicrobial peptides (microsine, bacteriocin) that inhibit the growth of pathogenic bacteria. Although there are several micro-organisms that produce bacteriocins, those produced by the LAB are of particular interest to the dairy industry. LAB have long been used in a variety of food fermentations by converting lactose to lactic acid, as well as producing additional antimicrobial molecules such as other organic acids, diacetyl, acetoin, hydrogen peroxide, antifungal peptides, and bacteriocins.[37] Lactic acid bacteria bacteriocins are often active across a range of pH values, resistant to high temperatures and active against a range of food pathogenic and spoilage bacteria.[38] In addition, LAB bacteriocins are sensitive to digestive proteases such as pancreatin complex, trypsin and chymotrypsin, and thus do not impact negatively on the gut microbiota.[37] Research shows that *S. boulardii* suppresses the reproduction of *Candida albicans, Salmonella typhi, Shigella, Escherichia coli*. They prevent adhesion of pathogens; probiotic bacteria make it difficult for pathogens to enter the epithelial cells of the intestine and urogenital system thanks to their number and volume advantage. They strengthen the epithelial barrier and prevent translocation of pathogens. They inhibit the reproduction of pathogens by consuming the nutrients they need to reproduce; *S. boulardii* inhibits the reproduction of *C. difficile* by consuming up the monosaccharides needed by colostridium difficile. They affect intestinal enzyme activity: probiotic bacteria increase lactase, maltase and sucrase activity. *S. boulardii* polyamines allow the maturation of aminopeptidases.[39]

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