International Archive of Applied Sciences and Technology
Int. Arch. App. Sci. Technol; Vol 9 [1] March 2018: 01-05

[ISO9001: 2008 Certified Organization]
www.soeagra.com/iaast.html

IAAST
ONLINE ISSN 2277- 1565
PRINT ISSN 0976 - 4828

CODEN: IAASCA ORIGINAL ARTICLE

DOI: .10.15515/iaast.0976-4828.9.1.15

Enumeration of microflora of kefir grains and kefirprepared from cow milk and goat milk

Vyshnavi Manthani[a*], Srikanth keerthi[a] and PrabhaRao[b]
a Department of Dairy Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru-24, Karnataka, India
a Department of Dairy Technology, PV Narasimharao Telangana Veterinary University, Hyderabad, Telangana, India
b Department of Dairy Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru-24, Karnataka, India
Corresponding author's Email address: aishu14teddy@gmail.com

ABSTRACT
Kefir is a thick, sour and slightly spritzy fermented milk drink produced through the action of lactic acid bacteria and yeasts present in the milk kefir grains. Kefir grains are elastic, gelatinous masses of uneven surface looking like a waxy cauliflower substance, yellow-white in colour and have a slimy but firm texture with a characteristic smell.Within kefir 'grains', a classic example of a SCOBY (Symbiotic Community of Bacteria and Yeasts) existed. Kefir grain cultured in irrespective of type of milk showed 7 log countof lactic acid bacteria while yeast was in 6 log count. Kefir grain cultured in cow milk showed slightly more count of lactobacilli among lactic acid bacteria compared to that of grains in goat milk.The viable lactic counts in both cow milk and goat milk kefir were nearly 8 log counts except leuconostoc (around 9 log) only in case of goat milk kefir. Yeast counts were around 5 log but counts were slightly more in goat milk kefir compared to cow milk kefir. There was no significant difference existed between the viable counts of lactic and yeast in cow and goat milk kefir as per statistical analysis.
Key words: Kefir grain; SCOBY; Lactic acid bacteria; Fermented milk drink

Received 22/11/2017 Revised 20/12/2017 Accepted 09/02/2018

Citation of this article
V Manthani, Srikanth keerthi and Prabha Rao· Enumeration of microflora of kefir grains and kefirprepared fromcow milk and goat milk. Int. Arch. App. Sci. Technol; Vol 8 [4] December 2017. 01-05.

INTRODUCTION

Fermentation of milk using lactic cultures as inoculum increases the shelf life and also improves nutritive value. Fermented milk products made with certain strains of lactic acid bacteria and lactose fermenting yeasts or the milk product obtained through yeast-lactic fermentations normally showed presence of lactic acid, ethanol and carbon dioxide having consistency of butter milk and fizziness due to carbon dioxide produced by yeast fermentation. Major alcoholic fermented milk products included kefir, kumiss and acidophilus-yeast milk [1].

Kefir is an acidic, viscous, self-carbonated dairy beverage. The fermentation of milk to prepare kefir is induced by small, white, cauliflower-shaped masses named kefir grains and considered to possess high nutritional and functional properties. Kefir has a smooth and creamy texture with an acidic, slightly alcoholic and yeasty taste; the presence of carbon dioxide gives a varying degree of effervescence. Kefir is a natural probiotic drink containing live bacteria, yeasts with sharp acidic taste and yeasty flavour. Lactose hydrolysis during fermentation was due to simultaneous action of bacteria and yeasts contained in kefir grains [2].

Microflora of kefir grains

Kefir grains are referred as small living communities of bacteria, yeast and kefiran, which existin solid, gummy, grain-like masses those multiply when properly cared. When the grains were seeded in raw cow milk, pasteurized, whole fat, low fat milk, after successive fermentations, they grow, propagate and breakup into new generation grains, pass the same characteristics as the old grains[3];[4].Culturing cow's milk produces a thick, smooth milk kefir and even **g**oat milk is also becoming popular for culturing. As per Polish standards, grains should consist of 80% lactobacilli, 12% yeasts and 8% lactococci.The genus *Lactococcus* tends to grow faster than yeast in milk because of its high capacity to metabolize lactose. This genus hydrolyzes lactose, produces lactic acid and a suitable environment is created for lactobacilli and yeast growth [5]. Yeasts and lactobacilli genus are mutually dependent and grow in balanced proportions in kefir grains, and symbiosis between yeasts, lactobacilli and lactococci was observed during the production of kefir [6].Yeasts synthesize complex B vitamins and hydrolyze milk proteins, by using oxygen to produce CO_2 and ethanol [6].

They kefir grains microflora when analyzed found them as variable, depending on the source of the grain[7]. *Lactobacillus kefiranofaciens* produce kefiran, aheteropolysaccharide composed of equal proportions of glucose and galactose.*Lactobacillus kefiranofaciens*and *Lb. kefiri*were the major bacterial populations in all kefir grains as identified by [8]. When the whole kefir grain was examined the researcherfound that the outer layer of the grain consists of lactococci and yeasts in the inner layer of the grain, the quantity of lactobacilli were much higher[6].Chen *et al.*(2015) enumerated the microflora of kefir grains as mixture of lactic acid bacteria (10^8cfu/g), yeast (10^6–10^7cfu/g) that stick to a polysaccharide matrix with symbiotic association[8]. They also found that the microbial composition of kefir and kefir grains vary depending on geographic, climatic and cultural conditions as well as the diversity of local species of wild yeasts and bacteria.

MATERIALS AND METHODS

Milk samplesand milk kefir grains:

Cow milk samples were collected from Dairy farm, Department of ILFC, Veterinary College, KVAFSU, Hebbal, Bengaluru-24. While goat milk samples were collected from Sinchana Private Goat farm located in Baglur, Bengaluru North. The samples were transported to the laboratory under refrigerated condition. The milk kefir grains purchased from HSR layout, Bengaluruwere preserved in cow and goat milk separately and analyzed for the microflora.

Enumeration of cultured kefir grains and kefir

Kefir grains cultured in cow and goat milk separately and kefir prepared using cow and goat milk were serially diluted using sterile 2% sodium citrate diluent for the enumeration of lactobacilli and leuconostoconMan Rogosa and Sharpe medium (MRS,Himedia); lactococcion M17 medium (Himedia) at incubation of 30°C in candle jar for 24-48 h. Yeast counts were carried out on Malt extract agar medium (pH-3.5) at incubation temperature of 30°C under aerobic conditions for 3 to 5 days respectively.After the completion of the incubation period, the colonies were counted in countable plates ranging between 30 -300 and average count was expressed as cfu/g of product.

Statistical analysis

The data obtained in the present study was analyzed using one way ANNOVA and multi linear Regression analysis using R software (R. version 3.1.3 (2015-3-09) to calculate mean and F values to prove significant or non-significant effect of parameters adopted.

RESULT AND DISCUSSION

Enumeration of lactic and yeast counts present in commercial kefir grains cultured in cow and goat milk

Kefir grains purchased from HSR layout, Bengaluru appeared as clumps of cooked rice with cauliflower texture and slimy naturehaving diameter of 2 cm (Plate 1). In agreement to the above statement [6] also considered that kefir grains look like small clumps of cauliflower, gelatinous to touch and whitish to yellowish in colour. A similar observation by [8] also revealed kefir grains as elastic, gelatinous masses of uneven surface looks like a waxy cauliflower substance, yellow-white in colour, and have a slimy but firm texture with a

characteristic smell. The grains are water insoluble and vary in size ranging from few millimeters to 4 cm in diameter.

The viable counts of lactic and yeast counts on an average in both cow milk cultured kefir grain and goat milk cultured kefir grains were around 7 logs and 6 logs respectively.Among the viable log count of lactic acid bacteria, lactobacilli in cow milk kefir grain was found to be slightly more accounting for 7.47 followed by lactococci of 7.35; leuconostocs of 7.30 while yeast count was 6.20 $\log_{10}$cfu/g whereas goat milk kefir grain revealed slightly more viable count of lactococci (7.35) followed by lactobacilli of 7.17, leuconostocs of 7.12 while yeast count was 6.43$\log_{10}$cfu/g.The viable counts of leuconostocs and lactobacilli showed significant difference (p<0.05) between cow and goat milk kefir grains, whereas the differences between viable counts of lactococci and yeast were insignificant (Table 1).

On par with the present study, [6] showed lactic acid bacterial count ranging from $6.4x10^4$ to $8.5x10^8$ and yeast levels present in kefir grains varied widely, ranging from and $1.5x10^5$ to $3.7x10^8$cfu/g. The microflora of Turkish kefir grains had ratio of 10^9:10^6 of lactic acid bacteria and yeasts, with lactobacilli species predominating, and no significant fluctuation during storage as enumerated by[8]. In researchthe scientists found lactobacilli species to be the predominant lactic acid bacteria type (78%) in Brazilian kefir grains, with lactococci comprising the majority of the remaining 28 % of lactic acid bacterial species[2]. As per [5], the microflora of kefir grains contain mixture of lactic acid bacteria (10^8cfu/g), yeast (10^6–10^7cfu/g), that stick to a polysaccharide matrix with symbiotic association. They also found that the microbial composition of kefir and kefir grains vary depending on geographic, climatic and cultural conditions as well as the diversity of local species of wild yeasts and bacteria.

Enumeration of lactic and yeast counts in kefir prepared from cow and goat milk

The cow and goat milk kefir prepared using commercial kefir grains were subjected for the enumeration of lactic acid bacteria and yeast. The samples were enumerated for lactic acid bacteria such as lactococci, leuconostocs and lactobacilli and also for yeast counts. The cow milk kefir showed slightly higher viable log count of lactobacilli of 8.78 followed by lactococci of 8.48, leuconostocs of 8.36 and yeast of 5.13.

The goat milk kefir surprisingly showed predomination of viable log count of leuconostoc as 9.02 followed by lactobacilli of 8.67 and lactococci of 8.64.The yeast count was slightly lower accounting for 5.93$\log_{10}$cfu/g (Table 2). All the viable lactic counts in both cow milk and goat milk kefir were nearly 8 log counts except leuconostoc (around 9 log) only in case of goat milk kefir. Yeast counts were around 5 log but counts were slightly more in goat milk kefir compared to cow milk kefir. There was no significant difference existed between the viable counts of lactic and yeast in cow and goat milk kefir as per statistical analysis.

Most of the research studies on kefir microflora found viable counts of lactobacilli, lactococci and yeast; only few have observed the presence of leuconostoc. But in the present study, leuconostoc counts were more in goat milk kefir. But viable counts of remaining lactic acid bacteria and yeasts were almost similar to the present study. In six samples of kefir from Iraq, after fermentation of the grains, the total counting of bacteria reported by [9] was of 10^9cfu/gfor *Lactococcus*, 10^8cfu/gfor *Leuconostoc,* 10^5cfu/gfor *Lactobacillus*, 10^5cfu/gfor acetic acid producers and 10^5cfu/gfor yeasts. In South African household kefir, yeast levels as high as 8$\log_{10}$cfu/g were found [2]. The viable population of 10^8cfu/g of lactobacilli and lactococci and 10^5cfu/g of yeasts after 24 h of fermentation[4]. The amounts of yeast in kefir vary, with reported values ranging from 10^3 to 10^6[10].

Plate 1: Purchased kefir grains

Table 1: Enumeration of lactic and yeast counts in commercial kefir grains cultured in cow and goat milk

Type of kefir grain	Type of count			
	Lactococci	Leuconostoc	Lactobacilli	Yeast
	log_{10}cfu/g			
Cow kefir grain	7.35±0.97[a]	7.30±0.88[a]	**7.47±1.31[a]**	6.20±0.48[a]
Goat kefir grain	**7.35±0.54**[a]	7.12±0.46[b]	7.17±0.57[b]	6.43±0.15[a]
CD (p≤0.05)	1.32 (NS)	0.97*	1.18*	0.58(NS)

Note:

- Mean ± SD(Standard Deviation)
- Differing superscripts along the column indicates significant differences
- CD – Critical Difference
- Higher count value among other counts is made bold
- NS – not significant

Table 2: Enumeration of lactic and yeast counts in kefir prepared from cowand goat milk kefir

Type of milk kefir	Type of count			
	Lactococci	Leuconostoc	Lactobacilli	Yeast
	log_{10} cfu/g			
Cow milk kefir	8.48±0.36	8.36±0.51	**8.78±0.30**	5.13±0.72
Goat milk kefir	8.64±0.52	**9.02±0.26**	8.67±0.12	5.93±1.09
CD(p≤0.05)	1.13 (NS)	1.62 (NS)	0.36 (NS)	2.82 (NS)

Note:

- Mean ± SD(Standard Deviation)
- CD – Critical Difference
- Higher count value among other counts is made bold

CONCLUSION

Kefir grain cultured in either cow or goat milk revealed 7 log count of lactic acid bacteria while yeast was 6 log count. Kefir grain cultured in cow milk showed slightly more count of lactobacilli among lactic acid bacteria compared to that of grains in goat milk, which showed more lactococcal count. All the lactic counts were in 8 log, only slight increase in leuconsotoc was noticed in goat milk kefir than in cow milk kefir. There was no significant difference existed between the viable counts of lactic and yeast in cow and goat milk kefir. As per the present study, even cow milk could be used to prepare kefir as well as to maintain kefir grains as the microflora were almost similar to that found in goat milk kefir.

ACKNOWLEDGEMENT

The present study had been a part of post-graduation research work taken up in the Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Bengaluru – 24, Karnataka, India. We take this opportunity to thank the staff of the department for supporting in this study.

REFERENCES

1. S. Otles and O. Cagindi, "Kefir: A Probiotic Dairy-Composition, Nutritional and Therapeutic Aspects," *Pakistan J. Nutr.*, vol. 2, no. 2, pp. 54–59, 2003.
2. S. Arslan, "A review: chemical, microbiological and nutritional characteristics of kefir," *CyTA - J. Food*, vol. 13, no. 3, pp. 340–345, 2014.
3. E. Simova, D. Beshkova, a Angelov, T. Hristozova, G. Frengova, and Z. Spasov, "Lactic acid bacteria and yeasts in kefir grains and kefir made from them.," *J. Ind. Microbiol. Biotechnol.*, vol. 28, no. 1, pp. 1–6, 2002.
4. A. Irigoyen, I. Arana, M. Castiella, P. Torre, and F. C. Ibáñez, "Microbiological, physicochemical, and sensory characteristics of kefir during storage," *Food Chem.*, vol. 90, no. 4, pp. 613–620, 2005.
5. M. R. Prado *et al.*, "Milk kefir: Composition, microbial cultures, biological activities, and related products," *Front. Microbiol.*, vol. 6, no. OCT, pp. 1–10, 2015.
6. T. Pogačić, S. Šinko, Š. Zamberlin, and D. Samaržija, "Microbiota of kefir grains," *Mljekarstvo*, vol. 63, no. 1, pp. 3–14, 2013.
7. K. Zajsek and A. Gorsek, "Experimental Assessment of the Impact of Cultivation Conditions on Kefiran Production by the Mixed Microflora Imbedded in Kefir Grains," 2011.
8. M. R. Prado *et al.*, "Milk kefir: Composition, microbial cultures, biological activities, and related products," *Front. Microbiol.*, vol. 6, no. OCT, 2015.
9. J. Lim, "Hedonic scaling: A review of methods and theory," *Food Qual. Prefer.*, vol. 22, no. 8, pp. 733–747, 2011.
10. T. Submitted, "FLOW CHART ON GRADING GROUP 9.pdf," no. August, 2011.