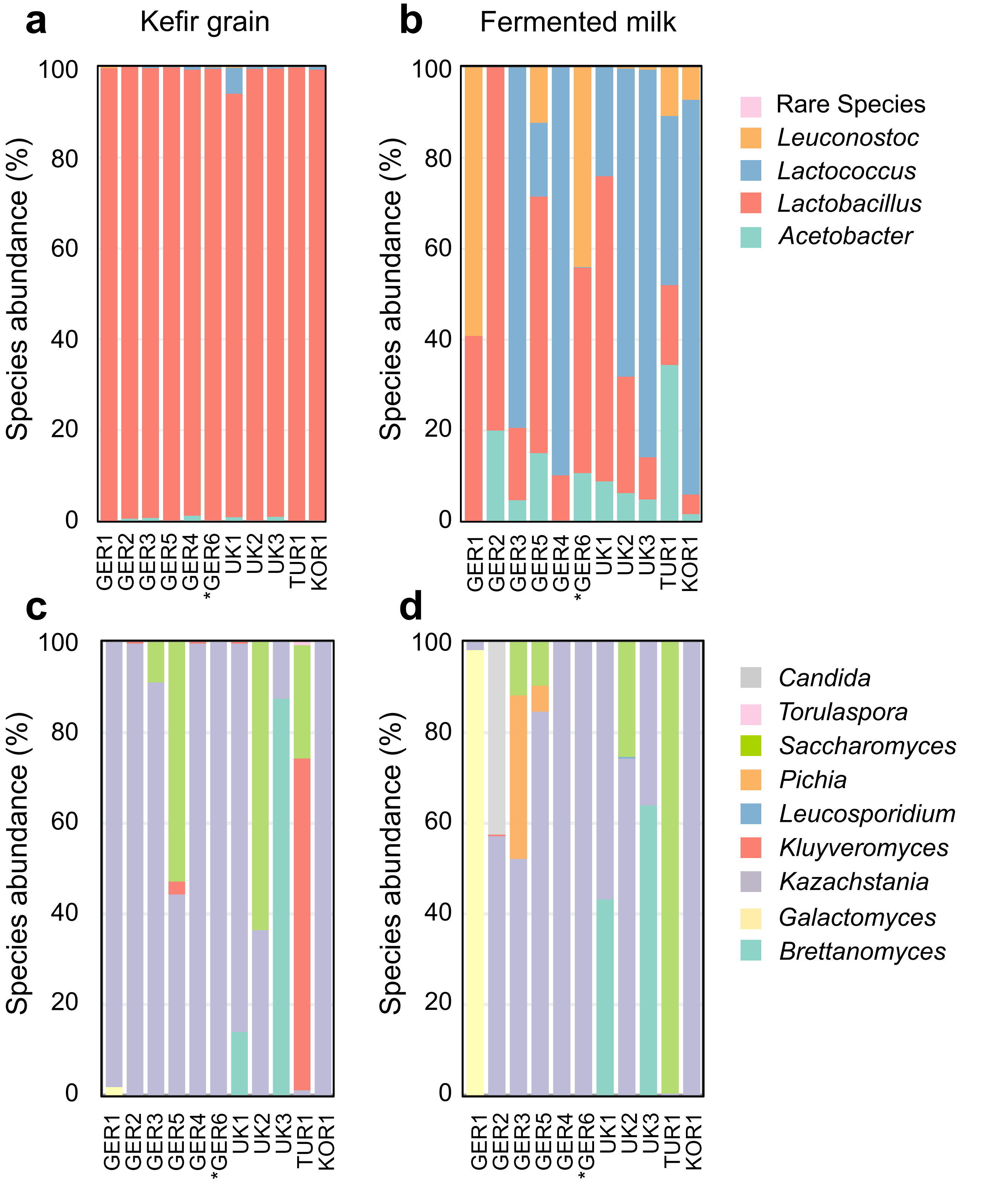
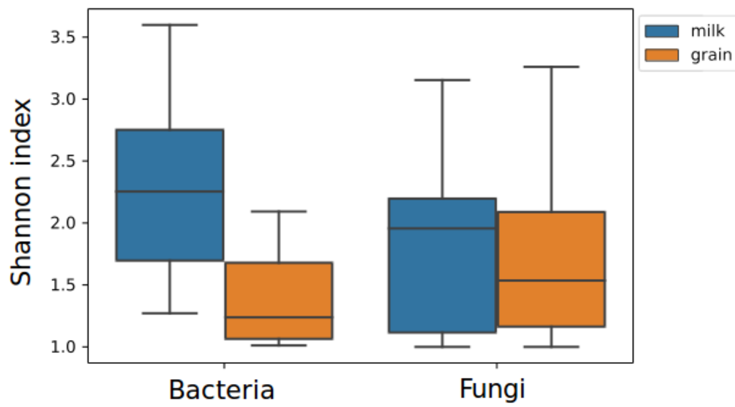
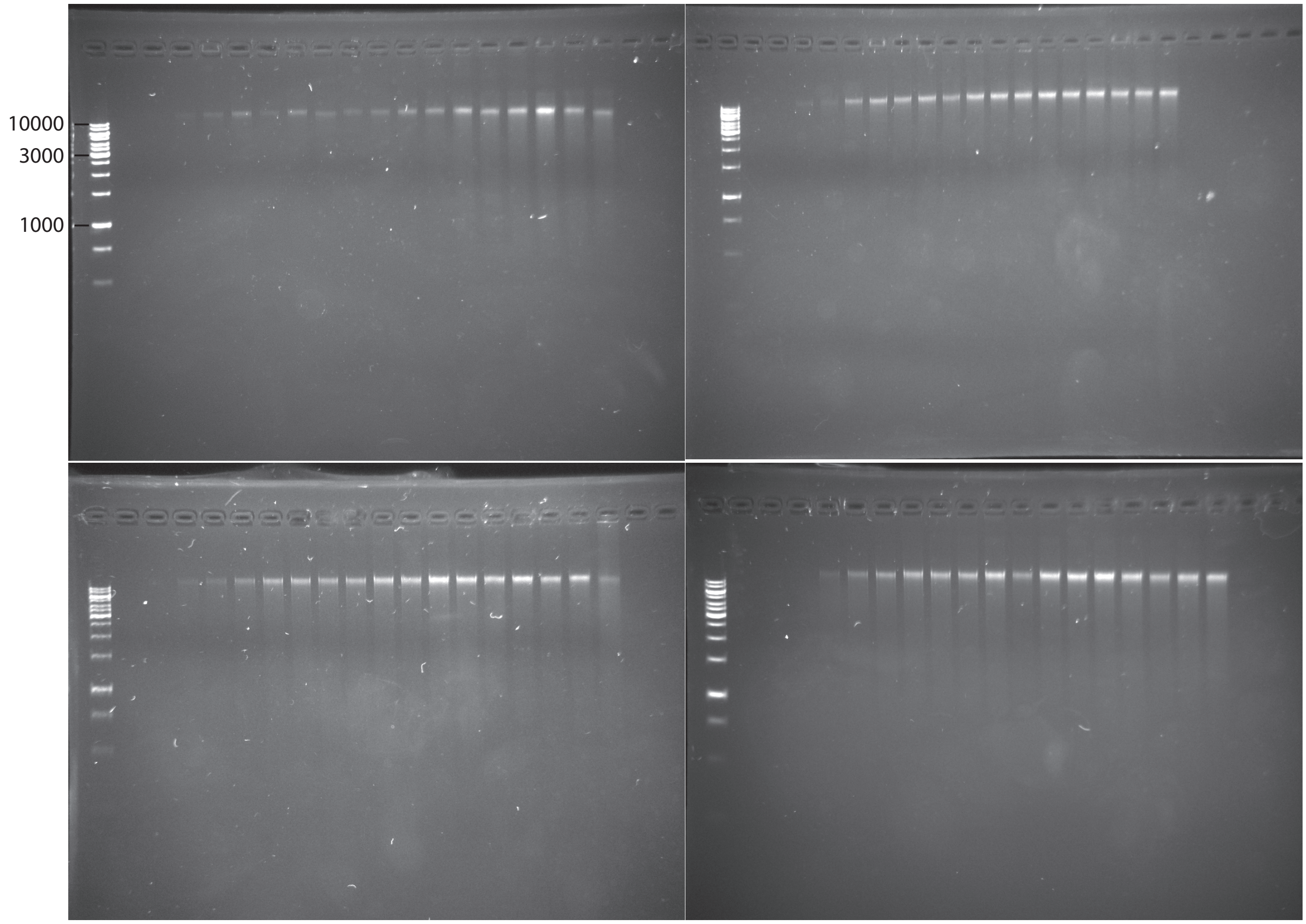


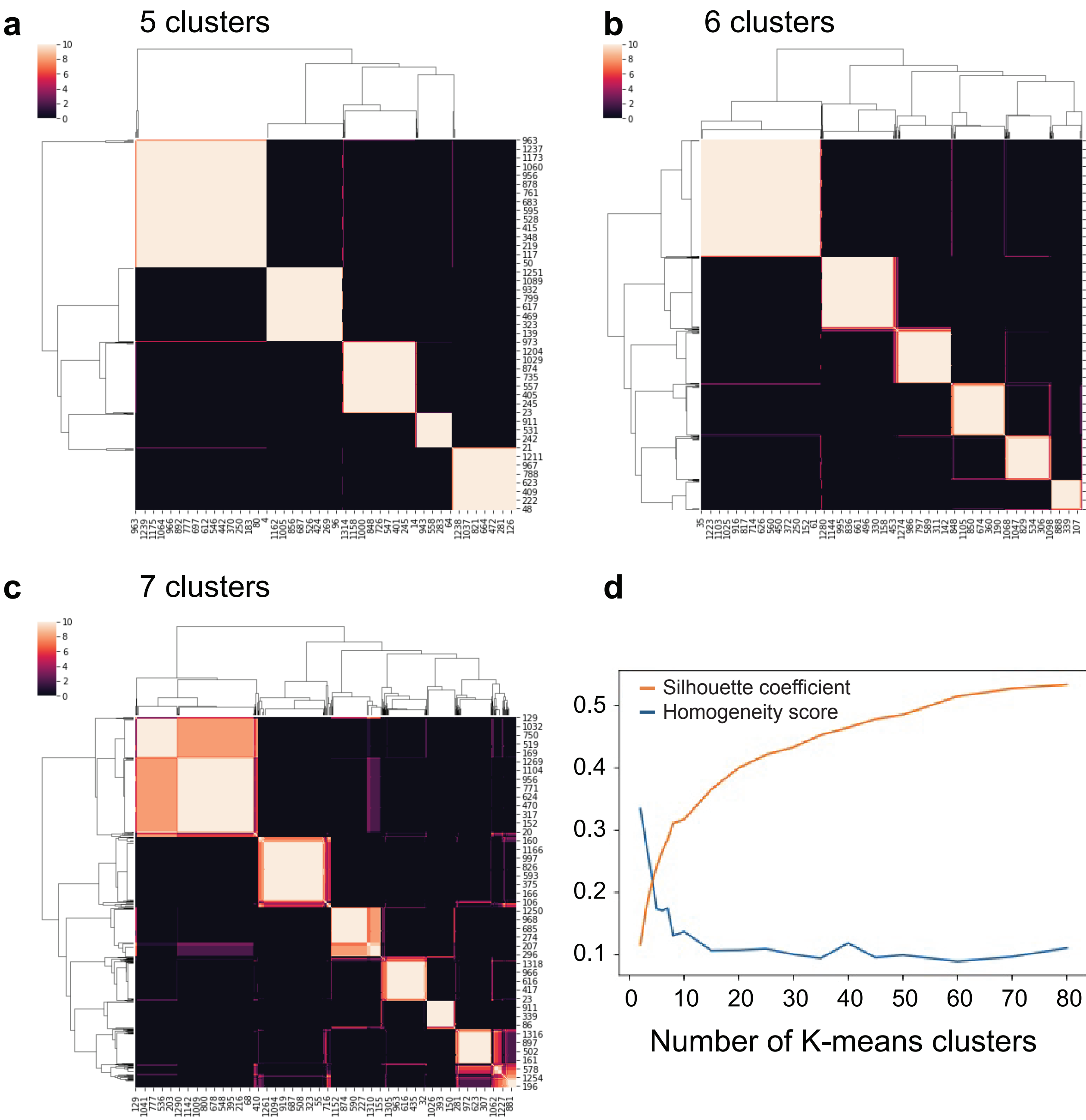
Supplementary Fig. 1 | Microbial composition of the kefir grain (a, c) and corresponding fermented milk (b, d), obtained from diverse geographical locations. Bacterial (**a, b**) and fungal (**c, d**) populations were measured using 16S rDNA and ITS amplicons, respectively. Rare species = relative abundance <0.01 %; GER6* = reference kefir grain (GER6, OG2) used for the subsequent analyses in this study.



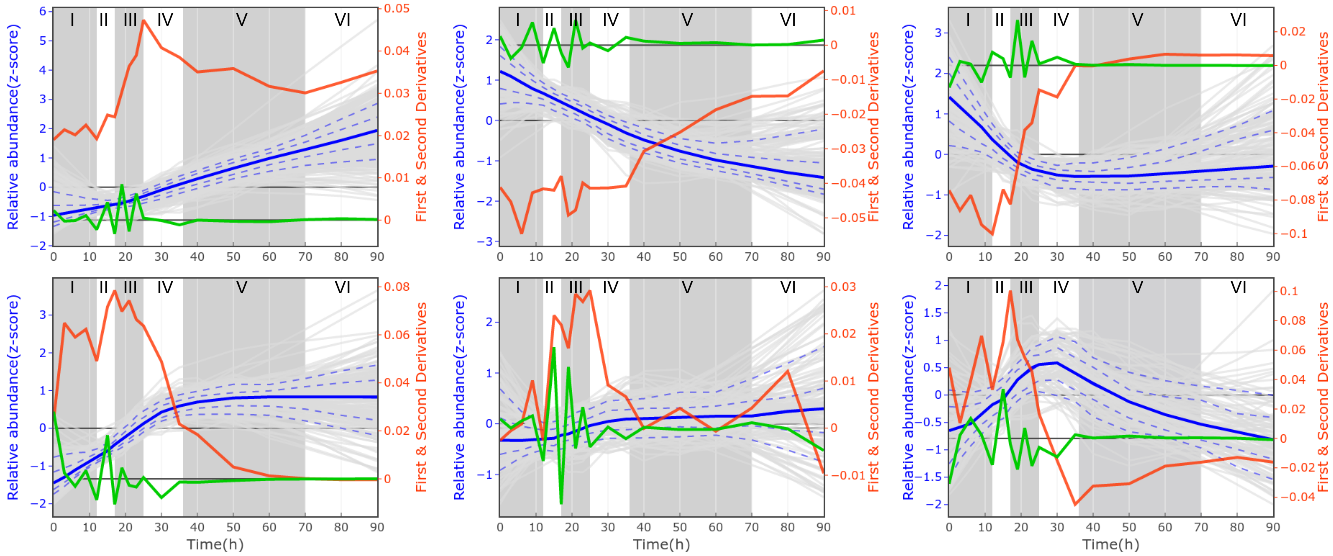
Supplementary Fig. 2 | Variation in the bacterial and fungal Shannon diversity index of the kefir grain and kefir fermented milk. N=4 biologically independent samples. The boxplot represents the interquartile range including the median, and the ends of the whiskers represent ±1.5× interquartile range.



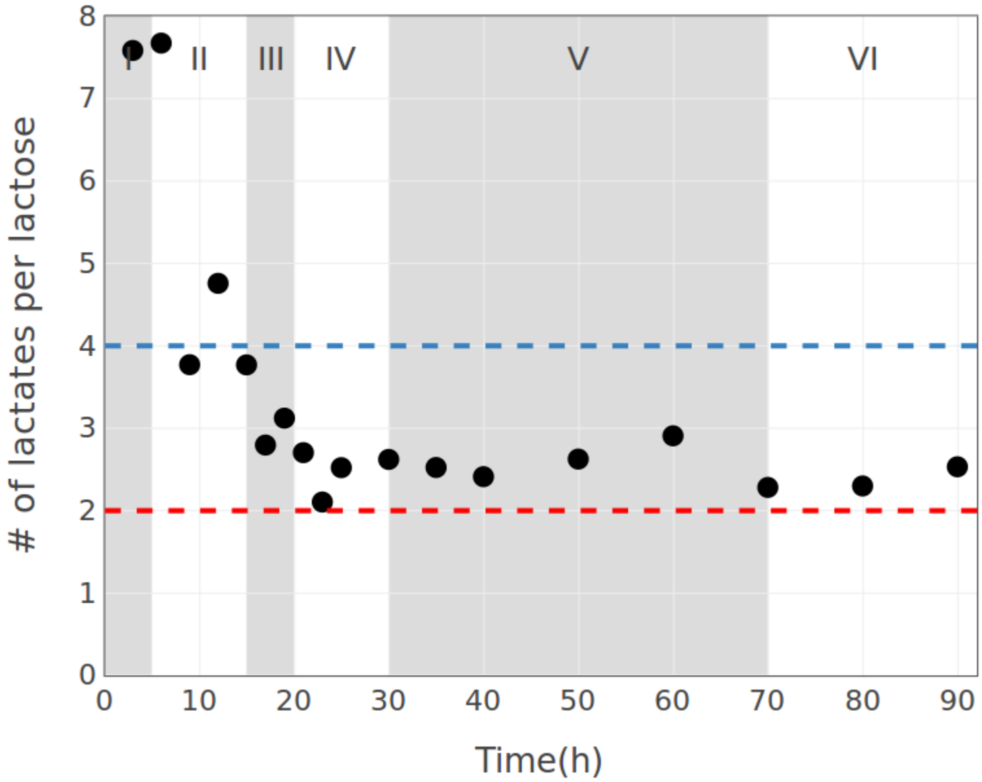
Supplementary Fig. 3 | Original agarose gel images related to Extended Data Fig. 1.



Supplementary Fig. 4 | K-means clustering of untargeted metabolomics data. a, 5 clusters, **b**, 6, clusters and **c**, 7 clusters. **d**, The graph shows the change in silhouette coefficient and homogeneity score as a function of the number of clusters. Separation in six clusters was found to provide a clear grouping of metabolites with similar dynamics.



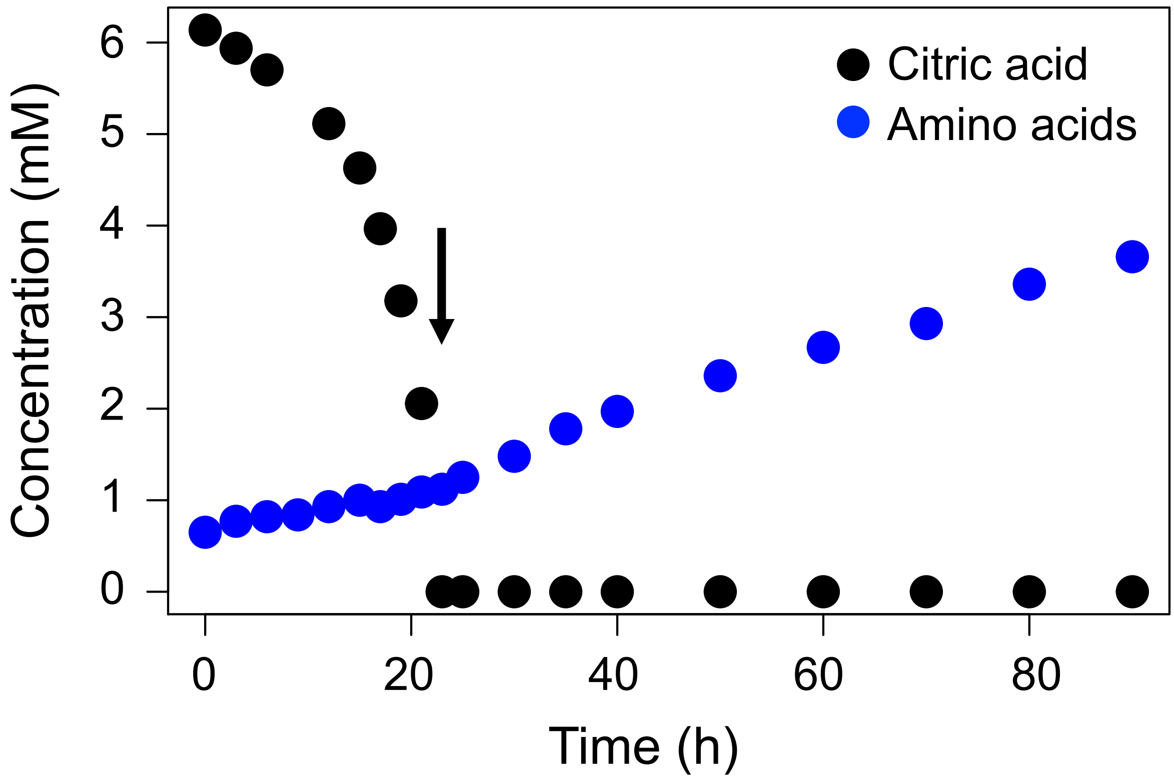
Supplementary Fig. 5 | First and second derivatives of metabolite dynamics match kefir fermentation stages. Data from FIA-qTOF MS analysis. Gray lines show ion profiles in each cluster (relative abundance, left y-axis). The solid blue lines denote the median ion profile for each cluster, while the dotted blue lines mark the quartiles. Red lines represent the first derivatives (right y-axis), and the green lines the second derivatives (right y-axis), of the median ion profile in the respective clusters.



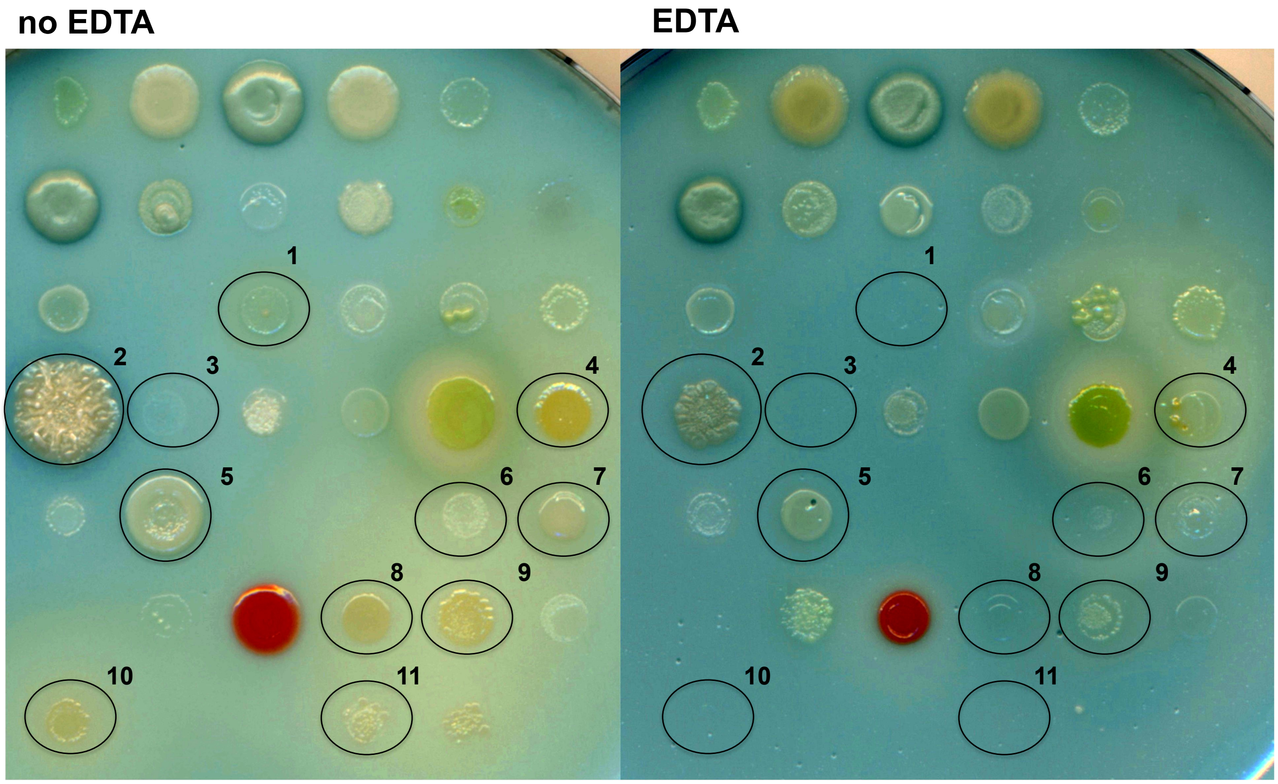
Supplementary Fig. 6 | Conversion of lactose into lactate during kefir fermentation. The dotted blue and red lines mark four and two lactates per lactose molecule, respectively.

Lactic acid fermentations resulting in a single, or multiple, end products are termed homofermentative and heterofermentative, respectively. The fermentation of lactose in a homofermentative manner results in four lactic acid molecules per molecule of lactose, whereas the heterofermentation produces two lactic acid molecules plus other products like acetic acid, ethanol and CO2.

We note that the higher apparent yields at two first time points are due to lactate carryover from kefir grain inoculum into fresh, lactate free, milk.

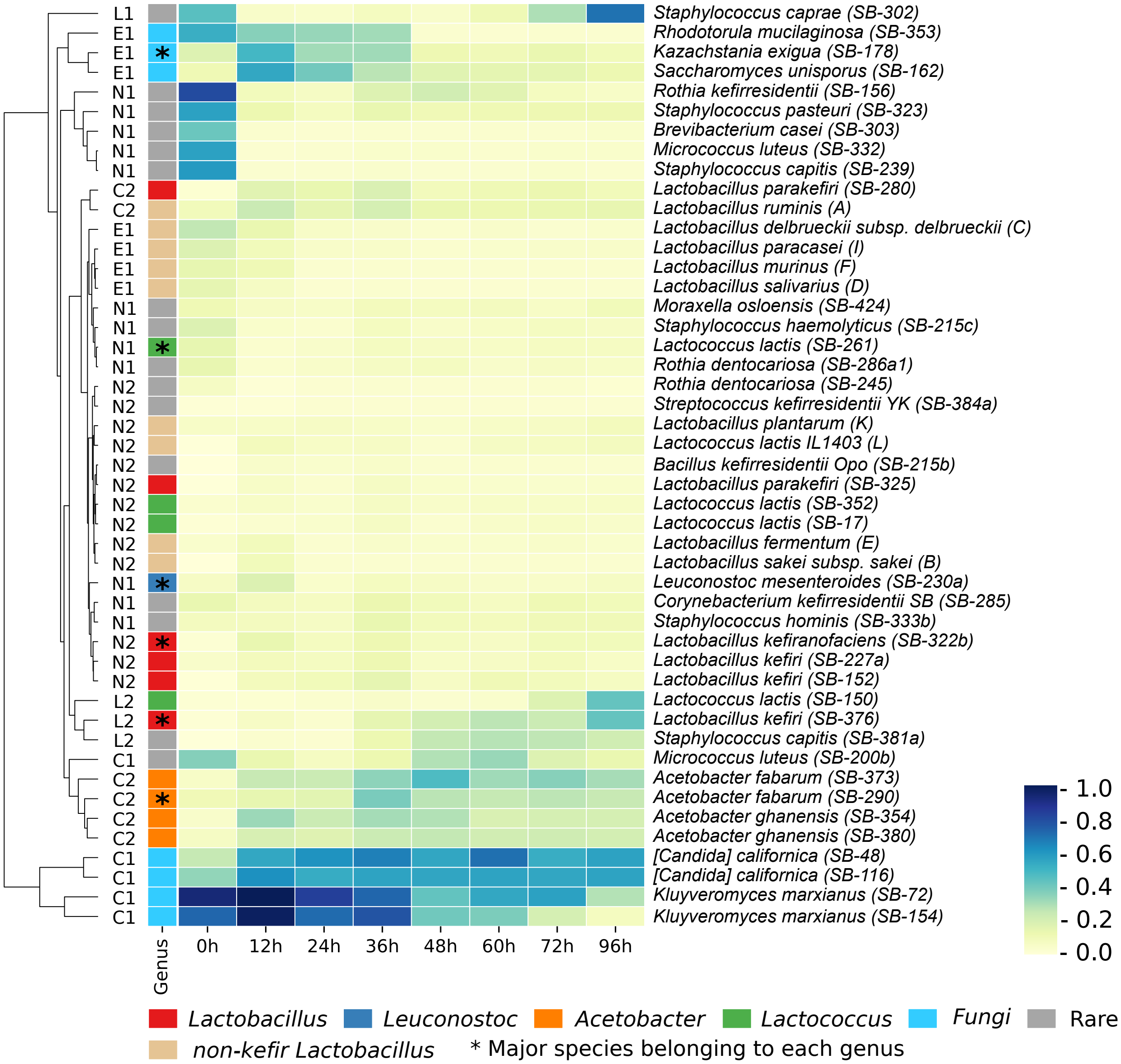


Supplementary Fig. 7 | Increase in free amino acids after citrate depletion during kefir fermentation. Amino acids = total amount of free amino acids in kefir fermented milk.

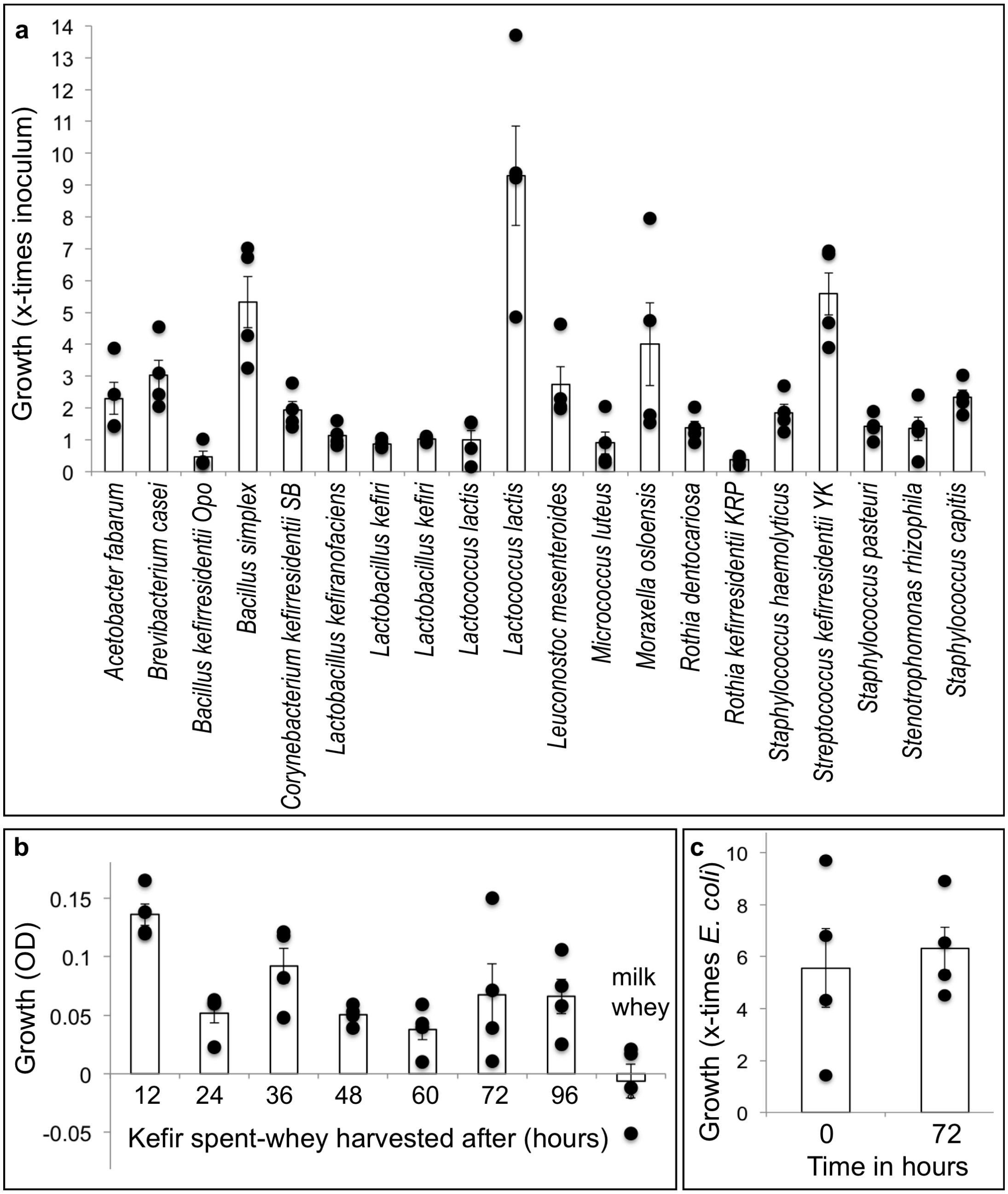


1	*Leuconostoc mesenteroides* (SB-230a)
2	*Bacillus simplex* (SB-271)
3	*Lactobacillus parakefiri* (SB-280)
4	*Acetobacter fabarum* (SB-290)
5	*Brevibacterium casei* (SB-303)
6	*Lactobacillus parakefiri* (SB-325)
7	*Micrococcus luteus* (SB-332)
8	*Acetobacter ghanensis* (SB-354)
9	*Acetobacter fabarum* (SB-373)
10	*Acetobacter ghanensis* (SB-380)
11	*Moraxella osloensis* (SB-424)

Supplementary Fig. 8 | Inhibition of kefir species by EDTA on milk-agar colored with bromocresol green. Most affected species are circled and listed (bottom).

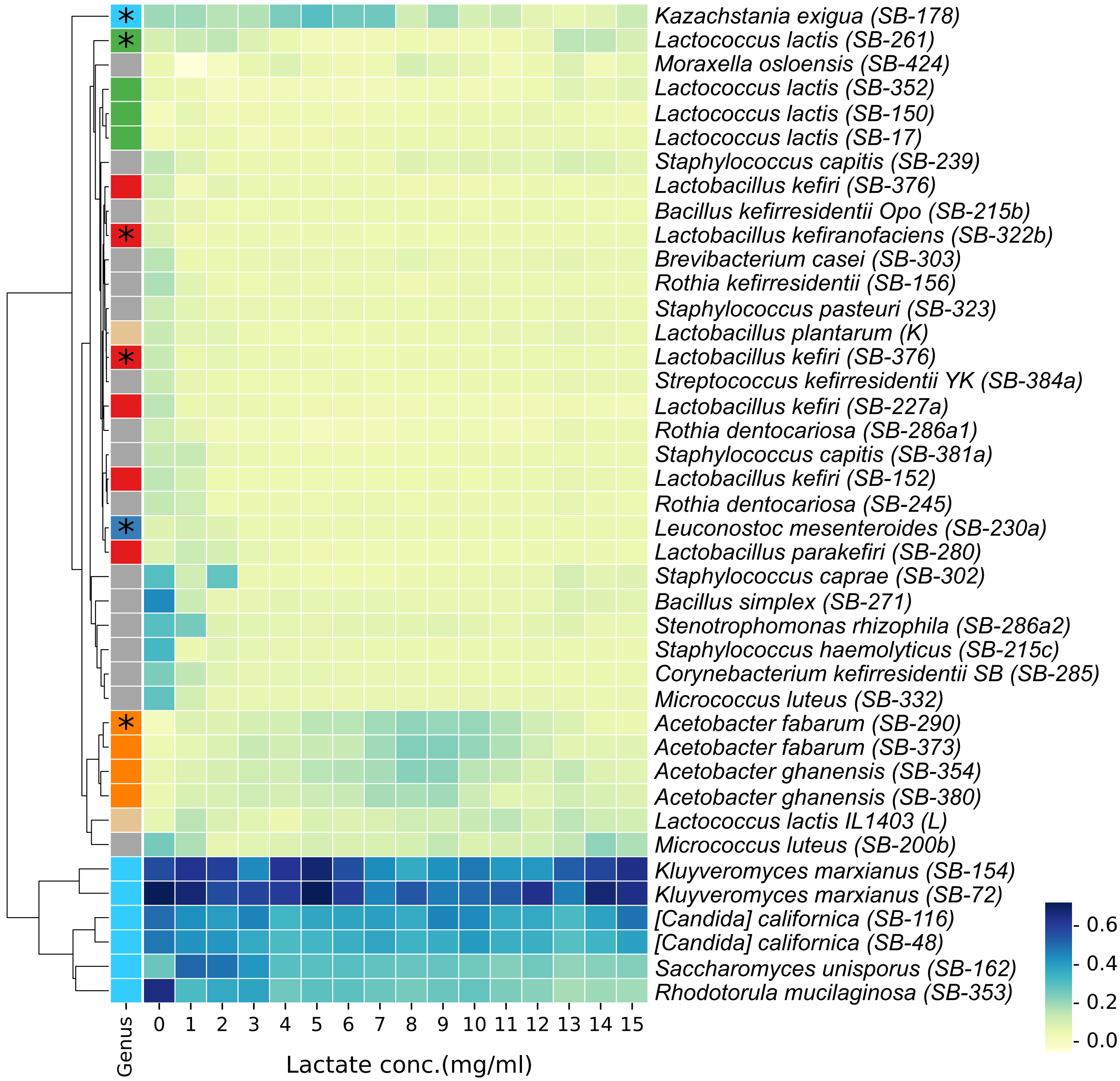


Supplementary Fig. 9 | Growth (OD_{600}) of individual isolates in kefir spent whey harvested at different fermenting times. "0h" refers to non-fermented milk whey at pH 6.5. Groups (Row labels on left): E=early, L=late, C=constant, N= non- or decreased growth, subgroup: 1=preferred growth in milk whey, not in kefir whey, 2=no growth in milk whey.

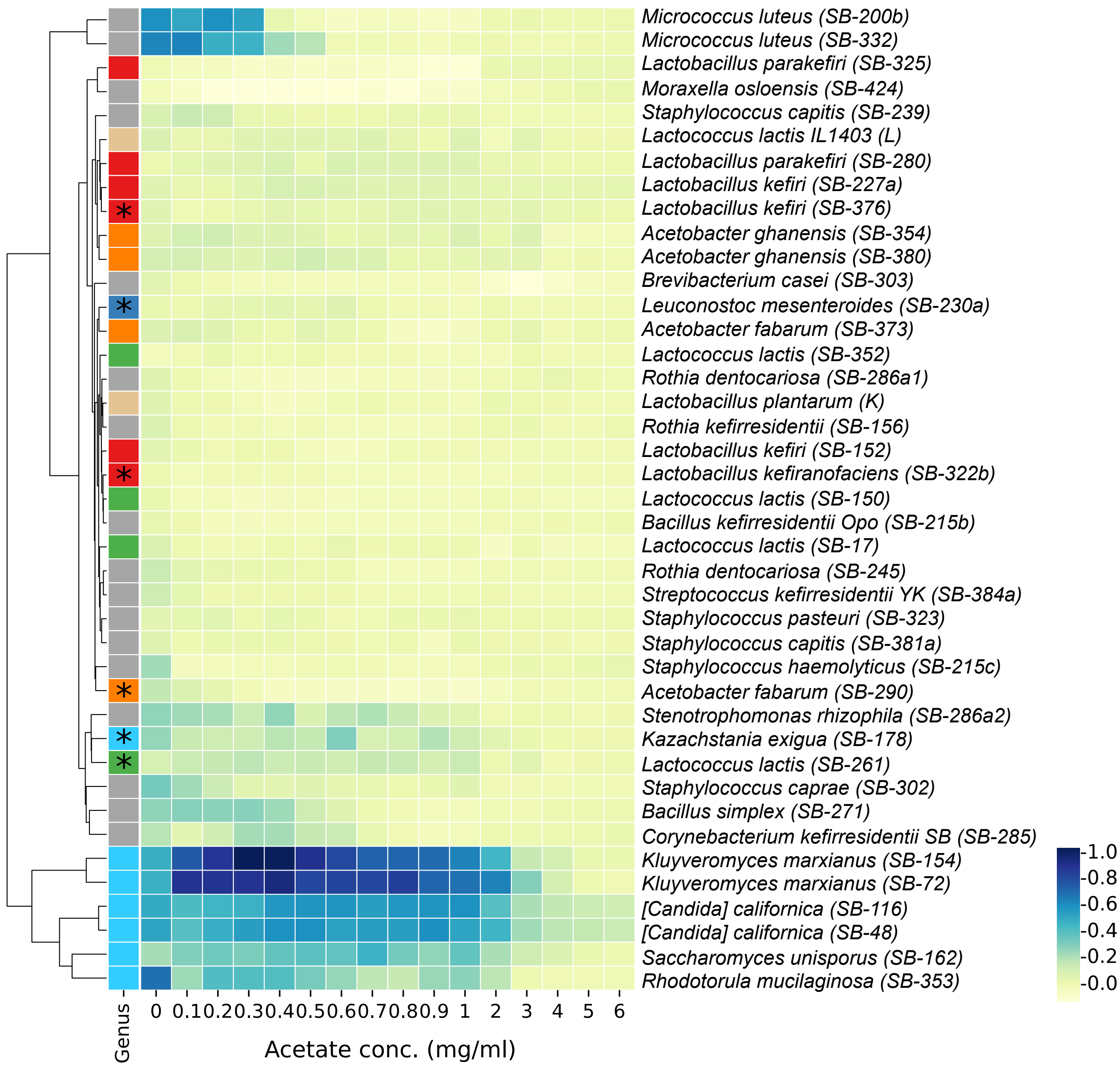


Supplementary Fig. 10 | a, Growth of kefir species in milk after 72 hours determined by 16S amplicon sequencing and *E. coli* standard. Significant growth (two-sided t-test) was observed mainly for rare species like *B. simplex* SB-271 (p-value 0,0004), *R. kefirresidentii KRP* SB-156 (p-value 0,0027), *S. kefirresidentii* YK SB-384a (p-value 0,011) and *S. saccharolyticus* SB-239 (p-value 0,028) as well as for one main kefir species: *L. lactis* SB-261 (p-value 0,001). **b**, Growth of *L. kefiranofaciens* in kefir spent whey harvested at different time points and milk whey (pH= 6.5), measured after 7 days. We tested whether *L. kefiranofaciens* would grow significantly better at 12 h, compared to the whey harvested at any other time point. This was the case for all other time points when compared with the whey harvested after 12 hours (one-sided t-test). p-values: 24h - 0,0005, 36h - 0,0365, 48h - 0,0001, 60h - 0,00027, 72h - 0,0378, 96h - 0,0062 and milk whey - 0,00018. **c**, Growth of *L. kefiranofaciens* in milk at time of inoculum (0 hours) and after 72 hours. No significant growth was observed after 72 hours in milk (two-sided t-test, p-value - 0,72)

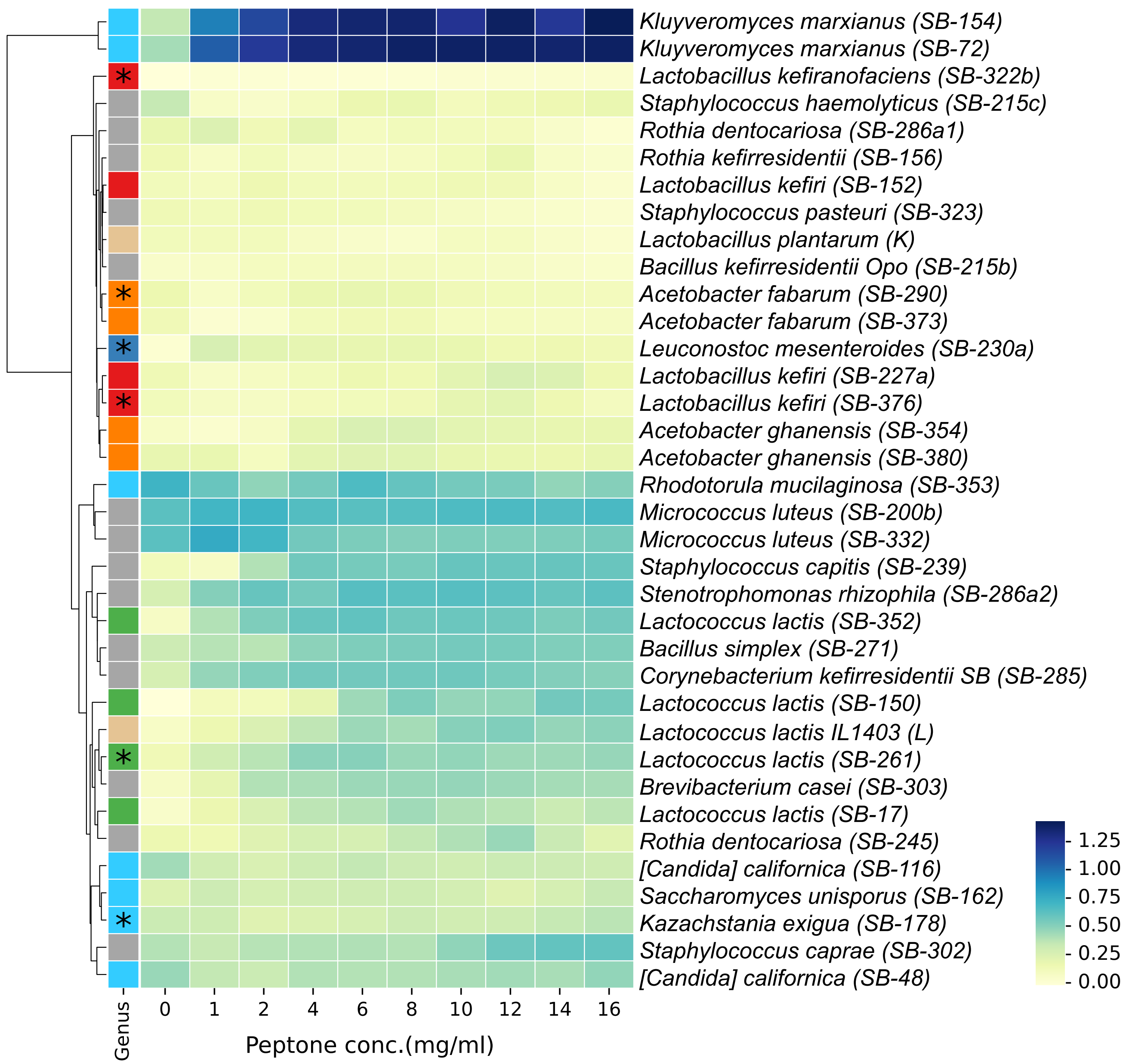
All panels: N=4 biologically independent samples, error bars =SD. Data are presented as mean values +/- SD.



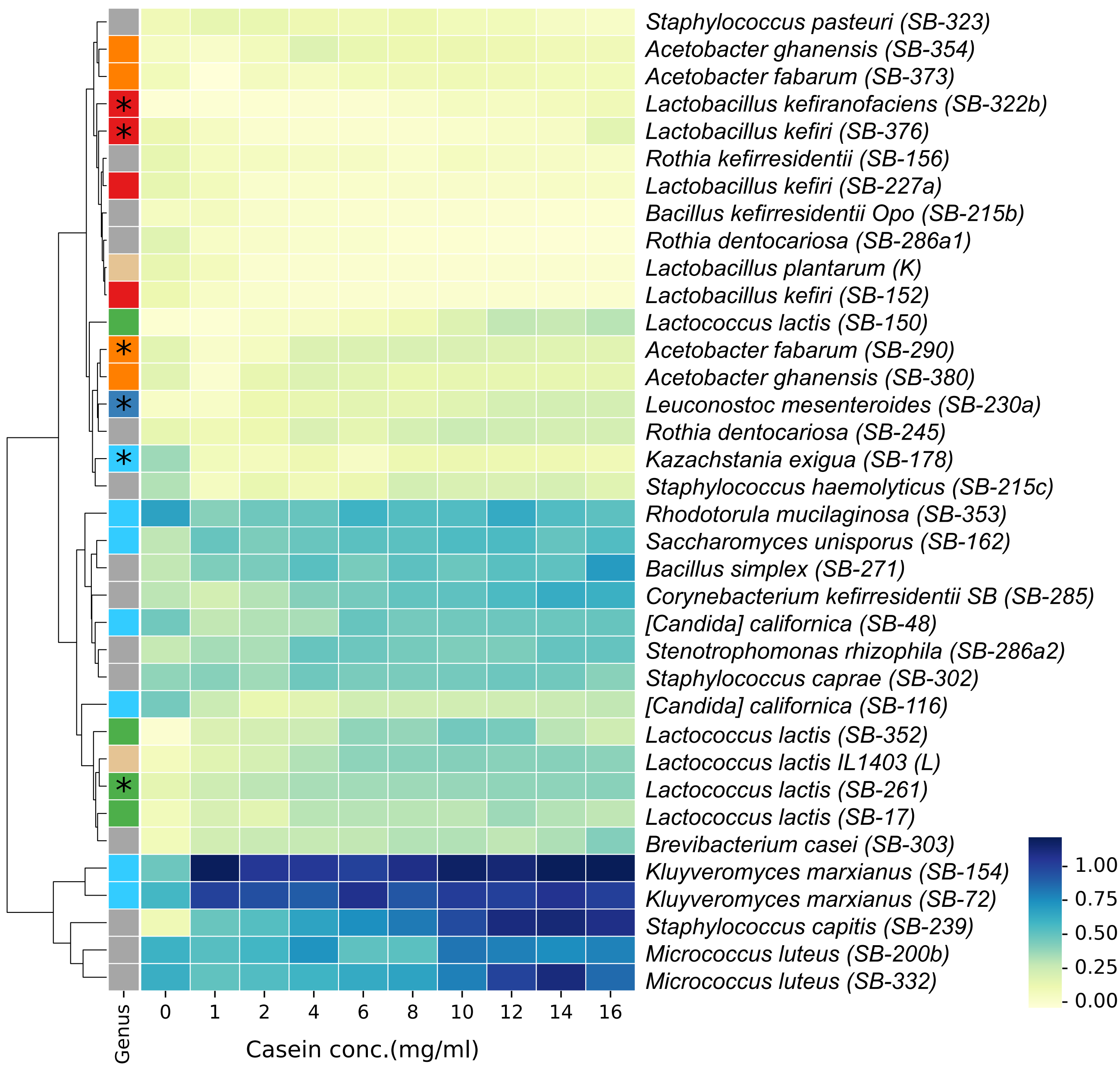
Supplementary Fig. 11 | Growth of different species in milk whey supplemented with lactate. Genus color: grey = rare species, other colors = main kefir species, * = main kefir species in kefir GER6 (reference kefir community). See Supplementary Table 20 for data underlying this figure.



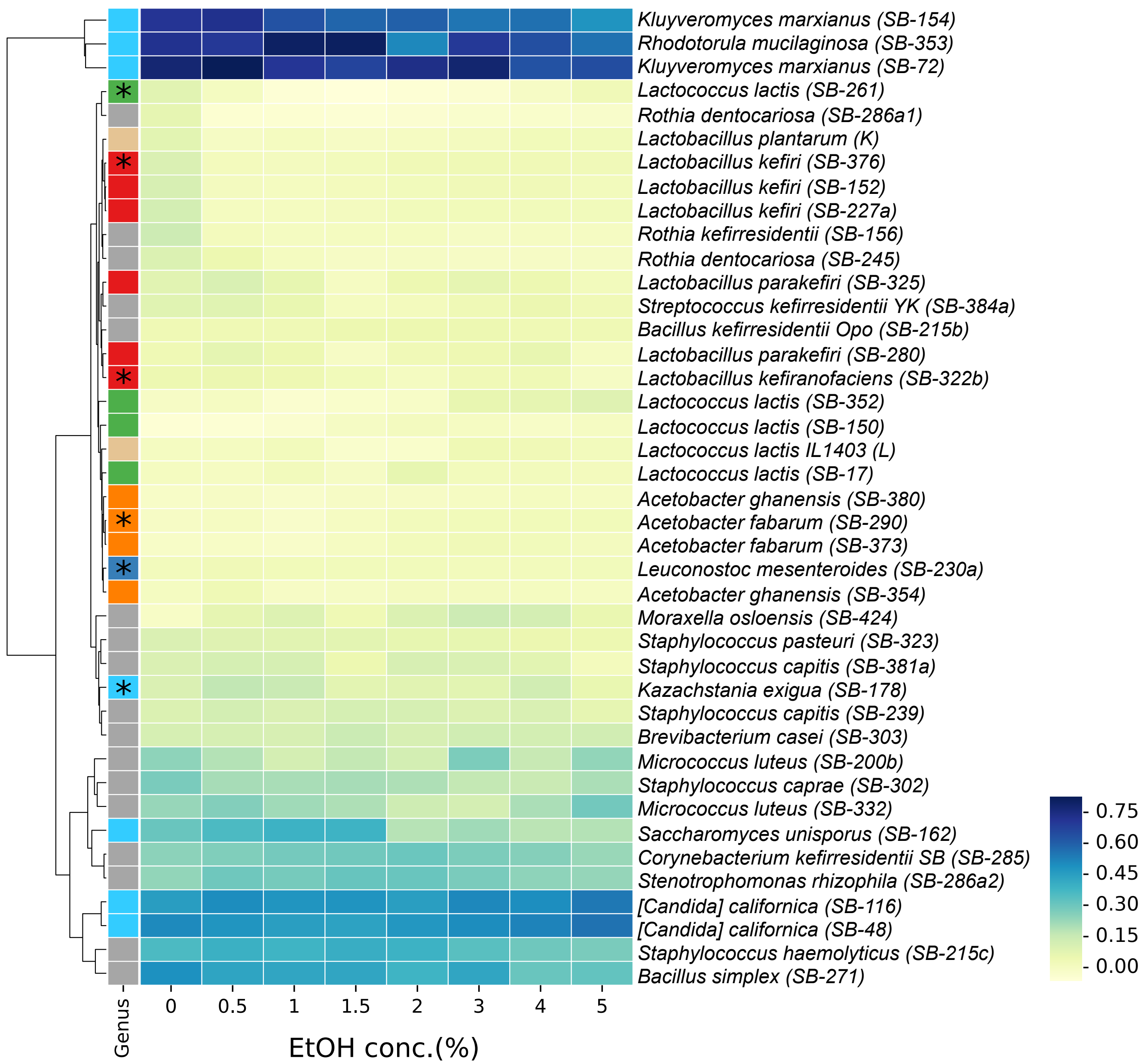
Supplementary Fig. 12 | Growth of different species in milk whey supplemented with acetate. Genus color: grey = rare species, other colors = main kefir species, * = main kefir species in kefir GER6 (reference kefir community). See Supplementary Table 20 for data underlying this figure.



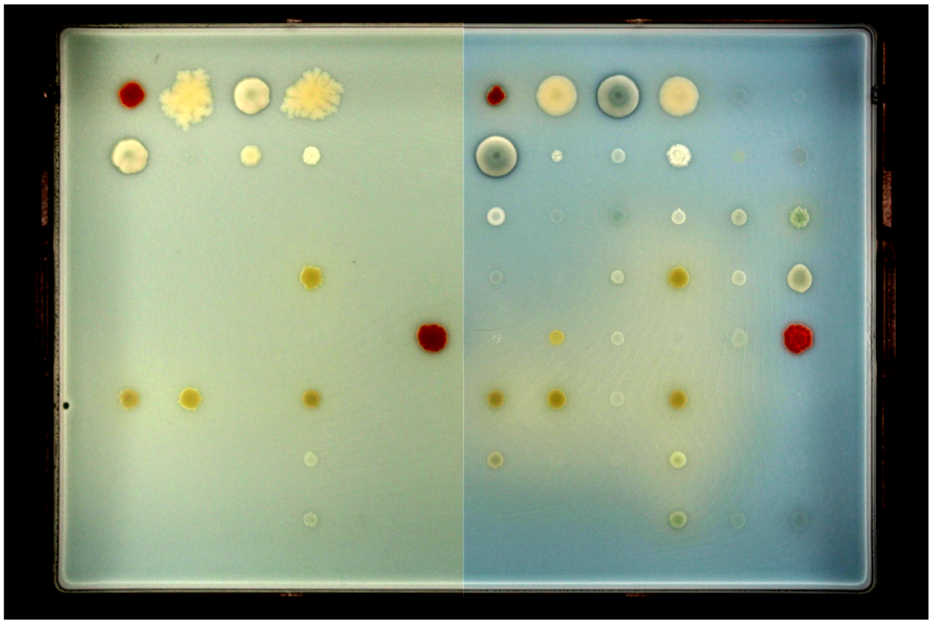
Supplementary Fig. 13 | Growth of different species in milk whey supplemented with peptone. Genus color: grey = rare species, other colors = main kefir species, * = main kefir species in kefir GER6 (reference kefir community). See Supplementary Table 20 for data underlying this figure.



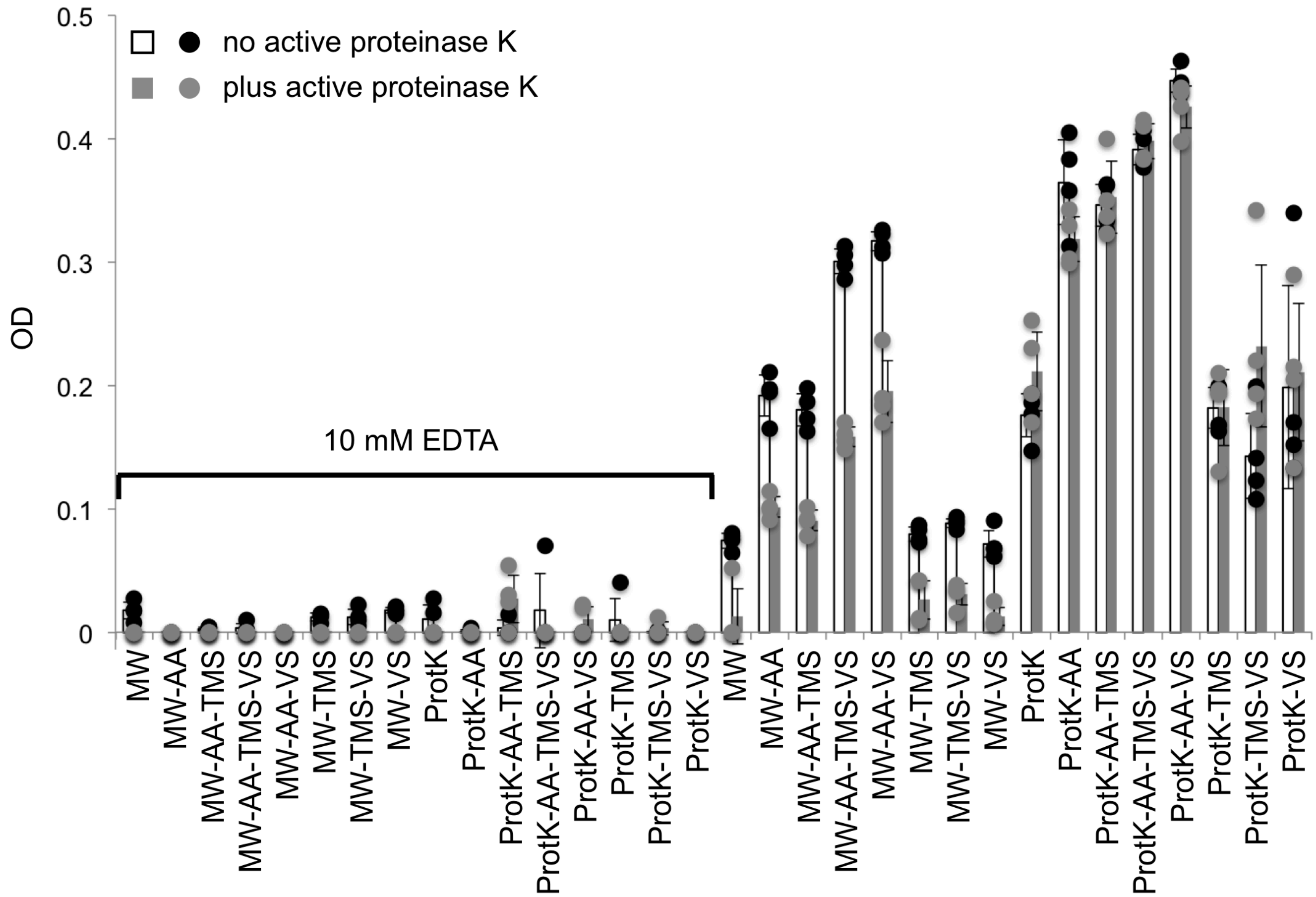
Supplementary Fig. 14 | Growth of different species in milk whey supplemented with casein.
Genus color: grey = rare species, other colors = main kefir species, * = main kefir species in kefir GER6 (reference kefir community). See Supplementary Table 20 for data underlying this figure.



Supplementary Fig. 15 | Growth of different species in milk whey supplemented with ethanol. Genus color: grey = rare species, other colors = main kefir species, * = main kefir species in kefir GER6 (reference kefir community). See Supplementary Table 20 for data underlying this figure.

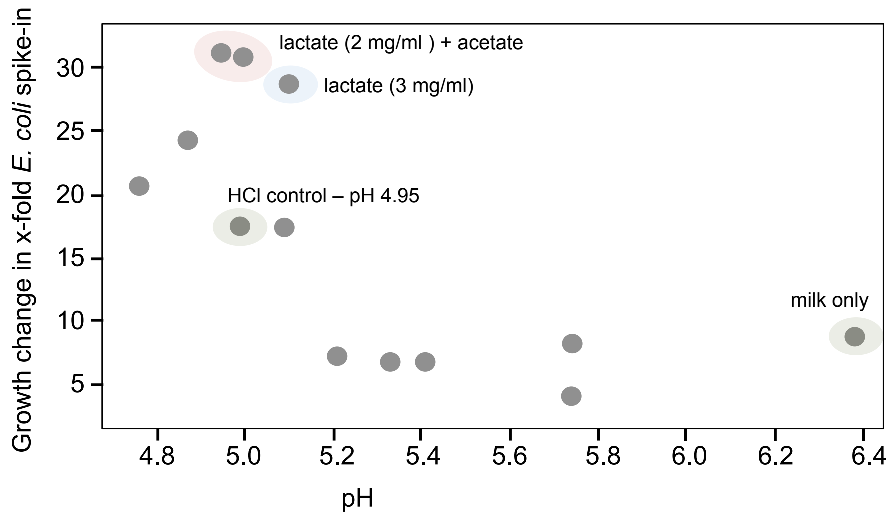


Supplementary Fig. 16 | *L. lactis* is a potent inhibitor of other kefir species on milk plates (80 % milk, 20 % water agar and bromocresol green). Left: growth of kefir species with *L. lactis* plated as background lawn. Right: growth of kefir species without any background species.

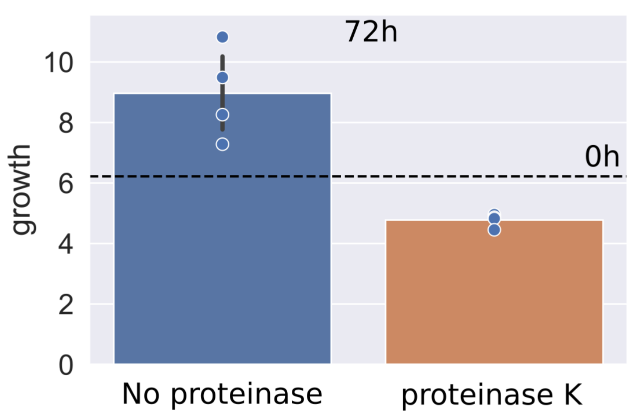


Supplementary Fig. 17 | *L. mesenteroides* monoculture growth on milk whey produced from proteinase K predigested (ProtK) and untreated (MW) milk with and without addition of additional active proteinase K (0.2 mg/ml) and EDTA. Milk whey medium (MW) and protein rich whey medium (ProtK) was supplemented with different combinations of trace metals (TMS), vitamins (VS) and amino acids (AA). N=4 biologically independent samples, data are presented as mean values +/- SD.

a

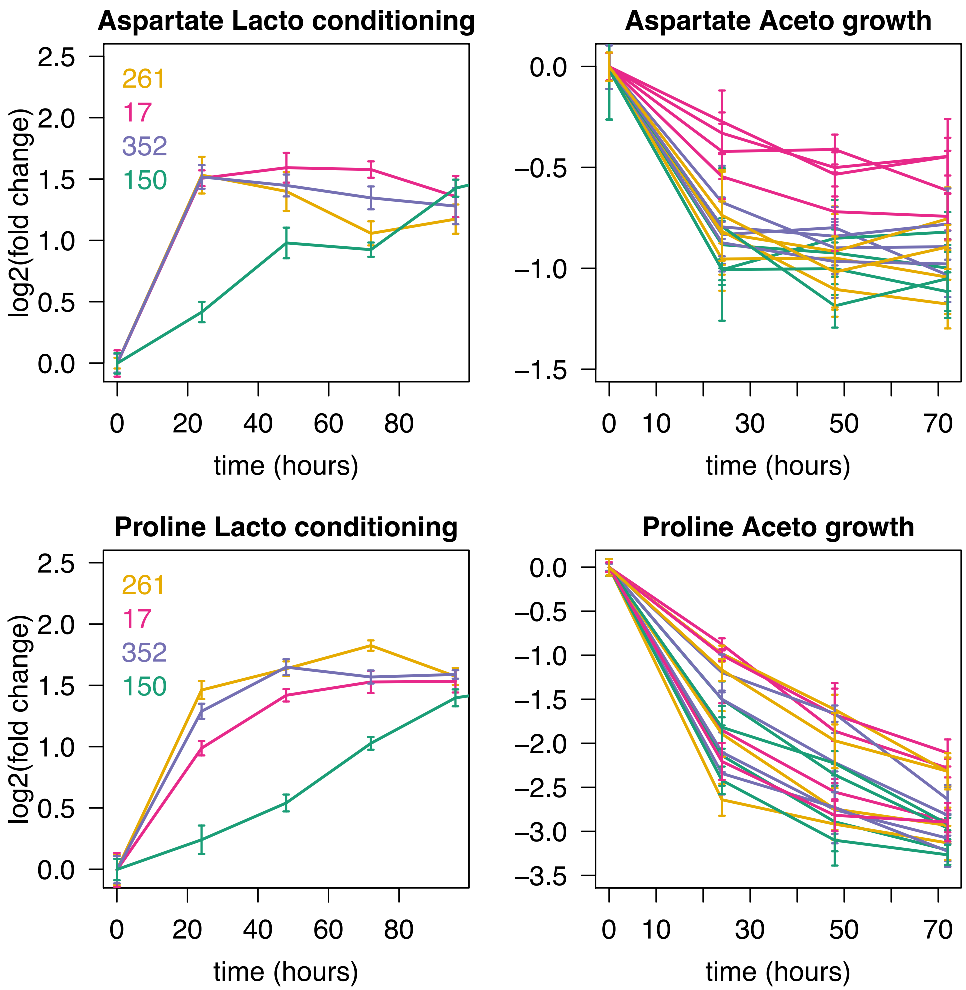


b

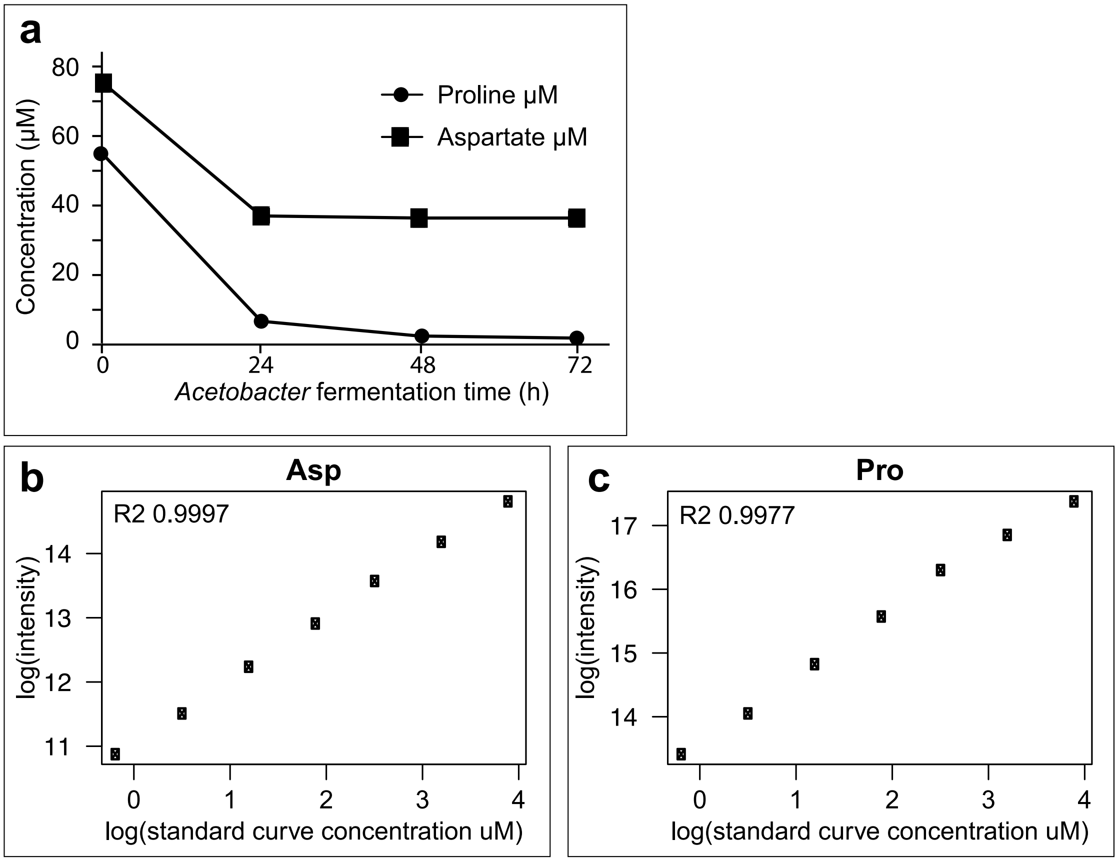


Supplementary Fig. 18 | *L. kefiranofaciens* profits from lactate and acetate when added to milk. **a**, *L. kefiranofaciens* growth in milk after 72 h plotted against pH. There is a clear preference for growth around pH 5, but it cannot explain the effect of lactate supplementation fully, since pH adjustment with HCl or acetate does not result in comparable growth as lactate containing samples. **b**, Addition of proteinase K to milk had no effect on *L. kefiranofaciens* growth. N=4, biologically independent samples, error bars = mean values +/- SD.

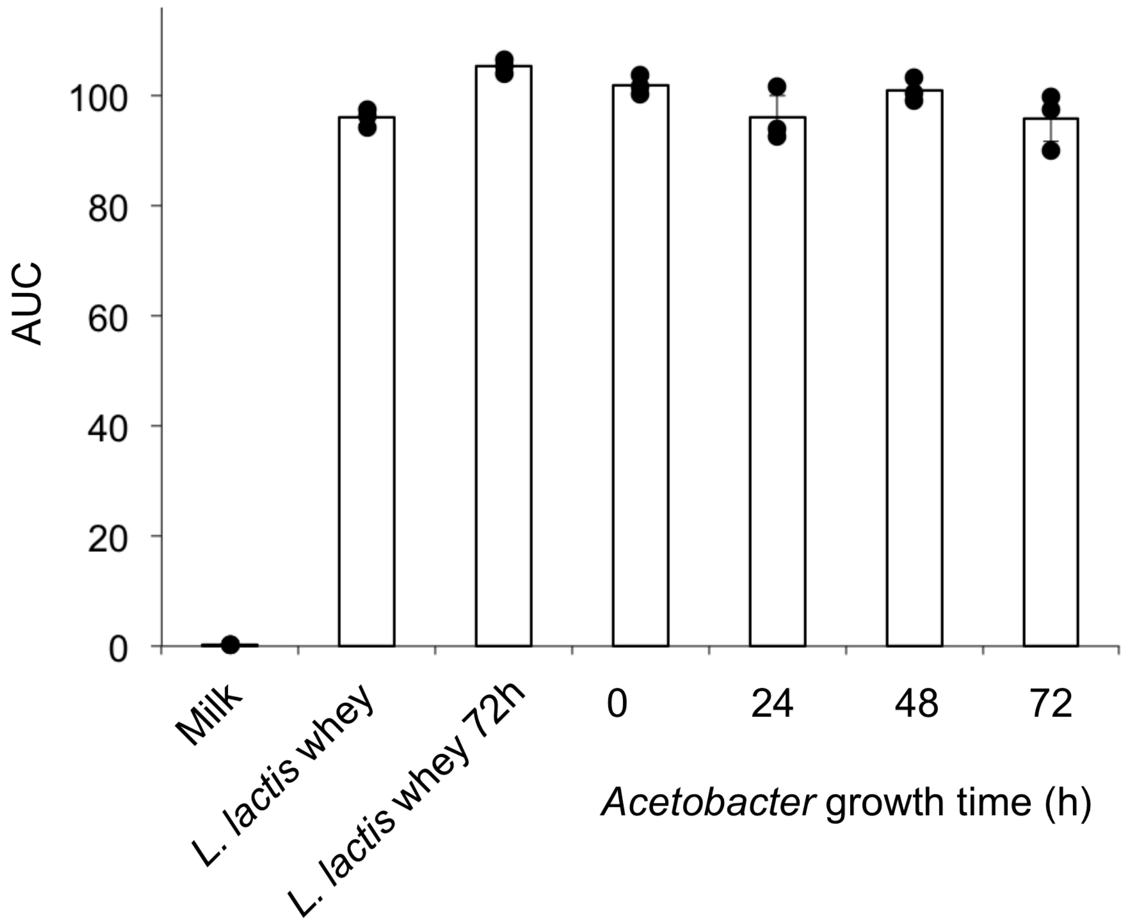
Untargeted FIA-TOF



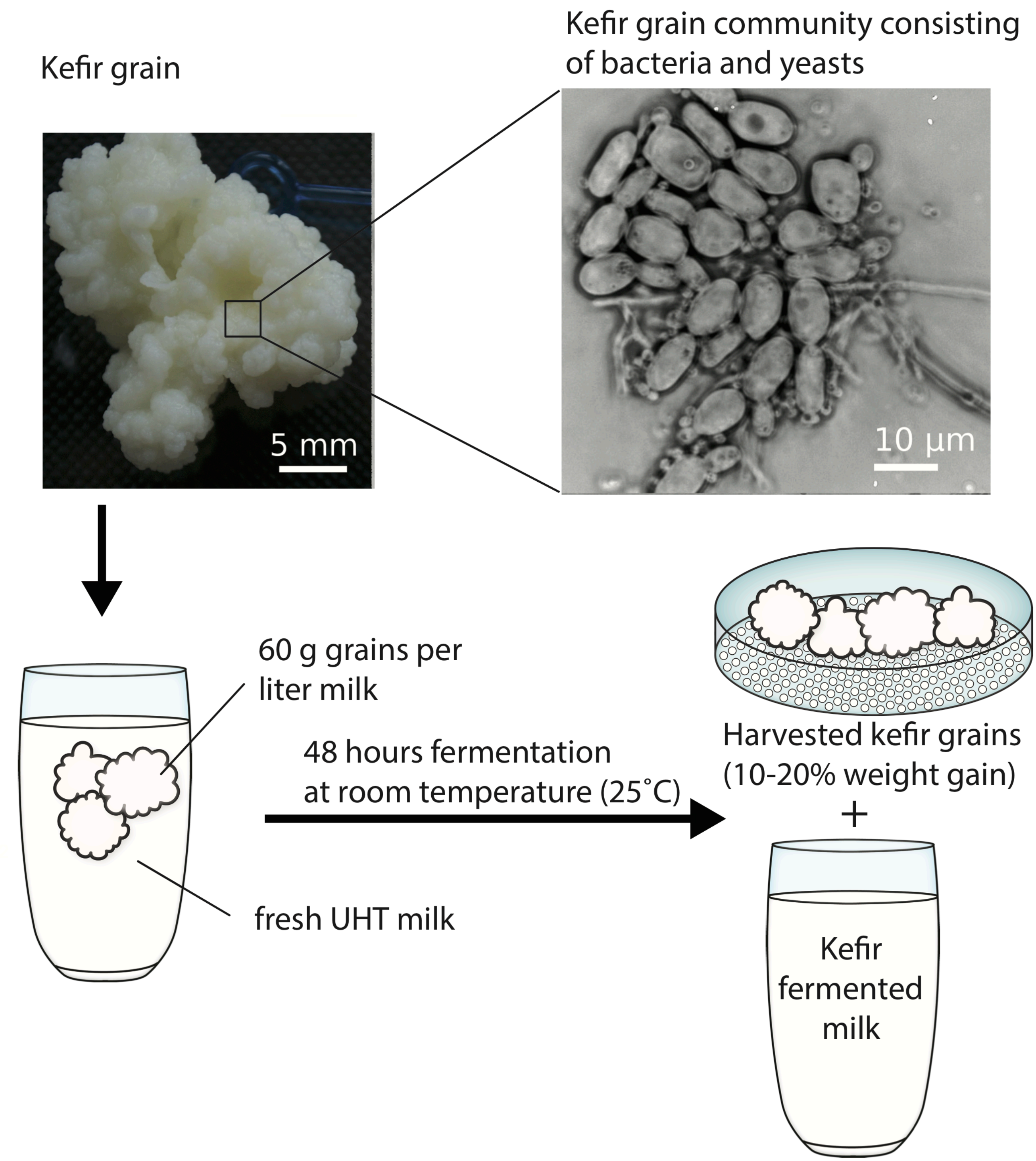
Supplementary Fig. 19 | Putative aspartate and proline consumption by *Acetobacter* in *L. lactis* spent medium as revealed by untargeted FIA-TOF mass-spectrometry analysis based on exact masses (Methods). Left panels: Aspartate (top) and proline (bottom) concentration change during conditioning of milk by four *L. lactis* strains isolated from kefir (SB-17, SB-150, SB-261 and SB-352). Right panels: Aspartate (top) and proline (bottom) concentration change during growth of four *Acetobacter* strains (*A. fabarum*: SB-290, SB-373 and *A. ghanensis*: SB-354, SB-380), isolated from kefir, in spent whey. N=3 biologically independent samples, each measured twice (2 technical replicates per biological one), data are presented as mean values +/- SD.



Supplementary Fig. 20 | a, Quantification of aspartate and proline consumption by *A. fabarum* in *L. lactis* spent whey using HILIC-qTRAP. **b-c**, Calibration curves for aspartate and proline against pure compound standards.



Supplementary Fig. 21 | Relative lactate concentration (AUC: Area Under Curve, GC-MS data in Supplementary Table 13) in spent whey after *L. lactis* fermentation and during *A. fabarum* growth in *L. lactis* spent whey. *A. fabarum* does not consume considerable amounts of lactate during 72 h of growth in spent whey. N=3 biologically independent samples, data are presented as mean values +/- SD.



Supplementary Fig. 22 | Overview of kefir fermentation and grain propagation process. 60 grams of kefir grains containing the kefir community are inoculated per liter of UHT milk to start the fermentation. Kefir fermentation is usually done for 48 h at room temperature (we used a 25˚C incubator for our studies) without shaking. After the fermentation, fermented milk and grains are separated in a loose-knit sterile sieve. On average, the grain wet-weight (i.e. weight of the grains after washing with sterile water and draining) increased around 10-20 % at the end of fermentation. Harvested grains can directly be used to start a new fermentation batch or for storage. Note: kefir fermented milk made in the laboratory is not advised for consumption.