



# Corrigendum: Cell-Free Spent Media Obtained From *Bifidobacterium bifidum* and *Bifidobacterium crudilactis* Grown in Media Supplemented with 3'-Sialyllactose Modulate Virulence Gene Expression in *Escherichia coli* O157:H7 and *Salmonella* Typhimurium

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Keywords: *Bifidobacterium bifidum*, *Bifidobacterium crudilactis*, bovine milk oligosaccharide, *Escherichia coli* enterohemorragic O157:H7, *Salmonella enterica* serovar Typhimurium, virulence expression, 3'-sialyllactose, whey

### A Corrigendum on

Cell-Free Spent Media Obtained from *Bifidobacterium bifidum* and *Bifidobacterium crudilactis* Grown in Media Supplemented with 3'-Sialyllactose Modulate Virulence Gene Expression in *Escherichia coli* O157:H7 and *Salmonella* Typhimurium

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In the original article, there was an error. "The Escherichia coli O157:H7 ATCC 43890 strain mentioned in the original manuscript is not the one used, since it was the Escherichia coli O157:H7 ATCC 35150 strain."

A correction has been made to the section MATERIALS AND METHODS, subsection Bacterial Strains and Growth Conditions:

*Bifidobacterium bifidum* BBA1 was isolated from feces from a breast-fed child (CHU - Hôpital des Bruyères, Liège, Belgium) and *B. crudilactis* FR/62/B/3 from Saint-Marcellin, a raw cow milk cheese from Vercors (France). Both strains were stored at  $-80^{\circ}$ C and grown on De Man, Rogosa, and Sharpe (MRS) medium (Oxoid, Hampshire, UK) supplemented with cysteine-HCl (0.5 g/l) and mupirocin (0.08 g/l) at 37°C for 48 h in an anaerobic workstation (Led Techno, Heusden-Zolder, Belgium) containing 10% H<sub>2</sub>, 10% CO<sub>2</sub>, and 80% N<sub>2</sub>. Several successive cultures, in the same conditions as described previously, have been realized in MRS broth, prior to use. Pathogenic enterohaemorrhagic *E. coli* (EHEC) strain O157:H7 ATCC 35150 ( $stx_2^+$ ) and *S. enterica* serovar Typhimurium strain ATCC 14028 were stored at  $-80^{\circ}$ C and grown in Luria Bertani (LB) media

(Sigma-Aldrich, Diegem, Belgium). Two reporter mutants, E. coli O157:H7 ATCC 43888 (stx<sup>-</sup>, LEE:lux) containing plasmid LEE1luxCDABE and resistant to ampicillin (Ampr) and kanamycin (Kan<sup>r</sup>) and S. Typhimurium SA 941 256 containing plasmid pSB377 (hilA::luxCDABE; Ampr) were designed by Medellin-Pena et al. (2007) and Bayoumi and Griffiths (2010), respectively. Both strains were from the Canadian Research Institute for Food Safety Collection and were grown under aerobic conditions at 37°C in brain heart infusion (BHI) broth (Bio-Rad, Marnesla-coquette, France) supplemented with ampicillin (50 mg/l). A medium optimized for B. crudilactis FR/62/B/3, called MRS2 (Tanimomo et al., 2016) was considered as the reference medium for this study (Table 1) and was modified by removing or replacing glucose: MRS2 without any glucose (MRS2G) (control), MRS2 with a mix of glucose and whey (MRS2-Wh) and MRS2 with 3'SL (MRS2-3'SL) as the only source of carbohydrate (Table 1). Whey was collected at the beginning of a curdling process of a Belgian cheese factory (Liège area, Belgium). The quantity of lactose in MRS2-Wh medium was estimated to 25 g/l, based on lactose concentration of sweet whey (50 g/l of lactose; Food and Agriculture Organization/Organisation Mondiale de la Santé, 1998). However, mature bovine milk contains only traces of BMO (Kelly et al., 2013). The 3'SL, added to MRS2-3'SL, was provided by Carbosynth laboratory (Berkshire, UK). The concentration of 0.85 g/l was chosen to be close to natural concentrations found in colostrum (Nakamura et al., 2003). B. bifidum BBA1 and B. crudilactis FR/62/B/3 were grown in three independent experiments under the same anaerobic conditions as previously at 37°C for 48 h. Five log/ml of bifidobacteria from a fresh 48 h culture of bifidobacteria were inoculated into the fresh media (1% v/v). The concentration of 5 log/ml was confirmed by plating several dilutions of bifidobacteria at day 0 post inoculation. Bacterial growth was determined by viable counts after 48 h incubation. Cell free spent media (CFSM) were obtained after two centrifugation steps at 5000 × (Eppendorf Centrifuge 5804, Hamburg, Germany) for 10 min. Supernatants were then sterilized by filtration (Minisart<sup>®</sup> 0.45 µm and 0.2 µm, Sartorius, Vilvoorde, Belgium). Next, CFSM were freezedried (Virtis Benchtop 3.3 EL, SP Scientific, Suffolk, United-Kingdom) and rehydrated with sterile distilled water to obtain a 10x concentration. The same treatment was applied to nonfermented culture media (controls). The pH of rehydrated CFSM was adjusted to 7 using 1 M NaOH.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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