

# Volatile Fatty Acids Effective as Antibacterial Agents against Three Enteric Bacteria during Mesophilic Anaerobic Incubation

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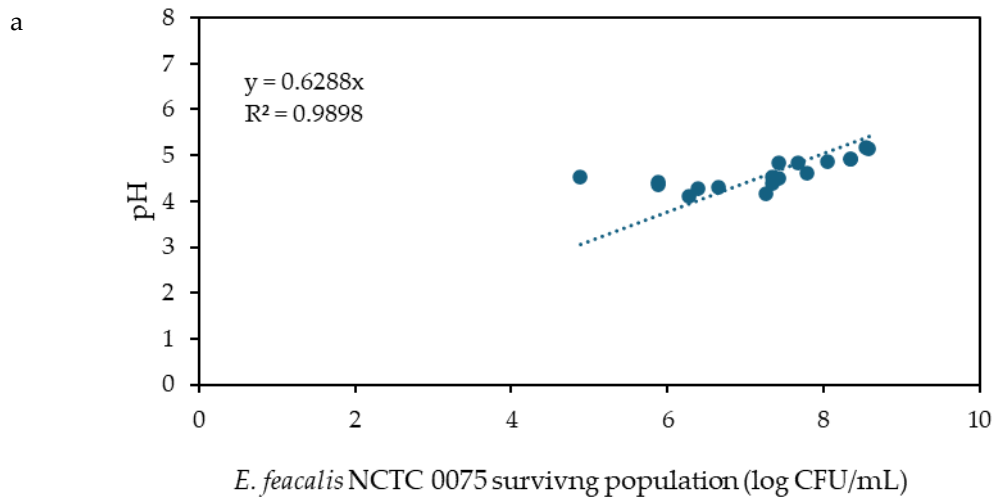
**Table S1.** Inferential statistics to determine significant difference using one-way and two-way ANOVA, and Tukey Honest post hoc tests.

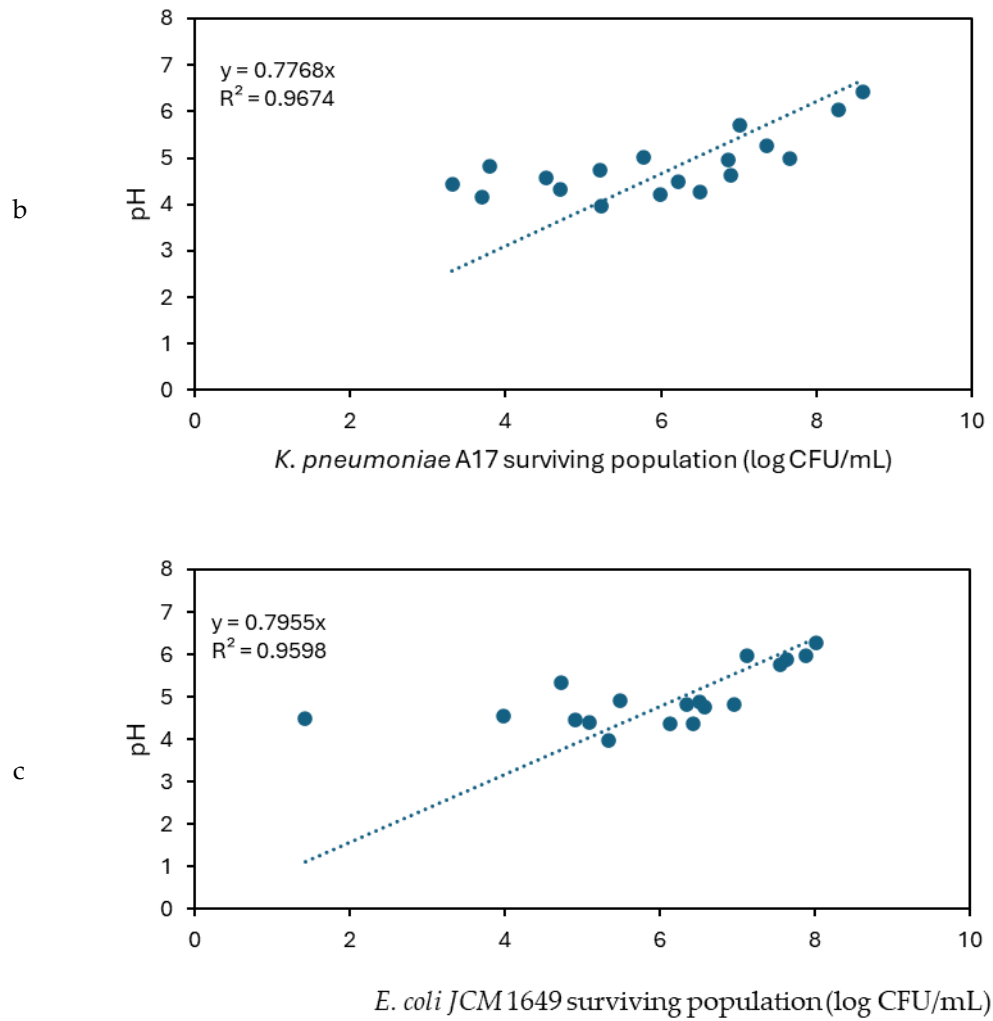
|                     |                     | <i>E. coli</i> JCM 1649 | <i>K. pneumoniae</i> A17 | <i>E. faecalis</i> NCTC 00775 |                    |                |                    |
|---------------------|---------------------|-------------------------|--------------------------|-------------------------------|--------------------|----------------|--------------------|
| VFA type            | Concentration (g/L) | log10 (CFU/mL)          | HSD Tukey Post Hoc       | log10 (CFU/mL)                | HSD Tukey Post Hoc | log10 (CFU/mL) | HSD Tukey Post Hoc |
| Acetic acid (C2)    | 0                   | 8.46                    | a                        | 8.66                          | a                  | 8.70           | a                  |
|                     | 1                   | 6.97                    | b                        | 6.89                          | b                  | 7.78           | b                  |
|                     | 2                   | 6.13                    | b                        | 6.50                          | b                  | 7.27           | b                  |
|                     | 4                   | 3.67                    | c                        | 4.61                          | c                  | 6.27           | c                  |
| Propionic acid (C3) | 0                   | 8.46                    | a                        | 8.66                          | a                  | 8.70           | a                  |
|                     | 1                   | 7.55                    | ab                       | 7.66                          | ab                 | 8.05           | b                  |
|                     | 2                   | 6.57                    | b                        | 6.30                          | b                  | 7.35           | c                  |
|                     | 4                   | 4.50                    | c                        | 3.51                          | c                  | 6.66           | d                  |
| Butyric acid (C4)   | 0                   | 8.46                    | a                        | 8.66                          | a                  | 8.70           | a                  |
|                     | 1                   | 7.55                    | ab                       | 7.25                          | a                  | 8.35           | a                  |
|                     | 2                   | 6.57                    | b                        | 5.44                          | b                  | 7.35           | b                  |
|                     | 4                   | 4.50                    | c                        | 2.02                          | c                  | 5.89           | c                  |
| Valeric acid (C5)   | 0                   | 8.46                    | a                        | 8.66                          | a                  | 8.70           | a                  |
|                     | 1                   | 7.88                    | ab                       | 7.03                          | b                  | 8.58           | a                  |
|                     | 2                   | 6.52                    | b                        | 5.79                          | b                  | 7.67           | a                  |
|                     | 4                   | 3.28                    | c                        | 2.37                          | c                  | 4.67           | b                  |
| Caproic acid (C6)   | 0                   | 8.46                    | a                        | 8.66                          | a                  | 8.70           | a                  |
|                     | 1                   | 8.01                    | a                        | 8.60                          | a                  | 8.55           | a                  |
|                     | 2                   | 5.56                    | b                        | 5.09                          | b                  | 7.43           | a                  |
|                     | 4                   | 2.39                    | c                        | 3.16                          | b                  | 4.10           | b                  |
| VFA                 | 0                   | 8.46                    | a                        | 8.66                          | a                  | 8.70           | a                  |

|          |   |      |   |      |    |      |   |
|----------|---|------|---|------|----|------|---|
| cocktail | 1 | 7.91 | a | 7.49 | ab | 7.87 | a |
|          | 2 | 5.42 | b | 6.08 | b  | 7.42 | a |
|          | 4 | 2.42 | c | 2.24 | c  | 4.31 | b |

**Table S2.** Antibigram of bacterial strains used for antibacterial analysis of VFAs. Gram-positive and Gram-negative antibiotic MAST rings were used for the test.

| Antibiotic                    | <i>E. faecalis</i> NCTC0075 | <i>E. coli</i> JCM 1649 | <i>K. pneumoniae</i> A17 |
|-------------------------------|-----------------------------|-------------------------|--------------------------|
| Ampicillin (AP) 10ug          | S                           | S                       | R                        |
| Cephalotin (KF) 5 ug          | R                           | R                       | R                        |
| Collistin Sulphate (CO) 25 ug | R                           | S                       | S                        |
| Gentamicin (GM) 10ug          | R                           | S                       | S                        |
| Streptomycin (S) 10ug         | R                           | R                       | R                        |
| Sulphatriad (ST) 200ug        | R                           | R                       | R                        |
| Tetracycline (T) 25ug         | S                           | S                       | S                        |
| Cotrimoxazole (TS) 25ug       | R                           | S                       | S                        |
| Chloramphenicol (C) 25 ug     | R                           | S                       | S                        |
| Erythromycin (E) 5 ug         | R                           | R                       | R                        |
| Fusidic acid (FC) 10ug        | R                           | R                       | R                        |
| Oxacillin (OX) 5ug            | R                           | R                       | R                        |
| Novobiocin (NO) 5ug           | R                           | R                       | R                        |
| Penicillin G (PG) 1 unit      | R                           | R                       | R                        |





**Figure S1.** Correlation analysis of surviving population (log CFU/mL) (a measure of VFA toxicity) of *E. faecalis* NCTC 00775 (a) *K. pneumoniae* A17 (b) and *E. coli* JCM 1649 (c) against pH. VFA toxicity correlated positively with pH with  $R^2$  values of 0.9898, 0.9674 and 0.9598 respectively for the three strains.