

1 **Biological activity of the non-microbial fraction of kefir: antagonism against intestinal**
2 **pathogens**

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6 **Supplementary materials**

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8 **Materials and methods**

9 **Antagonic activity against *Salmonella* of the non-microbial fraction of kefir**

10 **1. *Salmonella* Enteritidis preincubation with non-microbial fraction of kefir**

11 *Salmonella* Enteritidis culture was centrifuged at 5000 xg for 5 min and resuspended in
12 phosphate-buffered saline solution (PBS). The suspension was adjusted to OD_{600nm} 0.36
13 ($\approx 1 \times 10^8$ CFU/ml). Then diluted to 2×10^6 CFU/ml in 5 ml of non-microbial fraction of kefir,
14 adjusting to pH=4.5 and incubated for 1 h at 37 °C. The control condition was incubated in
15 5 ml of PBS. Then, suspensions were centrifuged 5 min at 5000 xg, resuspended in the
16 same volume of cell culture medium, (Dulbecco's Modified Eagle's Medium, DMEM) base
17 and concentration of *S. Enteritidis* was confirmed by counting viable microorganisms on
18 nutrient agar plates followed by 24 h incubation at 37 °C in aerobiosis. The cell culture
19 plate was filled with 0.5 ml/well of pathogen's suspension and was incubated for 1 h at
20 37 °C in controlled atmosphere (5% CO₂). The association and invasion of *S. Enteritidis* was
21 determined as described Golowzycy *et al.* (2007).

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23 **2. Pre-incubation of cells with non-microbial fraction kefir**

24 The Caco-2/TC-7 cells seeded in 24-well plates were incubated for 1 or 16 h with the non
25 microbial fraction of kefir or with acid lactic solution 100 mM, neutralized and filtered,
26 diluted 1:1 in DMEM. The control condition was incubated with DMEM. Then the cells

27 were washed 3 times with PBS and were added 0.5 ml of the suspension of *S. Enteritidis*
28 (2×10^6 CFU/ml) prepared on DMEM. Association and invasion assays were performed as
29 indicated in the previous section.

30 **Table 1:** Effect of the pre-treatment of Caco-2/TC-7 cells with the non-microbial fraction
31 of kefir (NMFK) and lactate 100 mM on the interaction with *S. Enteritidis*. Results are
32 expressed in Log₁₀ CFU/well.

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	1 h pre-treatment		16 h pre-treatment	
	Association	Invasion	Association	Invasion
Control	4.87 ±0.10 ^a	3.55 ±0.27 ^b	4.87 ±0.10 ^a	3.55 ±0.27 ^b
NMFK	4.65 ±0.30 ^a	3.54 ±0.36 ^b	4.85 ±0.07 ^a	3.68 ±0.17 ^b
Lac 100mM	4.39 ±0.29 ^a	3.30 ±0.34 ^b	4.51 ±0.42 ^a	3.69 ±0.50 ^b

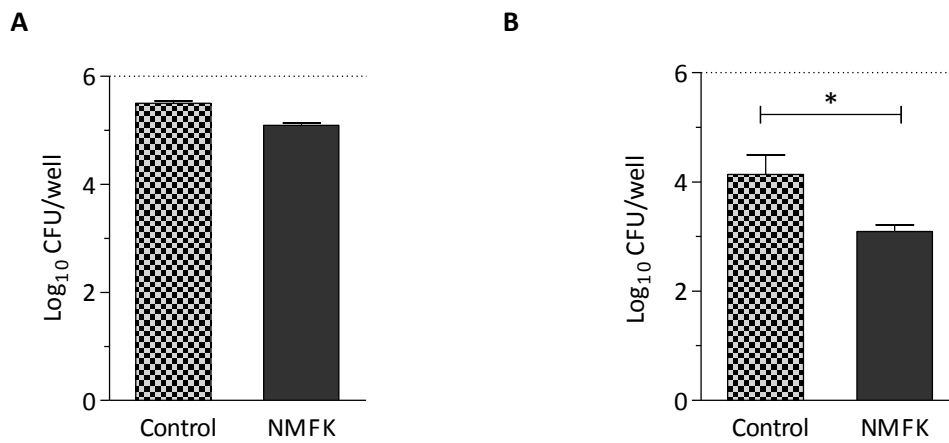
34 Different letters within columns indicates significant differences with $p < 0.05$.

35 **Figure 1: Effect of the pre-treatment of *S. Enteritidis* with the non-microbial fraction**
36 **of kefir (NMFK) on their interaction with Caco-2/TC-7 cells. (A) Association (B)**
37 **Invasion. Results are expressed in Log₁₀ CFU/well. * indicates significant differences**
38 **with $\alpha=0.05$.**

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40 **Figure 1:**

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