Kefiran, a Novel Polysaccharide
Produced in the Kefir Grain by *Lactobacillus brevis*

J. W. M. LA RIVIERE and P. KOOMAN

International Course in Sanitary Engineering and Laboratory of Microbiology; Laboratory of General and Technical Biology, Technological University, Delft, The Netherlands

KARIN SCHMIDT
Institut für Mikrobiologie der Universität, Göttingen, Germany

Received April 20, 1967

Summary. 1. Almost half of the material embedding the microbial population of the kefir grain consists of kefiran, a polysaccharide consisting of equal amounts of galactose and glucose with an $\left[\alpha\right]_{D}^{20\circ} = +68.0^\circ$ ($C = 1$, $H_2O$).

2. Kefiran is the capsular material of the large rod-shaped bacteria which predominate in the grains and have the properties of *Lactobacillus brevis*.

Kefir is an acidic, mildly alcoholic, effervescent drink from the Caucasus. It is prepared by subjecting milk to fermentation by kefir grains, i.e., conglomerates of bacteria and yeasts; after one day fermentation at room temperature the acidified milk is separated from the grains and left to ferment for another day before it is consumed. The grains can immediately be re-used by immersing them in a fresh batch of milk. During the process, which can be repeated *ad infinitum*, the grains gain in weight, their volume varying between 0.5--20 ml/grain. Asepsis is not observed during the procedure.

Kefir is a popular beverage in Russia, where it is manufactured and marketed on a large scale. Grains are also sold to the public for domestic kefir preparation. In some instances (e.g., SCHULZ, 1946) kefir grains are produced from whey to serve as supplement in foods or pharmaceutical preparations.

It appears from the literature that the macroscopic appearance of the grains as well as the microscopic pictures of crushed grains show little or no variation (e.g., KERN, 1881; BEIJERINCK, 1889; FEOFILOVA, 1958). This suggests that at least part of the microbial composition of the grain is specific and constant, i.e., capable of surviving among numerous different microbes that can develop as a result of the lack of asepsis. This is a common feature of many time-honoured procedures for making fermented foods (HESSELTINE, 1965); essentially, these are continuous enrichment
cultures based on continued propagation of mixed microbial populations on a medium of constant composition. The constancy of the population is not preserved by asepsis but by—often mysterious—manipulation of cultural conditions.

Besides a remarkable problem in selection, the kefir grain presents the peculiar problem of its inception which is not common to most fermented foods: the literature does not contain any report of a successful attempt at reconstitution of the grain from crude or pure cultures, which suggests that the conditions for genesis differ from those for propagation of the grain.

Ecological factors initiating and maintaining stable mixed populations in the face of continuous infection are of obvious importance, for—once sufficiently known—they may afford control of the performance of such populations. A study of the ecology of the kefir grains was therefore undertaken.

Already at an early stage our attention was drawn to the remarkable mechanical properties of the grains: superficial examination of the white, lobed, cauliflower-like kefir grains shows that they consist of a slimy but extremely resilient material, in which the microbial population is firmly embedded. This is obviously of great survival value for the specific microbial population: selection takes place each time the fermented milk is poured off, carrying with it all suspended microbes not embedded in grains.

We describe here the part of our studies\(^1\) concerned with the embedding material, almost half of which was found to consist of a new polysaccharide, kefiran.

**Materials and Methods**

*Organisms.* Kefir grains were designated by their place of origin. Delft kefir grains were those from the stock of the Delft Laboratory of Microbiology, received in 1953 from Ir. J. O. Elema, Assen, The Netherlands. Russian grains were bought in a shop in Moscow in July 1966. Zürich kefir grains were received in the dried state from the Vereinigte Zürcher Molkereien, Zürich. Further samples of grains were donated by Chr. Hansen's Laboratory, Copenhagen, and the Bundesanstalt für Milchforschung, Kiel. A strain of *Bacillus megaterium*, producing a capsular polysaccharide, was received from Dr. J.-P. Aubert, Institut Pasteur, Paris.

*Cultivation Procedures.* Kefir grains were propagated at room temperature (20—25°C) by daily transfer — Sundays excepted — in pasteurized whole milk and sometimes in sterile skim milk or in whey. Whey was prepared by precipitating the casein from skim milk by adding 0.75 ml of 71\% lactic acid per 100 ml, centrifuging and readjusting the pH to 6 with KOH. Revival of dried kefir grains was attempted by the method of Burkey (1948).

Lactic acid bacteria from kefir grains were cultivated on MRS medium (Den Man et al., 1960) containing 2\% of lactose or glucose. Isolates were kept as stabs

---

\(^1\) Preliminary brief reports on part of the work were published earlier (La Rivière, 1963; Kooiman and La Rivière, 1966).