**Kefir Grains Behave Like Magnets:**
**Frequent Visual Observations of Pair-Bonding Behavior**

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The supplemental data review presented here is provided for completeness of a previous research article [https://hal.archives-ouvertes.fr/hal-01253250] showing that milk kefir grains (KGs) can establish close bonds and become a pair. Milk is traditionally used to culture dairy KGs. In this study, the motility and grouping ability of KGs were determined under experimental conditions (for details of the procedure see above reference). Here we report new findings on pair-bonding behavior of KGs which confirms earlier observations. The quantitative data have been obtained by consulting a collection of photographic and video records from the primary source which describes for the first time, an entirely new family of KGs obtained from isolated bacteria and yeast cells, and maintained in culture. A specific dataset was analyzed, focusing solely on demonstrating close interactions between pair partners consisting of two grain specimens – labeled J and K for tracking purpose. Specific morphological characteristics allowed their identification with a high degree of certainty (Appendices A–C). Behavioral data were collected on a daily basis and consisted of focal observations of grains moving freely before and/or after brief agitation. First a series of photographs was taken that was focused on the surface, then the camera was moved against the side walls of the container. Additionally, short videos were made at random moments during the day. Frequency recording was obtained by counting the number of times a pairing behavior occurred on different days. The pairing events took place from September 14 through November 15, 2012. As more of these interactions were visualized over time, these events turned out to be frequent, and revealed a recognizable behavioral pattern. Specifically, a total of n=22 cases of pairing events were recorded for pair JK at irregular intervals within a 60-day period (Appendix D). The frequency of events (37 per cent) is remarkable considering that the best time for detection may sometimes have been missed. The grains J and K engaged in the pair were often visible on the periphery, partially submerged and changing position relative to one another. Notably, both grains became progressively older while still maintaining a close relationship. During their life-cycle, they exhibited a similar pattern of actively seeking close mutual proximity, often reaching to the periphery of the container at certain times during the day, and doing so regardless of culture conditions, i.e., grain density, extent of fizzing action, manual agitation. It is possible that similar interactions may exist in milk assuming the same grains are reused in subsequent fermentations. In conclusion, the present findings clearly demonstrate that a pair of KGs could align themselves like magnets under various culture conditions. It is amazing to think that what appeared as little brown masses could actually interact in situ and form close bonds regardless of the surroundings. We are still far from being able to understand why these many pairing interactions between KGs themselves occurred in this system. The question of which phenomenon(s) drove these grains to periodically form a close pair and maintain a pair bond during their life cycle remains open.

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# Supplementary Data from Research Article [https://hal.archives-ouvertes.fr/hal-01253250]

## APPENDIX A

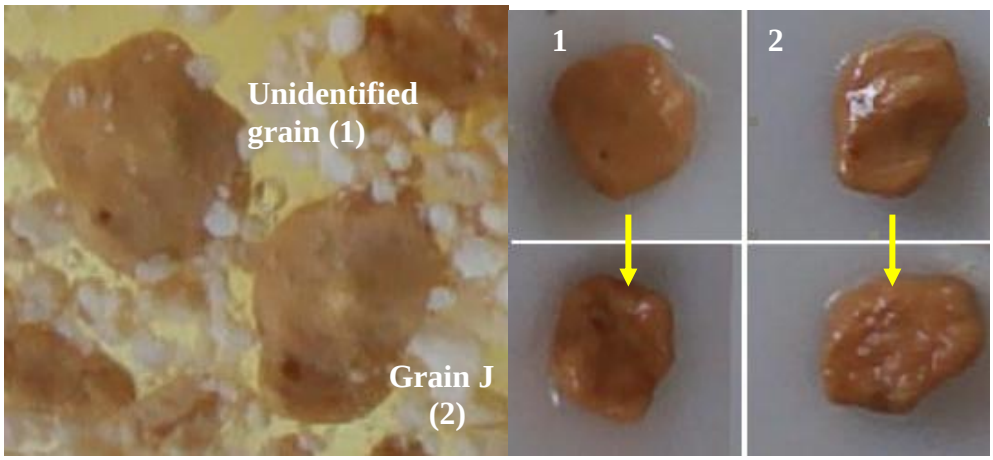

**Isolated kefir grains sorted from fine debris.** The arrows mark the presence of grain labeled 'J' (polygonal shape) and grain labeled 'K' (elongated shape). In spite of their small size, it was possible to photograph the individual grains before repeatedly transfering them to fresh medium. Fragmentation into smaller units, which is the usual fate of kefir grains was observed in ageing cultures. On average, each grain is about 1 cm in size or less. Photograph was taken October 5, 2012.

# APPENDIX B

## Grain J

**Morphology and growth behavior of grain J.** The composite images show the morphological transformation since its formation. The grain became progressively older when maintained in culture. An uneven accumulation of newly produced biomass spreading into thick sheets gave it a superficial resemblance to an ear. The photographs were taken days or weeks apart.

**Grain J visibly in motion.** Successive frames were extracted from a video within seconds.




**Grain growth and texture formation observed in situ and in vitro.** Notice the two grains having a great resemblance in form and color. Flipping the grains helps reveal what lies on the other side as illustrated here (arrows). Each grain specimen has a unique surface pattern that an expert eye can recognize.

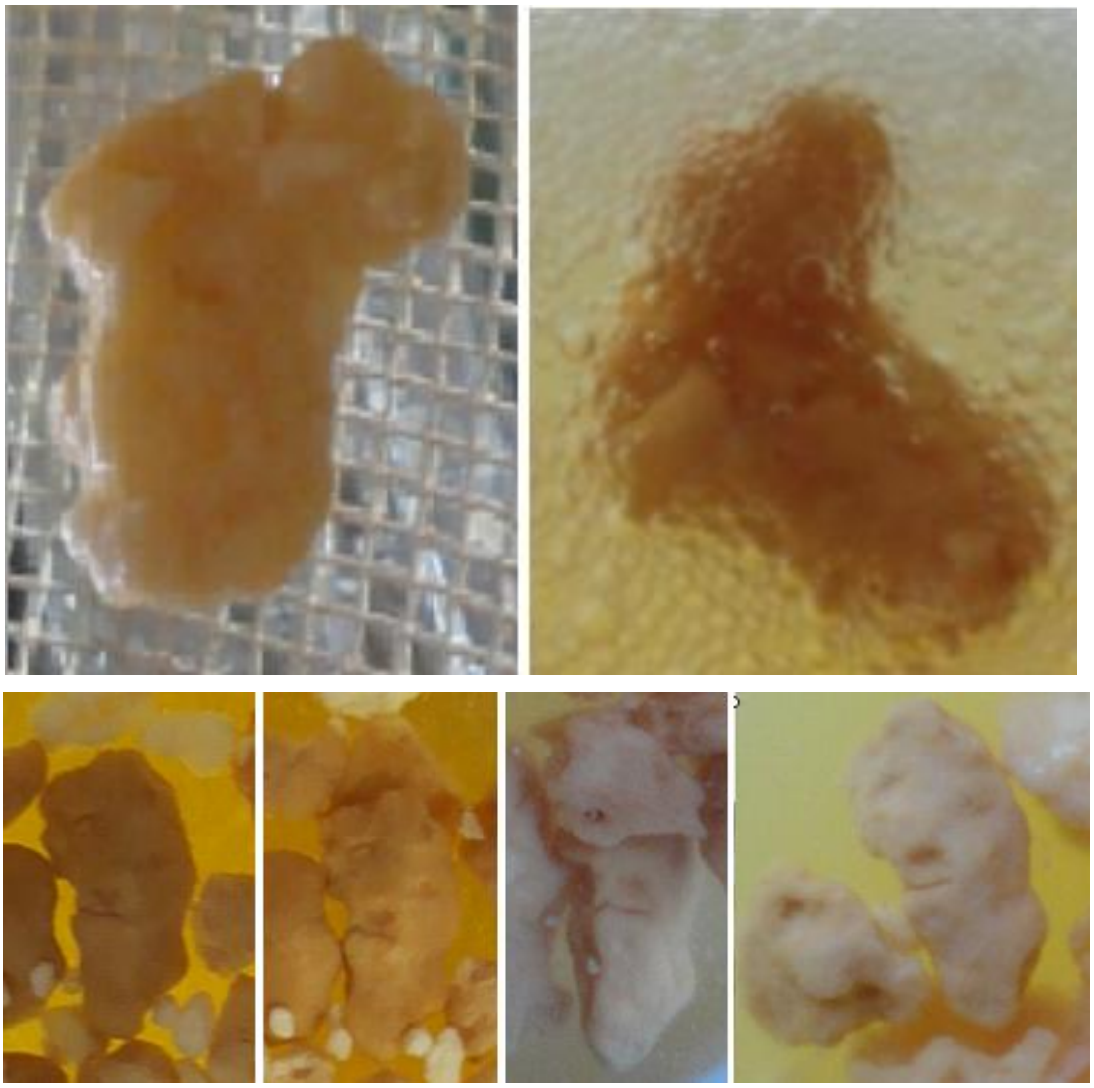
## Grain J (Reverse Side)

**Reverse side of grain J at various stages of development.** The grain became progressively older when maintained in culture. Grain identification was facilitated by flipping the image and looking at the grain upside down as illustrated in lower images.

Cleft side of grain J viewed from the surface (top images) or completely submerged beneath the surface (visible through the glass walls, lower image). New biomass was continually added and the grains became visibly much thicker.

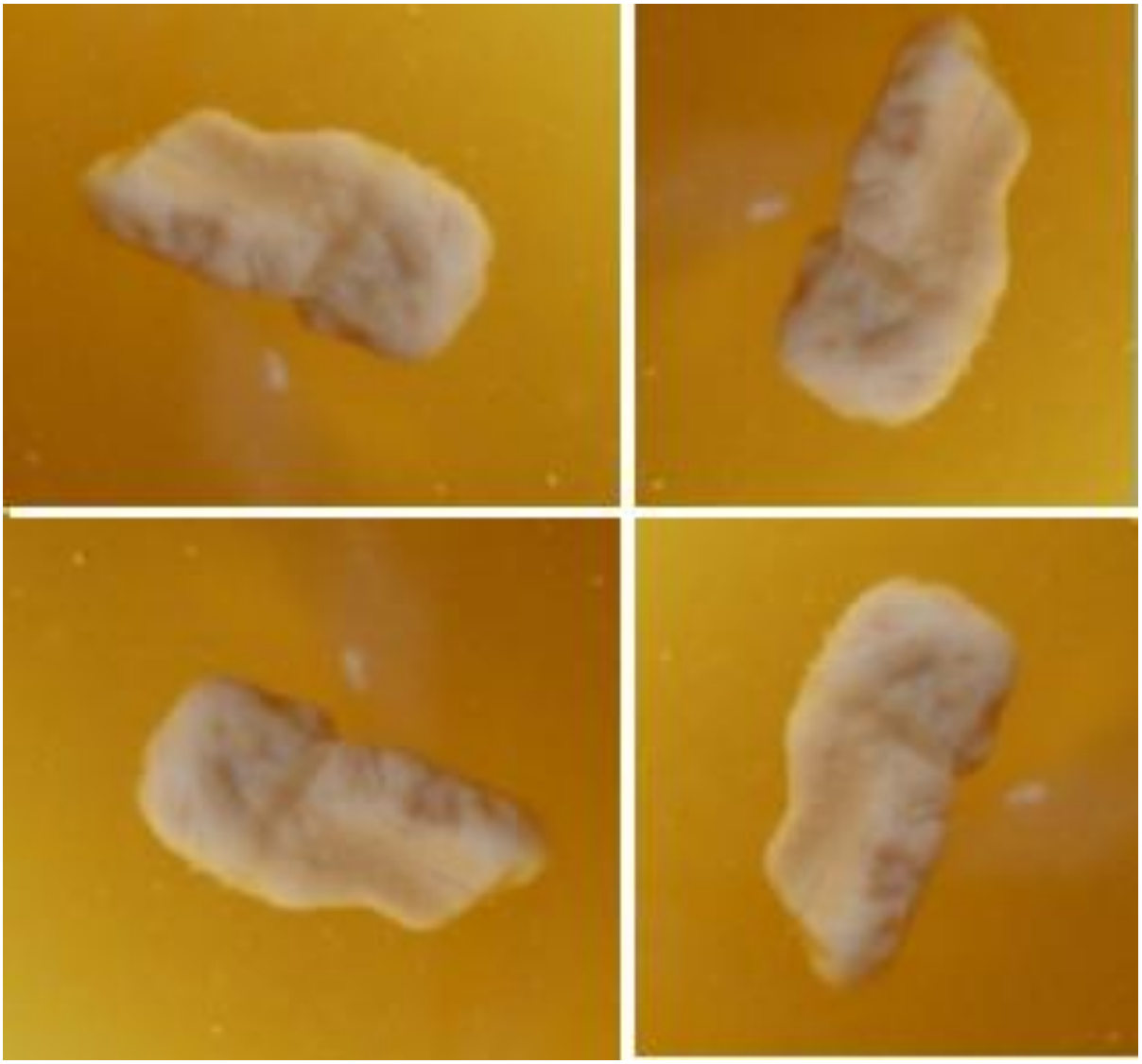
# APPENDIX C

## Grain K



**Morphology and growth behavior of grain K.** The grain became progressively older when maintained in culture The composite image shows laterally stretching, then folding and thickening of grain K in specific regions. Photographs were taken days or weeks apart.

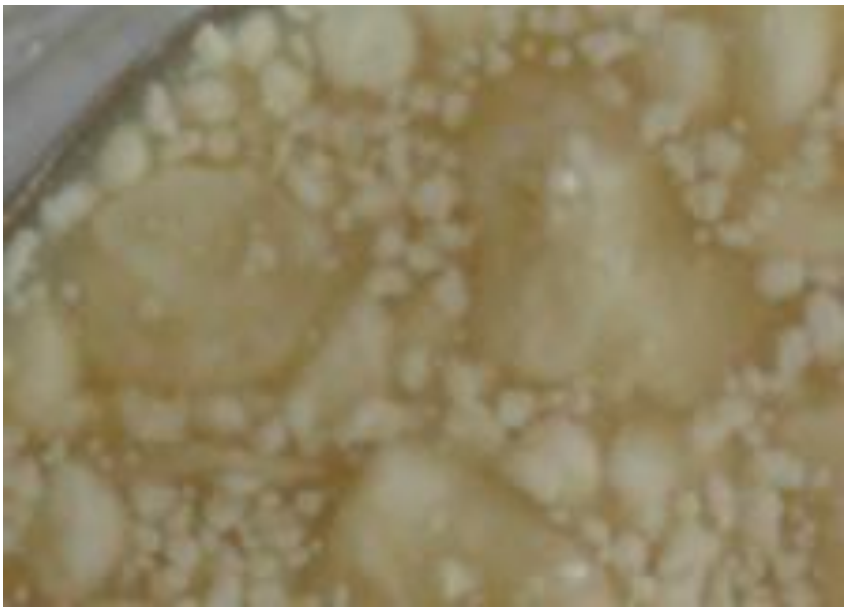
## Grain K (Reverse Side)



**Grain K (Side View)**

## APPENDIX D

**September 14**



**Pair-bonding began early in their lives**. Grains J and K were seen in close proximity even at this early stage of their development.

**September 20**

**Identified pair consisting of grains J and K.** The grains are brown due to the presence of molasses in the medium. Their surface is covered with a mass of small effervescing bubbles, also seen in the fermenting liquid.

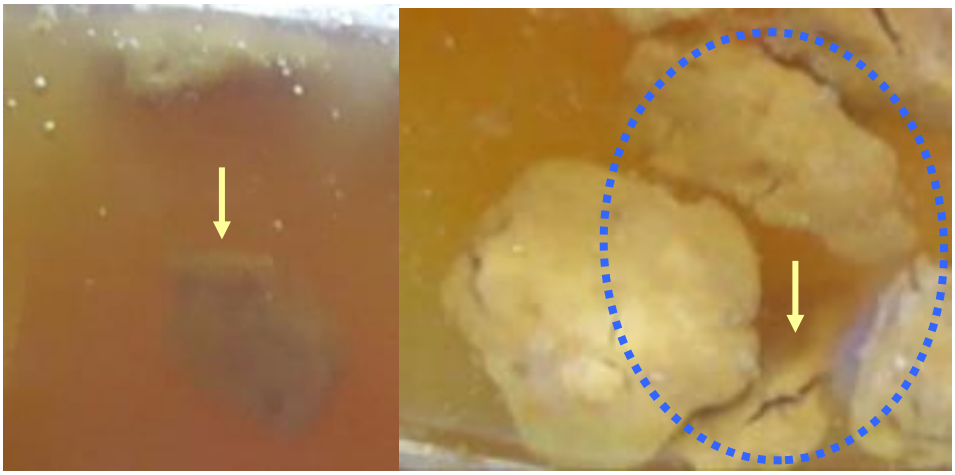
**September 23**

**September 26**

**October 2**

Pair JK is recognizable, despite changes in its appearance.

**October 8**



Remarkably, pair JK continues to be traceable against all expectations. It was on the periphery where grain J appeared in continual motion that the pairing took place. Specifically, grain J was seen moving downward, then resurfacing to perodically stay in close proximity to grain K. Frames were extracted from video within 5 seconds.

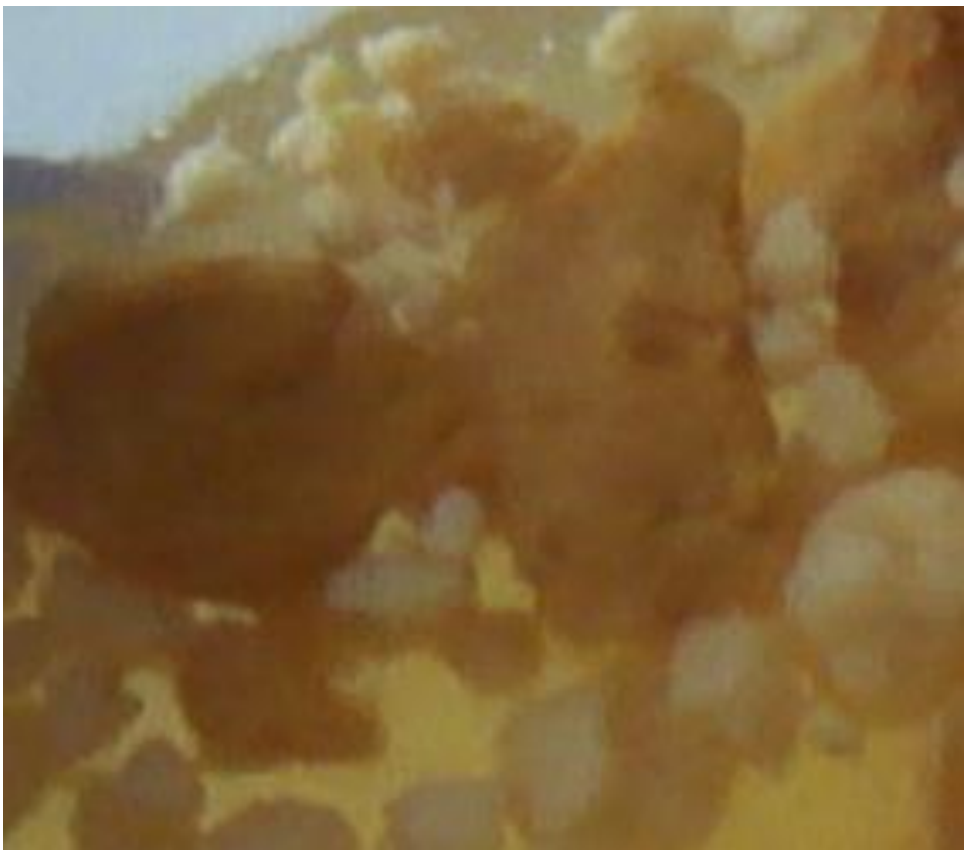
**October 13**


Undisturbed conditions


After agitation (swirling)


Enhanced view of pair JK

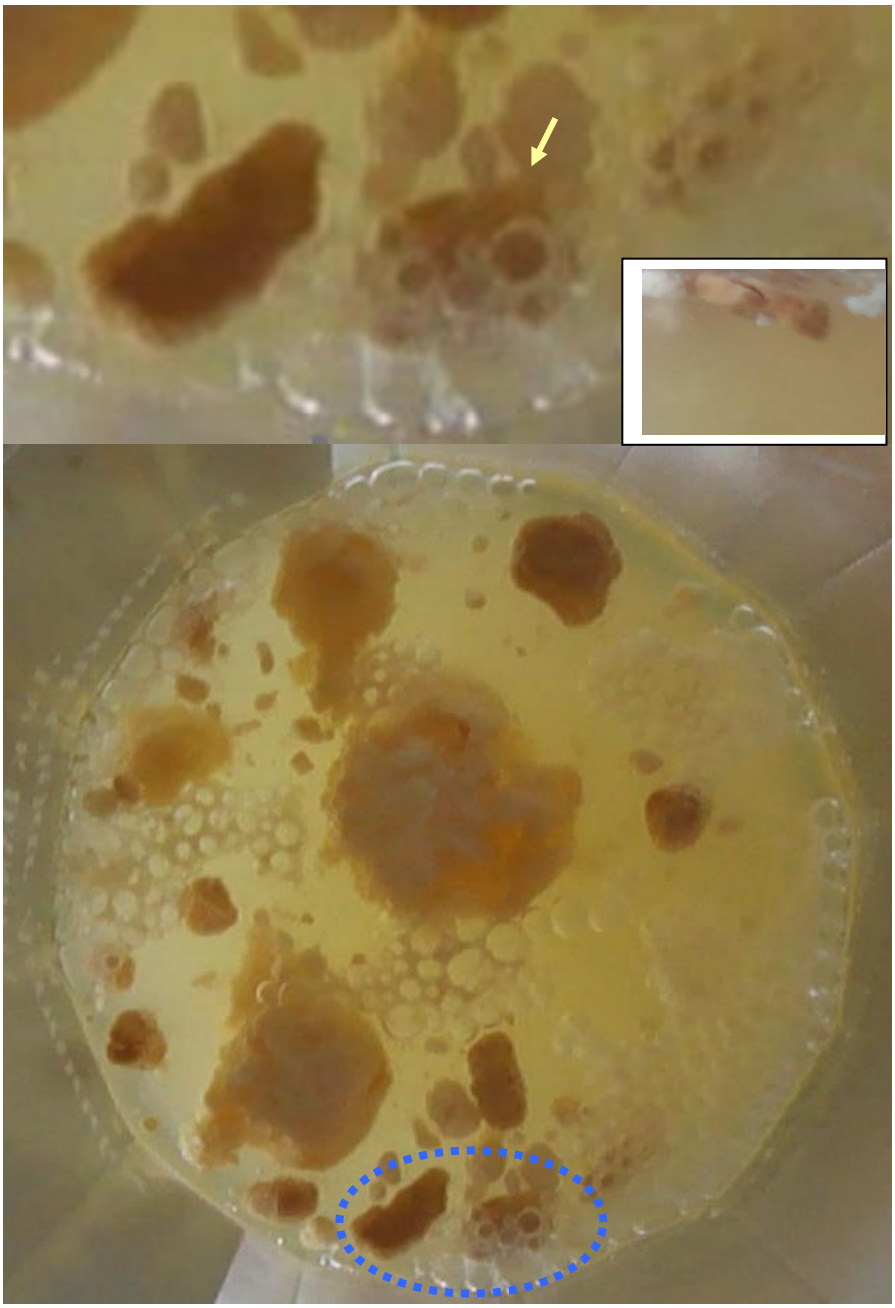
**October 16**



**October 21**

*Top:* Enhanced view of pair JK
*Botom:* Panoramic view of cultured grains from the surface. The dotted line marks the location of the pair.

**October 23**

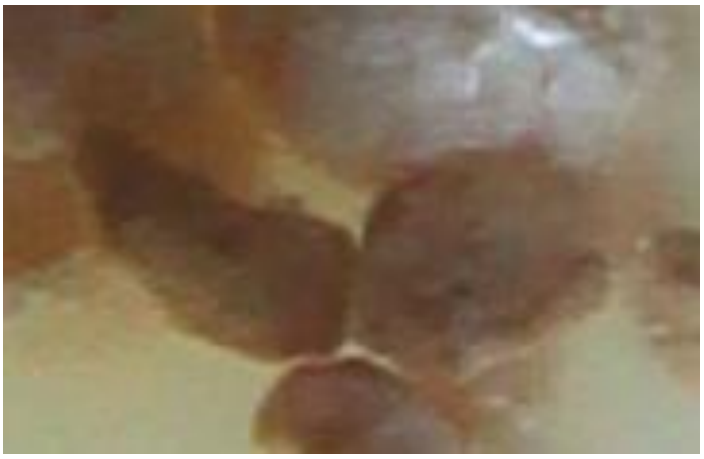


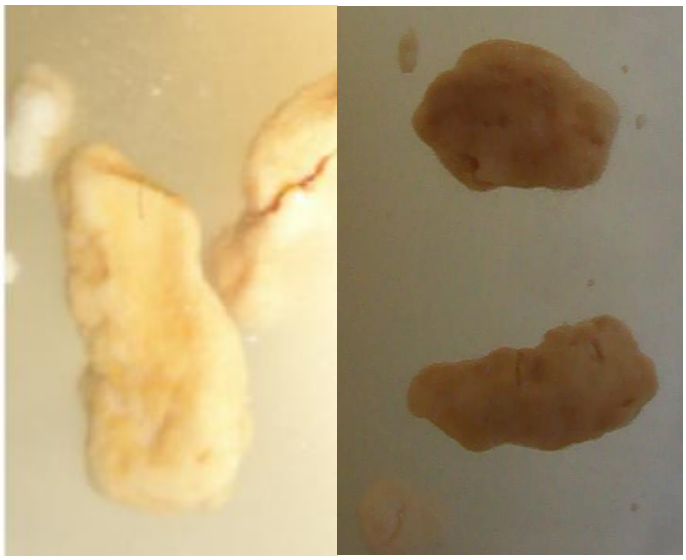
*Top:* Enhanced view of pair JK. Cleft side of grain J visible through the glass walls (shown in the inset).

*Botom:* Panoramic view of cultured grains from the surface. The dotted line marks the location of the pair.

**October 24 (after agitation)**

**October 25**

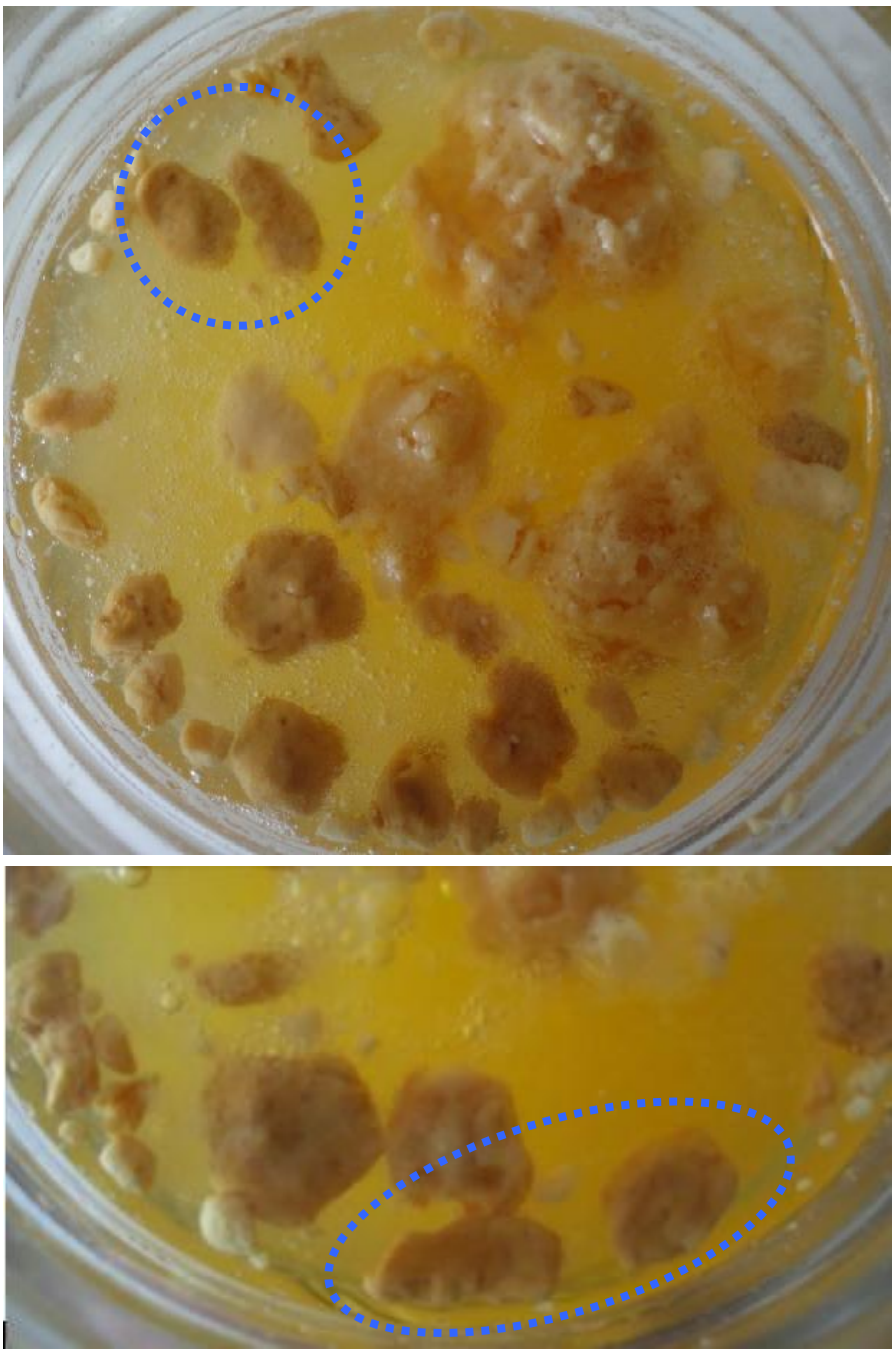




**October 29**

*Top:* Enhanced view of pair JK.
*Botom:* Panoramic view of cultured grains from the surface. The dotted line marks the location of the pair.

**October 31**

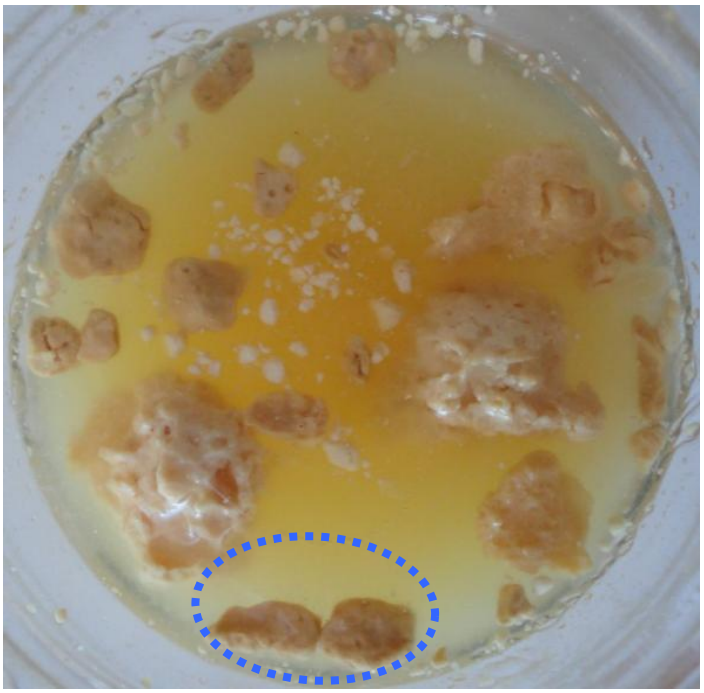


**Pair bonding between grains J and K was seen twice on the same day.** When comparing both images, one will notice that grain J has turned around showing the other side. Note the changing landscape and fizzing medium with a mass of small bubbles. The dotted lines mark the location of the pair on the periphery. Photographs were taken seven hours apart.

**November 1**

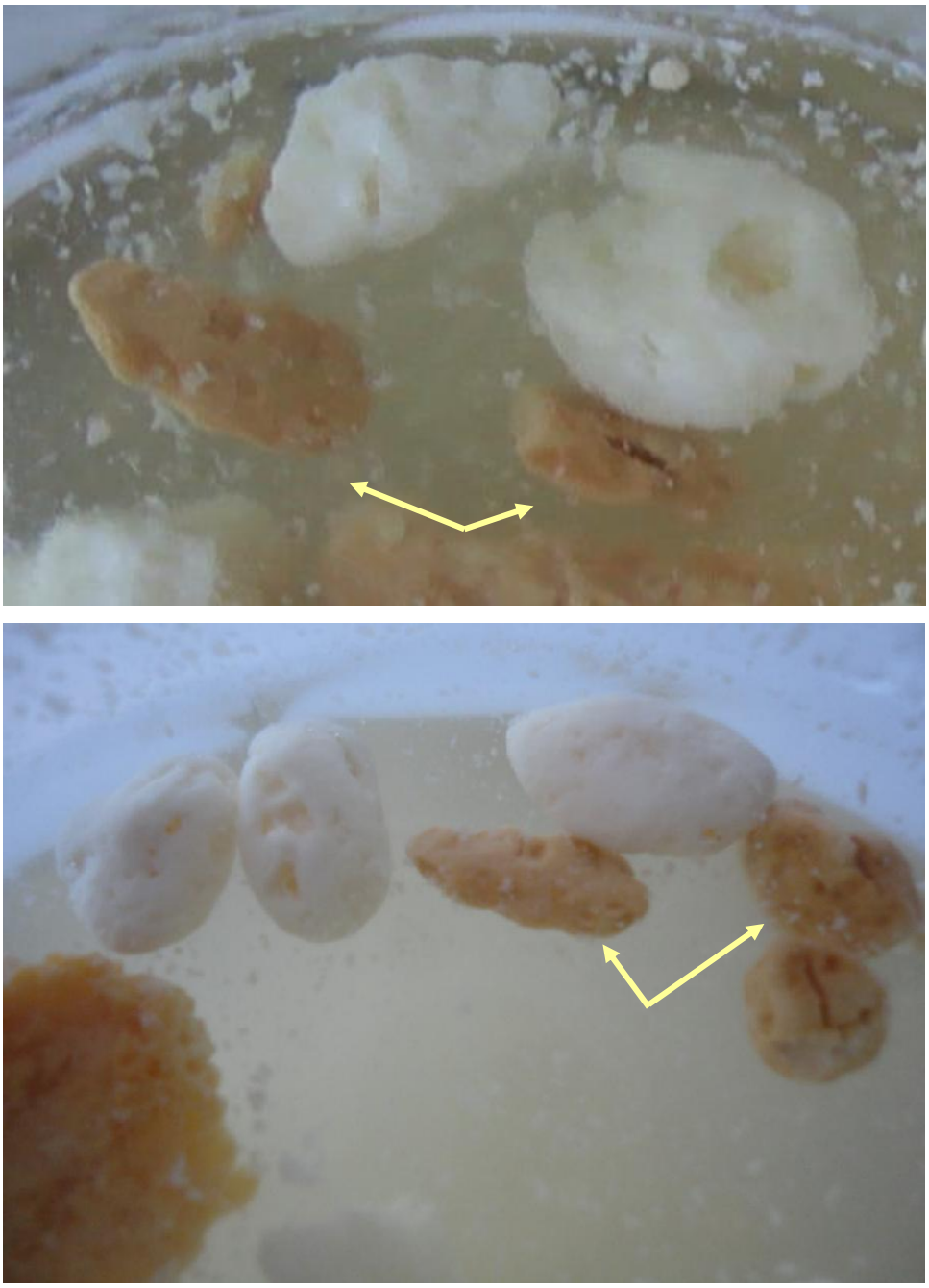
**November 3**

**November 4**



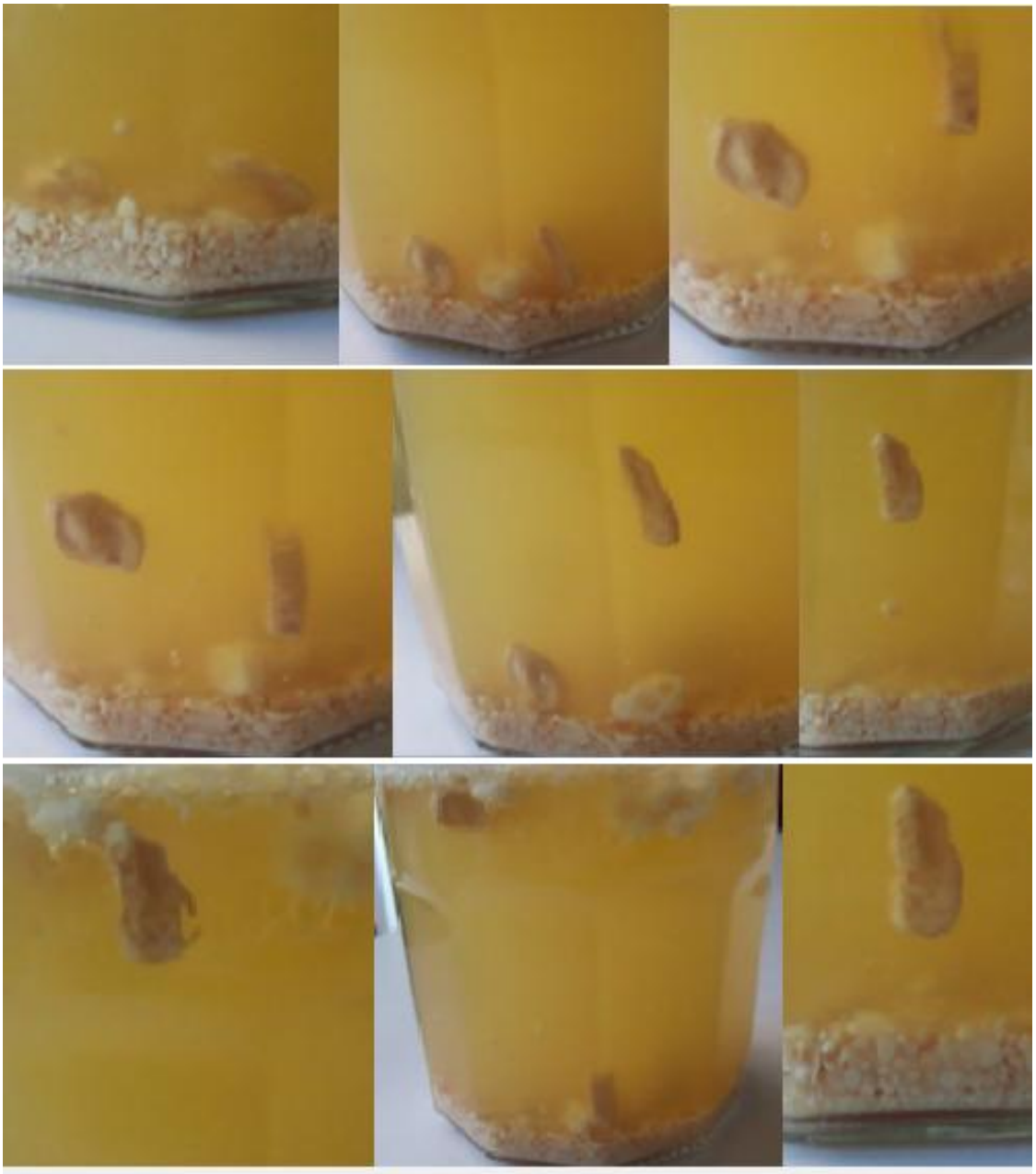
*Top:* Enhanced view of pair JK.
*Botom:* Panoramic view of cultured grains from the surface. The dotted line marks the location of the pair.

**November 5**



**Grains J and K seen at a different angle (arrows).** Cleft side of grain J is visible on the first image. Notice grain K viewed upside down as illustrated in lower image. Photographs were taken 10 seconds apart.

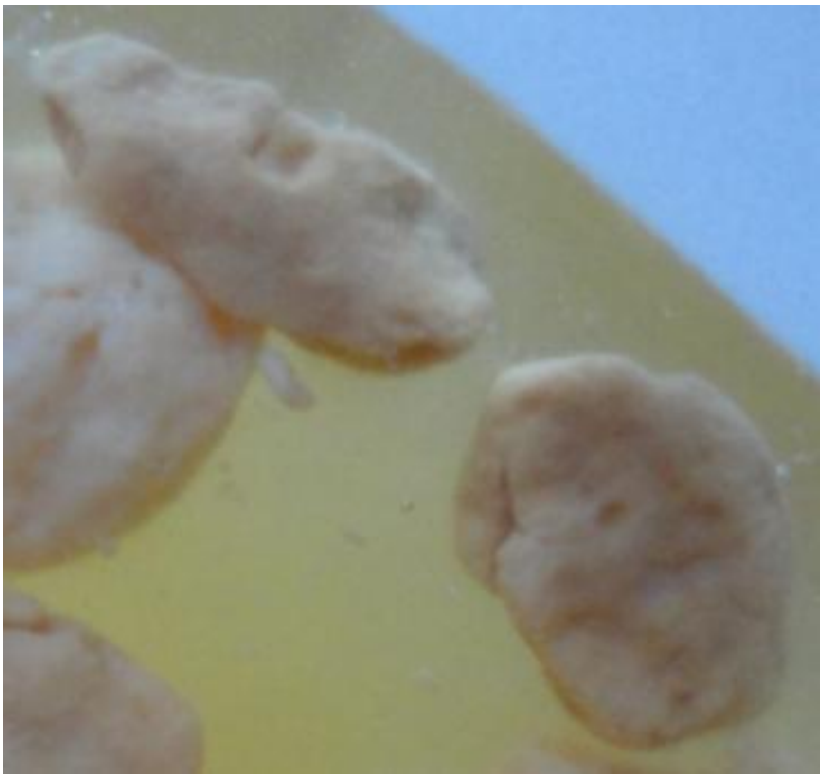
**November 7**



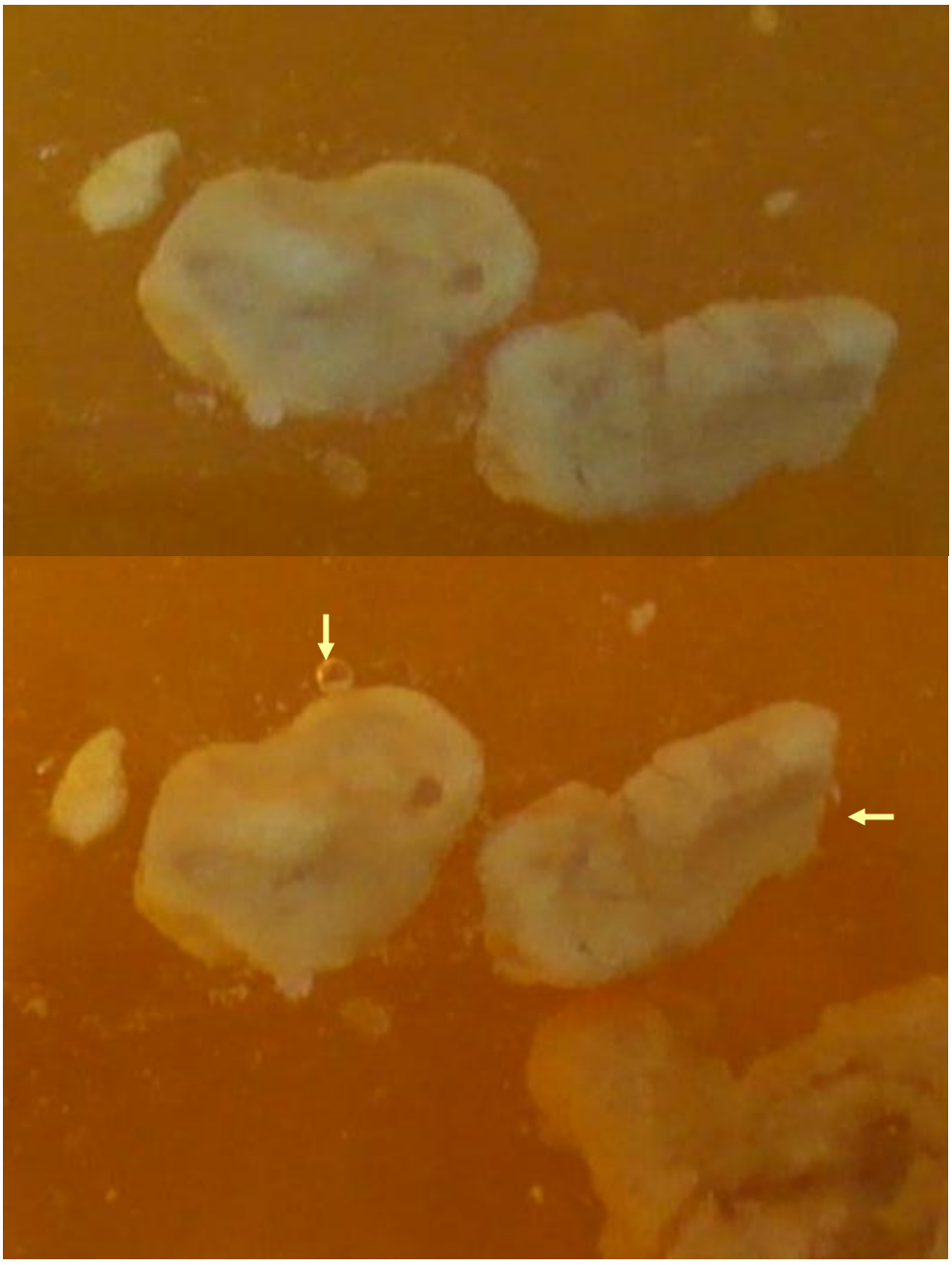
**The pairing took place between grains J and K in continual motion, passing each other in a crisscross fashion.** The composite image captures the whole scene: the grains seem to be receptive to the presence of one another stopping momentarily, as they continually bounce up and down. Successive frames were extracted from video every 20 seconds.

**November 10**

**November 11**



**November 15**



**Grains J and K have maintained a pair bond during all this time, demonstrating a sustained relationship as never seen before.** Notice a translucent viscous gel-like substance barely visible at the boundaries, and release of $CO_2$ in short bursts (arrows) presumably reflecting the metabolic activity of the microorganisms living inside them. Successive frames were extracted from video within seconds.