***Supplementary Image***

**Supplementary Figure 1. The growth curve of cocktail culture of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* at 0, 4, 8, 24 and 72 h in BHI and LJE at 37 ºC.** (A) The growth curve of cocktail culture of *E. coli* O157:H7 (3-log), *S. enteritidis* (3-log) and *L. monocytogenes* (6-log) at 0, 4, 8, 24 and 72 h in BHI. (B) The growth curve of cocktail culture of *E. coli* O157:H7 (3-log), *S. enteritidis* (3-log) and *L. monocytogenes* (6-log) at 0, 4, 8, 24 and 72 h in LJE. (C) The growth curve of cocktail culture of *E. coli* O157:H7 (3-log), *S. enteritidis* (3-log) and *L. monocytogenes* (4-log) at 0, 4, 8, 24 and 72 h in BHI. (D) The growth curve of cocktail culture of *E. coli* O157:H7 (3-log), *S. enteritidis* (3-log) and *L. monocytogenes* (4-log) at 0, 4, 8, 24 and 72 h in LJE.

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**Supplementary Figure 2. The number of reads and read lengths of MinION R9.4 Rev D direct metatranscriptome RNA-seq and RT-PCR amplicon sequencing for BHI and LJE samples collected from 4 h and 24 h. Results of direct metatranscriptome RNA-seq.** (A) BHI-24 h; (B) LJE-24 h; (C) BHI-24 h with non-rRNA mapping; (D) LJE-24 h with non-rRNA mapping. Results of RT-PCR amplicon sequencing of (E) BHI-4 h; (F) LJE-4 h.

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Supplementary Figure 3. Gel image of RT-PCR amplicons were used to verify the present of all three bacteria in the cocktail culture, and PCR was used to verify the complete removal of DNA contaminations using the protocol described above. (A) Non-multiplex RT-PCR of *E. coli* O157:H7 (*Ec*), *S. enteritidis* (*Se*) or *L. monocytogenes* (*Lm*) from24-hour cocktail culture in LJE. From left to right: 1. 100-bp DNA Ladder (NEB); 2. *E. coli* O157:H7 in LJE 24-hour cocktail culture; 3. *S. enteritidis* inLJE 24-hour cocktail culture; 4. *L. monocytogenes* inLJE 24-hour cocktail culture. 5. Negative control (NC) of *E. coli* O157:H7 in LJE 24-hour cocktail culture; 6. Negative control of *S. enteritidis* inLJE 24-hour cocktail culture; 7. Negative control of *L. monocytogenes* inLJE 24-hour cocktail culture. (B) Multiplex RT-PCR (Multi) of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* 4-hour cocktail in LJE. From left to right: 1. 100-bp DNA Ladder; 2. Multiplex RT-PCR of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes*;3.Negative control of *E. coli* O157:H7 (PCR without RT); 4. Negative control of *S. enteritidis* (PCR without RT); 5. Negative control of *L. monocytogenes* (PCR without RT)*.*

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