Observations of Kefir Grains and Their Structure From Different Geographical Regions: Turkey and Germany

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Summary: It is well known that kefir grains from different locations have various microbiological compositions and it is not certain that there is comparison between different samples. The mechanism of grain formation is not clear. In the previous studies no authors have reported the presence of kefir grains with a globular, sagy structure resembling small pouches . In this study we showed a distinct kefir grain formation exist after cauliflower-like structures and sheet-like structures.

Key words: Kefir, kefir grains, structure.

Farklı Coğrafi Bölgelerdeki Kefir Taneleri ve Kefir Tanelerinin Yapısının Gözlemlenmesi: Almanya ve Türkiye

Özet: Farklı bölgelerden elde edilen kefir tanelerinin değişik mikrobiyel kompozisyonları olduğu bilinmektedir ve farklı örnekler arasında benzerliğin olduğu da kesin değildir. Kefir taneciğinin oluşum mekanizması açık değildir. Önceki çalışmalarda, küçük cep şeklinde ve globüler tarzda kefir taneciklerinin varlığı rapor edilmemişti. Bu çalışmada, daha önceki çalışmalar da var olan karnabahar ve yaprak benzeri taneciklerin yanında, farklı bir kefir taneciği biçiminin de olduğunu gösterdik.

Anahtar kelimeler: Kefir, kefir taneciği, yapı

INTRODUCTION

In recent years there have been more interest in different fermented milk products known only to particular countries with a view to adapting them for commercial large-scale production in other parts of world. The best known of these in the Western world are yoghurt, cheese, acidophilus milk, kefir, koumiss and yakult. The names of the well-known fermented milks such as yoghurt, ayran/airan and probably koumys/koumiss come from Turkish Language (Gerhard, 1976; Kurmann, 1984), so too is the name of kefir which is thought to originate from Turkish word 'key(i)f' meaning 'good feeling' for the sense of well being experienced after drinking it (Novil, 1998).

Kefir is a self-carbonated, lactic sour, fermented milk beverage and produced by co-incident lactic acid and alcohol fermentation. It is made from whole or skim milk and produced by adding a unique culture 'kefir garin' to the fresh milk. The culture contains a group of micro-organisms dominated by *lactobacillus* spp. / yeast population in the forms of grains because the organisms are embedded in a resilient insoluble polysacharide matrix called 'kefiran' (La Riviere *et. al*., 1967). Traditional kefir cannot be prepared without kefir grains. These must be recovered in gelatinous form from kefir beverage after fermentation and they cannot be re-constructed from individual microbial components. This differs from other fermented milk products such as buttermilk and yoghurt which are made with a suspension of growing cell evenly distributed in milk.

Kefir beverage and kefir grains have a long history, consumed and produced across a wide geographical region (Yaman, 2000). The products and grain have therefore been studied in different regions (Angulo *et. al*., 1993; Beshkova *et. al*., 2002; Graciela *et. al*., 2001; Petterson *et. al*., 1985; Pintado *et. al*., 1996; Rea *et. al*., 1996; Wszolek *et. al*., 2001; Yoshida and Toyashima, 1994). It is not certain that there is comparison between different kefir grain samples. The published data, therefore on morphology and microbiological content of kefir and kefir grains used different samples from different sources can be expected to vary. The mechanism of grain formation is not clear and attempts at making kefir grains from pure or crude cultures have not been successful (Hirota and Kikuchi 1976; Koroleva, 1980; Lipatov, 1978).

In this study, we examined kefir grains obtained from two different geographical regions (Turkey and Germany) and compared them with the data available in the published literature.

MATERIALS AND METHODS

Kefir grains (100/g wet wt) were obtained from two different geographical locations. So called 'Turkish kefir grains' were purchased

from Ege University, Agriculture Faculty, Dairy Science and Technology Department in Izmir, Turkey. So called 'German kefir grains' were obtained in excange with Turkish kefir grains from a German veterinarian whose aunty has been making kefir at home for a long time in Germany**.** Both kefir grains were propagated daily in same brand UHT (ultra high temperature) whole milk sold in the market. Milk was incubated at 25°C for 24 hrs each time. The grains were filtered through cheesecloth for naked-eye and electron microscopes.

The specimens for electron microscopy were kept for 24 hours in glutaraldehyde-paraformaldehyde pref-fixing (pH 7.4) according to the Karnovsky's method (1965). It was then rinsed for 3 hours in a cacodylate buffer and fixed for a second time in a 1% (w/v) osmium solution for 2 hours at 25°C. Then, specimens were kept in 0.5 % uranyl acetate solution for 2 hours. After washing in distilled water, specimens were dehydrated in an ethanol series: 15%, 30%, 50% and 70% for 15 min each and were pass through propylene oxide. They were embedded in araldite M. Thin sections (1 micron) were stained with toludine blue. 300-400 angstrom thin sections were contrasted according to the method of Veneable and Coggeshall (1965), and examined in a Carl Zeiss EM 9S-2 model transmission electron microscope (Zeiss, Oberkochen, Germany).

RESULTS

Naked-eye examination of kefir grains showed two distinct structures in Turkish and German kefir grains. Turkish kefir grains were yellowish, cauliflower-like structures as previously described by La Rivière *et. al*. (1967), Kosikowski (1977), Molska *et. al*. (1980), Yoshida and Toyashima (1994). In addition to these, small thinner sheet-like structures and scroll-like forms were observed as found by Marshall *et. al*. (1984) in their samples. Figure 1 shows the varying types which ranges from thin flat sheets (Fig.1b) to the cauliflower floret forms (Fig.1a,c,d,e,f). These structures were elastic and quite tough. Marshall *et. al*. (1984) found that one side of the sheet was smooth and flat, the other side was convoluted and rough. Light and scanning electron microscopy showed this asymetry more clearly and revealed that a population of yeast and short *lactobacillus* spp. occupied the convoluted side in their samples. German kefir grains however showed a very different structure by naked-eye examination. They were much bigger than Turkish kefir grains in diameter and differed from Turkish kefir grains and the grains examined previously by other researchers (Bottazi and Bianchi, 1980;

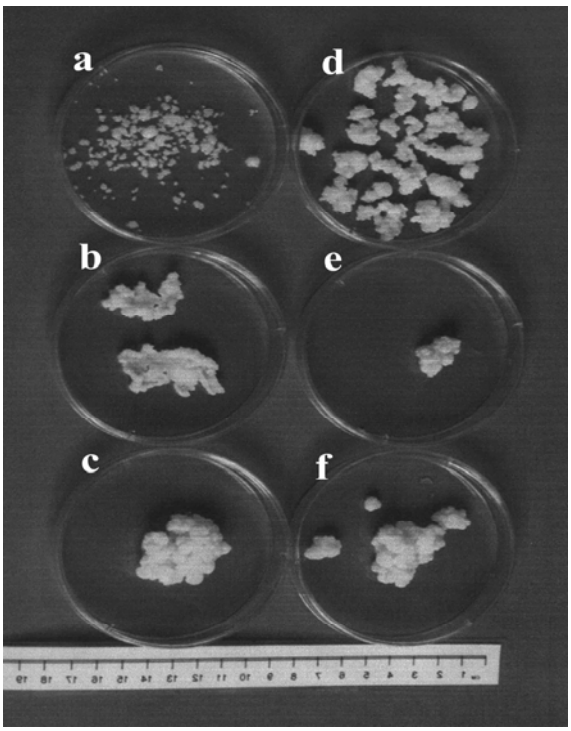

Figure 1. Various structures of Turkish kefir grains. a, c, d, e, f. Different sizes of Turkish kefir grains resembling cauliflower-like structures. b. Sheet-like structures of Turkish kefir grains.

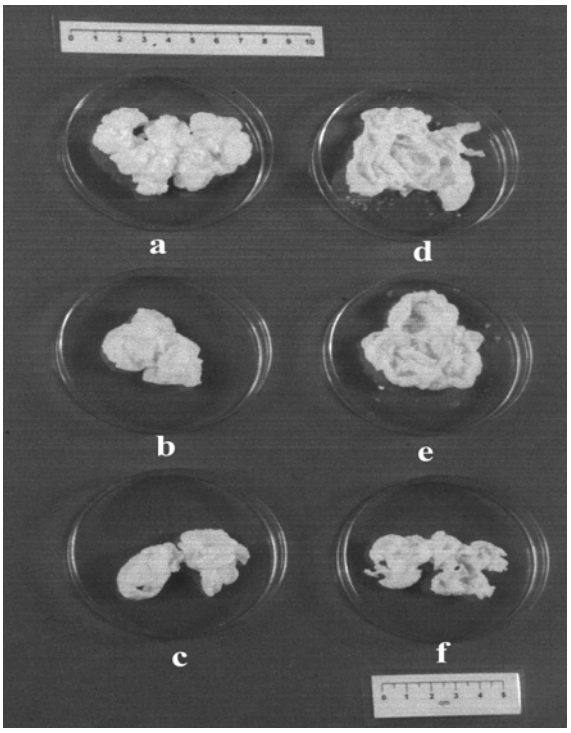

Figure 2. Samples of German kefir grains in sagy forms. a) A mature German kefir grain with two globular pouches, b) A mature German kefir grain with one pouch, c) A developing German kefir grain with two globular pouches, d), e), f) Cut-open structures of the samples a, b, c,respectively.

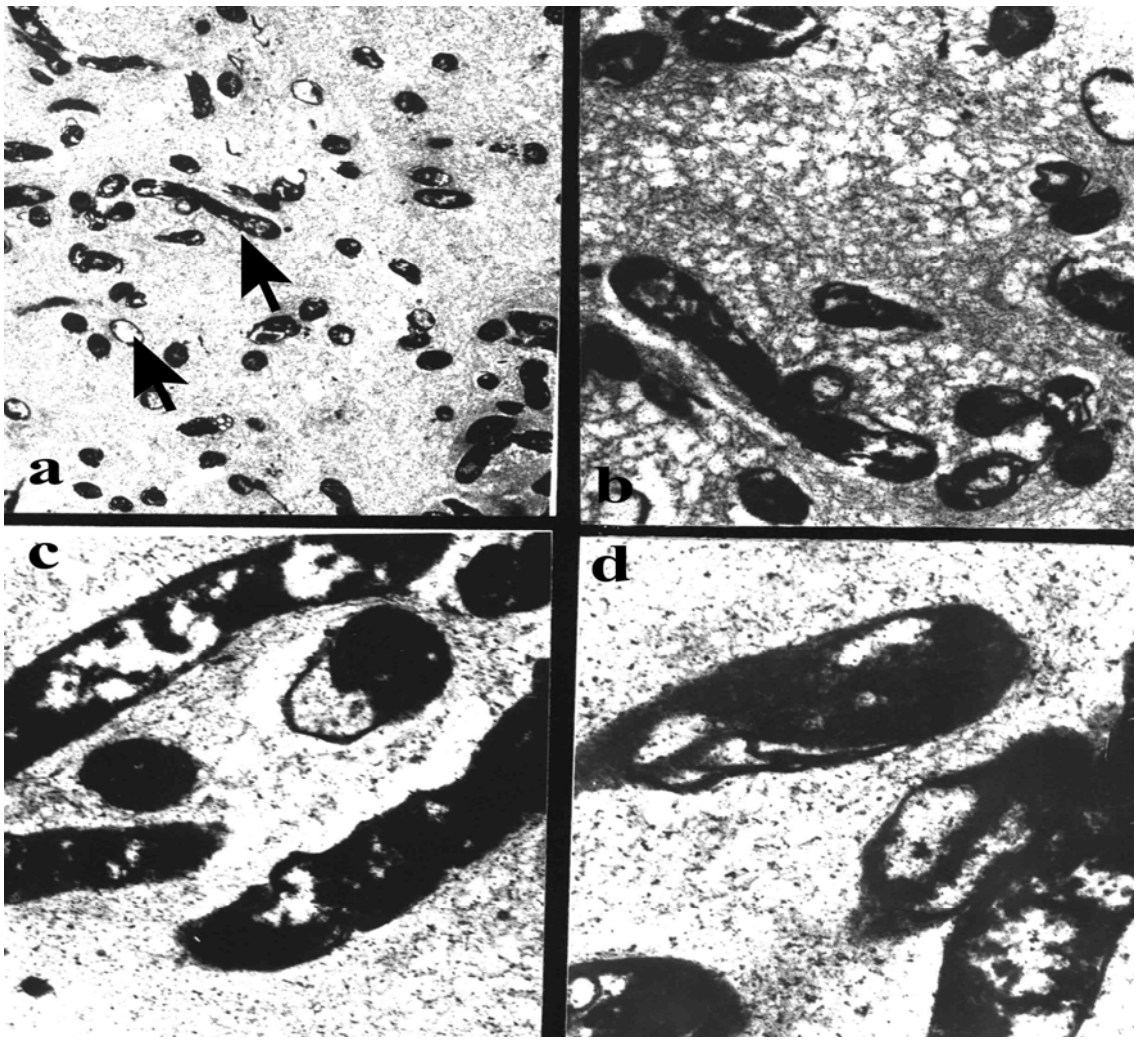

Figure 3. Transmission electron micrographs of German kefir grains. a) Arrow above showing long rod-shaped Gram (+) bacteria embedded in the fibrillar matrix, arrow below depicting vacuolated bacteria, X6500, b) X21000, c) X31000, d) X47000.

Marshall, 1984; Neve, 1992; Toba *et. al*., 1990) in terms of how these grains developed and formed globular, but sagy structure resembling a small pouch or a few pouches connected in the middle. This is the first time we came across with such a structure and there is no knowledge about this type of kefir grains in published literature so far in our knowledge. Figure 2 shows this different structure of kefir grains which happened to be a German kefir grain. Figure 2a had two pouches before cutting it open in a sheet-like form (Fig. 2d). Figure 2b shows a single and Figure 2c shows a smaller two pouched kefir grain. Their structures are presented in Figure 2e and Figure 2f after cutting them open respectively. It can be seen clearly that outside of the pouches are rough and the inner sides are smooth. This is similiar with the observations of Marshall *et. al*. (1984) in sheet-like flat forms. Transmission electron microscopy showed more details of their structure (Fig. 3). There were some long organisms possessing the cell wall structure of a Gram positive bacteria (Glauert and Thorley, 1969; Marshall, 1984). Some bacteria were intact as their cell walls were clearly defined whereas the cytoplasm of others appeared vacuolated (Fig. 3a). These bacteria were embedded in a dense fibrillar matrix (Bottazi and Bianchi, 1980; Marshall, 1984; Neve, 1992). This study did not show such a clear-cut distinction in the size of bacteria when different areas of the exterior and interior of the samples were investigated.

DISCUSSION

Under naked eye obsevations, kefir grains on recovery from milk are large, shiny and globular, with milk solids adhering to their surfaces. They are quite large, up to 6 cm, but on washing with water they are of various smaller sizes (0.5-3.5cm diameter) and characterized as an irregular form. Mature grains resemble miniature cauliflower florets in shape. They are yellowish in colour and have an elasticity when pulled apart. Turkish grains fit

into this defination. However, the German grains we observed differ from this known structure.

Kefir grains used for making kefir are a complex ecosystem which is stable, but not always the same. Different grains show different compositions, but at least a part of composition is stable in that many of the organisms isolated are common to all types. Visual examination of kefir grain is superficial and information obtained is limited compared to other methods available. The light microscopy (Dmitrichenko, 1976; Duitschaever *et. al*., 1988; Feofilova, 1958; Ottagalli *et. al*., 1973; Rosi, 1978) and scanning electron microscopy observations (Bottazi and Bianchi, 1980; Duitschaever *et. al*., 1988; Marshall, 1984; Molska *et al*., 1980; Neve, 1992) of the micro-structure of grains demonstrates an arranged microflora of rod-shaped and oval shaped-shaped micro-organism embedded in an extracellular spongy polymer. The peripheral part of the grain is densely populated by a micro-flora of short rod-shaped, but also, with long rod-shaped micro-organisms (*lactobacillus* spp.), while the inner part is filled with fibrous material in which oval-shaped micro-organisms (yeasts) are trapped. However, different arrangments have been found by different workers, some have reported that on propagation there are no particular discrete arrangements of the microflora (Bottazi and Bianchi, 1980; Molska *et. al*., 1980; Rosi, 1978; Toba *et. al*., 1990). These differences may be related to the ways of how grains are formed. Marshall *et. al*. (1984) suggested that kefir grains arise from the curling of flat sheet-like structures with subsequent folding and re-folding accompanied by increased thickening as the microflora multiplies and insoluble carbohydrate accumulates to form grain. Bottazi and Bianchi (1980) reported that *lactobacillus* spp. were predominant at the edge of the grain and the yeast at the centre, but the centre of the grain may have been the convoluted surface that had become inside as a consequence of folding. These suggestions however do not explain how these German kefir grains may have been formed in the shape of pouches and continue to do so. This is still to be determined. More detailed research is required to solve the mystery of kefir grain microbiology and its formation.

REFERENCES

Angulo, L., Lopez, E., Lema, C., 1993. Microflora present in kefir grains of the Galician region (North-West of Spain). J. Dairy Res., 60, 263-267.

Beshkova, DM., Simova, ED., Simov, ZI., Frengova, GI., Spasov, ZN., 2002. Pure cultures for making kefir. Food Microbiol., 19, 537-544.

Bottazi, V., Bianchi, F., 1980. A note on scanning Electron microscopy of micro organisms associated with the kefir granule. J. Appl. Bacteriol., 48, 265-268.

Dmitrichenko, MI., 1976. Microstructure and composition of kefir grains. Sovershentvovanie tekhnologicheskikh protsessor v molochnoi promyshlennosti. Tom I Chast' II. Leningard. U.S.S.R.: Leninradskii Tekhnologicheskii Institut Kkoldil'noi Promyshlennosti 43-46. In: Dairy Sci. Abst. 1976 38, 1066.

Duitschaever, CL., Kemp, N, Smith, AK., 1988. Microscopic studies of the microflora of kefir grains and kefir made by different methods. Milchwissensch., 43,8, 479-481.

Feofilova, EP., 1958. Microflora of kefir grains. Dairy Sci. Abst., 20, 1889.

Gerhard, JH., 1976. Alcoholic beverages and fermented foods. Indust. Microbiol., 1st ed., pp.165-191. USA.

Glauert, AM., Thorley, MJ. 1969. The topography of the bacterial cell wall. Ann. Rev. Microbiol., 23, 159-198.

Graciela, LG., Analía GA., Graciela, LA. 2001, Chemical and microbiological characterisation of kefir grains. J. Dairy Res. 68, 639-652.

Hirota, T., Kikuchi, T., 1976. Studies on kefir grains. I. Isolation and classification of micro-organisms from kefir grains and their characteristics. Reports of Res. Snow Brand Milk Products Co. Laboratory. No:74, 63-82.

Karnovsky, MJ., 1965. A formaldehyde-glutaraldehyde fixative of high osmality for use in electron microscopy. J. Cell Biol., 27, 137A-138A.

Koroleva, NS., 1980. Tiechniceskaja mikrobiologia kislomolocnych produktow. pišc. Prom., Moskwa, In: Molska, I., Kocen, I., Zmarlicki, S., 1980. Electron microscopic studies on structural and microflora of kefir grains. Acta Aliment. Polonica., Vol. VI, No.3, 145-154.

Kosikowski, F., 1977. Cheese and Fermented Products, 2nd ed., pp. 40-41, Ann Arbor, Michigan: Edwards Brothers Inc. USA.

Kurmann, JA., 1984. Fermented milks. In procedings of the International Dairy Federation (IDF) seminar in Avignon (France). Doc. 179, pp. 16-26.

Lipatov, NN., 1978. Fermented milks other than yoghurt. XXth Int. Dairy Congress, Paris, 43 ST.

La Riviere, JW., Kooiman, P., Schmidt, K., 1967. Kefiran; a novel polysaccharide produced in the kefir grain by *Lactobacillus brevis*. Arc. Microbiol., 59, 269-278.

Marshall, V., 1984. Observations on the structure of kefir grains and the distribution of the Microflora. J. Appl. Bacteriol., 57, 491-497.

Molska, I., Kocen, I., Zmarlicki, S., 1980. Electron microscopic studies on the structure and microflora of kefir grains. Acta Aliment. Polonica, Vol. VI, No.3, 145-154.

Neve, H., 1992. Analysis of kefir grain starter cultures by scanning electron microscopy. Milchwissensch., 47 (5), 275-278.

Novil, S., 1998. Kefir. Lifeway Foods, Inc, 7625 N. Austin Ave. Skokie, IL 60077, USA, 1998, Personal communication.

Ottagalli, G., Galli, A., Resmini, A., Volonterio, P., 1973. Microbiological and chemical composition and ultrastructure of kefir grains. Ann. Microbiol Enzy., 23:109-121.

Pettersson, HE., Christiansson, A., Ekelund, K., 1985. Making kefir without grains. Scandinavian J. Dairy Tech. and Know-How/Nm 2-85 (Nordisk Mejeriindustri 1985,12,8, 58-60.

Pintado, EM., Silva, LJA., Fernandes, BP., Malcata, FX., Hogg, TA., 1996. Microbiological and rheological studies on Portuguse kefir grains. J. Food Sci. Techn., 31, 15-26.

Rea, MC., Lennartsson, T., Dillon, P., Drian, FD., Rewille, WJ., Heapes, M., Cogan, TM., 1996. Irish kefir-like grains: their structure, microbial composition and fermentation kinetics. J. Appl. Bacteriol., 81, 83-94.

Rosi, J., 1978. I microorganismi del kefir: Gli acetobatteri (in Italian). Scienza Technica Lattiera-Casearia, 29, 221-227, In: Angulo, L., Lopez, E., Lema, C., 1993. Microflora present in kefir grains of the Galician Region (North-west of Spain). J. Dairy Res., 60, 263-267.

Toba, T., Arihara, K., Adachi, S., 1990. Distribution of micro-organisms with particular reference to encapsulated bacteria in kefir grains. Int. J. Food Microbiol., 10, 219-224.

Wszolek, M., Tamime, AY., Muir, DD., Barclay, NI., 2001. Properties of kefir made in Scotland and Poland using bovine, caprine, and ovine milk with different starter cultures. Lebensm.-Wiss. u.-Techn., 34, 251-261.

Veneable, JH., Coggeshall, R., 1965. A simplified lead citrate stain for use in electron Microscopy. J. Cell Biol., 25, 407-408.

Yaman, H., 2000 Partial characterisation of *lactobacilli* isolated from commercial kefir Grain. PhD thesis. Huddersfield University, Huddersfield, UK.

Yoshida, T., Toyashima, K., 1994. Lactic acid bacteria and yeast from kefir (in Japanese, English summary). J. Japanese Society Nut. Food Sci., 1994 47, 55-59.