microbial biotechnology

Open Access

Brief Report

Salmonella grows massively and aerobically in chicken faecal matter

Teresa Guerrero* **Diana Calderón, Sonia Zapata and Gabriel Trueba**
Microbiology Institute, Universidad San Francisco de Quito, Quito, Ecuador.

Summary

The use of wastewater for irrigation and animal manure as fertilizer can cause transmission of intestinal pathogens, conditions frequently observed in low- and middle-income countries (LMICs). Here, we tested the ability of *Salmonella* to grow in the faecal matter. We inoculated freshly isolated *Salmonella* strains (from chickens) in chicken faecal matter and incubated for 1 to 12 days, under aerobic and anaerobic conditions. We found that both *Salmonella* and *Escherichia coli* multiplied massively in faecal matter outside a host and significantly higher in aerobic conditions. Our results have critical implications in waste management, as we demonstrate that aerobic treatments may not be the best to reduce the number of *Salmonella* in the environment.

Introduction

Environmental transmission of intestinal pathogens is extremely important especially in low- and middle-income countries (LMICs) due to deficient sanitary infrastructure, unplanned urban growth, lack of wastewater treatment, etc. One of the main concerns in LMICs is the large proportion of untreated wastewater used for irrigation (Khalid *et al*., 2018) and the increasing use of animal manure as fertilizer without suitable treatment (Mandrell, 2009). Reports of grave enteric infections caused by environmental contamination of edible vegetables are also commonplace nowadays in industrialized countries (Callejón *et al*., 2015). Some of these outbreaks have been associated with high mortality, morbidity and large economic losses. The incidence of these infections is exacerbated by the increasing appeal to consume natural, non-processed fresh products (Mandrell, 2009).

Salmonella-contaminated water is responsible for a large number of outbreaks by the ingestion of water or produce (Mandrell, 2009); the sources for this contamination are human and non-human faecal matter (Medrano-Félix *et al*., 2017). The use of animal waste as fertilizer constitutes a serious risk that can be controlled by appropriate composting technology (Tiquia *et al*., 1998; Szogi *et al*., 2015). Human waste contamination, however, is much more difficult to monitor or control in LMICs where wastewater treatment or toilets are not available (Khalid *et al*., 2018). The fate of *Salmonella* in these conditions is not understood completely, although some researchers indicate that *Salmonella* enters into a viable non-culturable state outside the host (Winfield and Groisman, 2003). The reduction of the risk of this type of transmission requires an understanding of every aspect of *Salmonella* physiology in the environment outside the host (Mandrell, 2009). It is worth mentioning that *Salmonella*'s ability to grow in the faecal matter has been ignored.

It is known that *Salmonella* and other *Enterobacteriaceae* survive in faecal matter for some time and it has been shown that *Escherichia coli* (another member of the *Enterobacteriaceae*) also grows massively in faecal matter (Russell and Jarvis, 2001; Vasco *et al*., 2015; Sharma *et al*., 2019). Here, we tested *Salmonella*'s ability to grow in faecal matter in aerobic and anaerobic conditions and discuss the potential implications for faecal waste management.

Results and discussion

Two trials were performed with *Salmonella* Infantis inoculated in chicken faecal matter. In the first trial, we determined the growth of *Salmonella* by plate counting and by molecular detection after 0, 24, 48 and 72 h of incubation; in the second trial, we performed *Salmonella* plate counting daily, from day 0 to day 12 of incubation (Fig. 1).

Received 12 September, 2019; accepted 22 June, 2020.
*For correspondence. E-mail tguerrero@estud.usfq.edu.ec; Tel. +(593) 2 2971700; Fax +(593) 2 2890070.
Microbial Biotechnology (2020) **13**(5), 1678–1684
doi:10.1111/1751-7915.13624
Funding information
Universidad San Francisco de Quito (Postgraduate Funds).

In the first trial, *Salmonella* Infantis inoculated in chicken faecal matter multiplied in both aerobic and anaerobic conditions; however, the aerobic growth was significantly higher than the anaerobic growth at 48 h ($P = 1.28 \times 10^{-4}$) and 72 hrs ($P = 2.94 \times 10^{-4}$). Similarly, endogenous *E. coli* growth reached its peak after 48 h, predominantly in aerobiosis ($P = 1.92 \times 10^{-2}$) and from then on, its growth rate decreased (Fig. 2, Figs S1–S4). The growth curve of total endogenous coliforms was similar to that of *E. coli*, with a peak in aerobiosis at 48 h ($P = 1.30 \times 10^{-2}$), but their counts were higher (Fig. S5).

Escherichia coli had the highest specific growth rate (μ) during the second day in aerobiosis ($P = 8.14 \times 10^{-8}$), decreasing in the following 24 h; *Salmonella* started fast growth at 24 h and presented significantly higher values of μ in aerobiosis than in anaerobiosis at all time intervals (for $\Delta t1$, $\Delta t2$ and $\Delta t3$, $P = 7.49 \times 10^{-5}$, 6.93×10^{-7} and 9.73×10^{-3}, respectively). Likewise, endogenous coliforms presented higher μ values in aerobiosis than in anaerobiosis after 48 h ($P = 1.83 \times 10^{-2}$) (Fig. 3).

To determine whether the above growth pattern could be applied to other *Salmonella* serovars, in the first trial we run isothermal amplification 3M™ Molecular Detection Assay 2 – Salmonella (MDA2SAL) at different incubation times (under aerobiosis and anaerobiosis) with 5 *Salmonella* strains (belonging to different serovars) inoculated in chicken faecal matter. The molecular assay was performed daily until day 3 after incubation (0 to 72 h). For serovars Infantis, Heidelberg, Brandenburg and Stanley, the growth peak in aerobiosis was observed at 72 h ($P = 1.19 \times 10^{-3}$), while serovar Dublin growth peak occurred at 48 h (Fig. 4, Fig. S6).

In a subsequent experiment (trial 2), no colonies of *Salmonella* in XLD or XLD with NIT were observed in aerobiosis between days 2 and 6 of incubation, probably because of a massive growth of lactose-fermenting bacteria (yellow colonies) corresponding to the commensal *Enterobacteriaceae*. Increasing *Salmonella* counts were

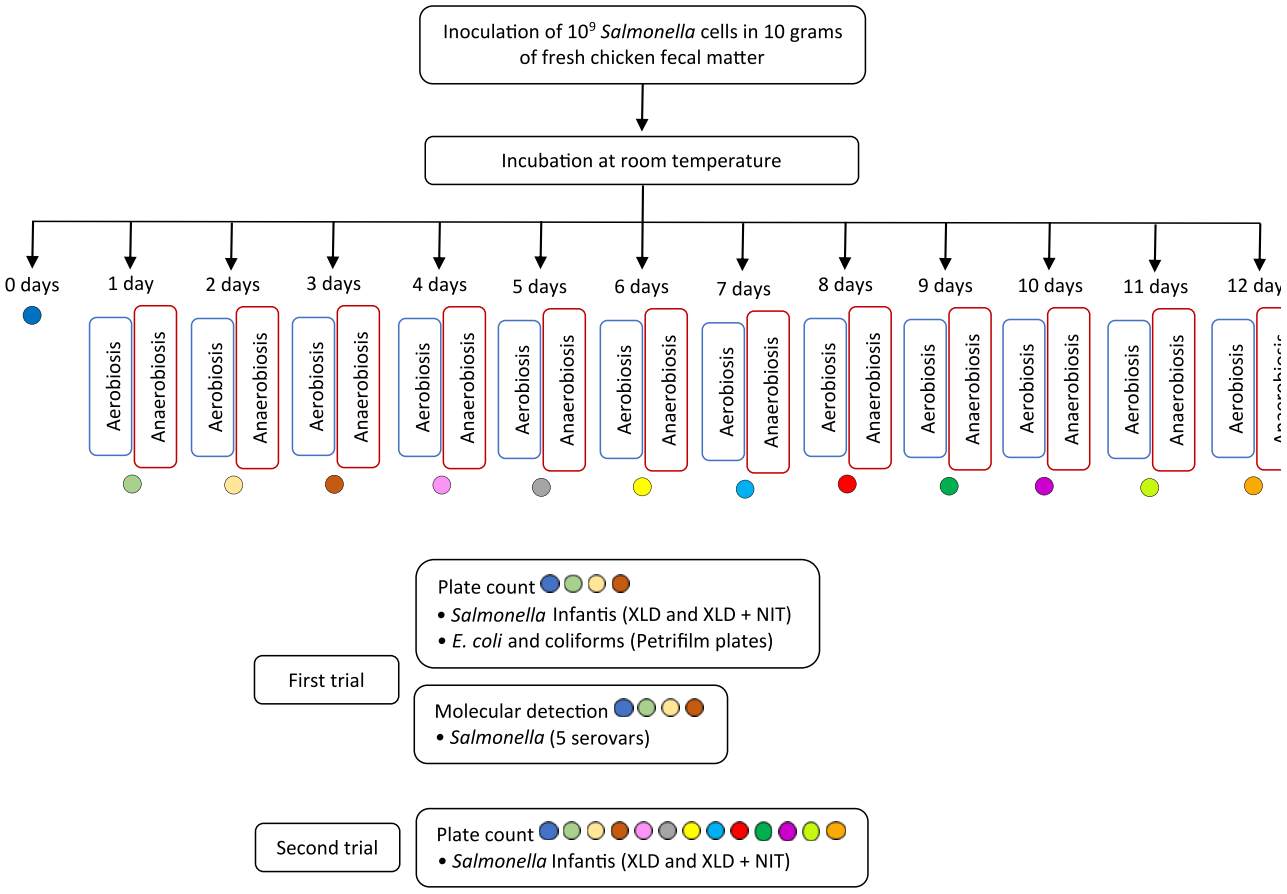

Fig. 1. Scheme of experimental procedures. The experiments performed in trial 1 and in trial 2 are indicated. In the first trial, we determined the growth of *Salmonella* by plate counting in XLD and XLD with nitrofurantoin (NIT), and by molecular detection after 0, 24, 48 and 72 h of incubation; in the second trial, we performed *Salmonella* plate counting daily, from day 0 to day 12 of incubation.

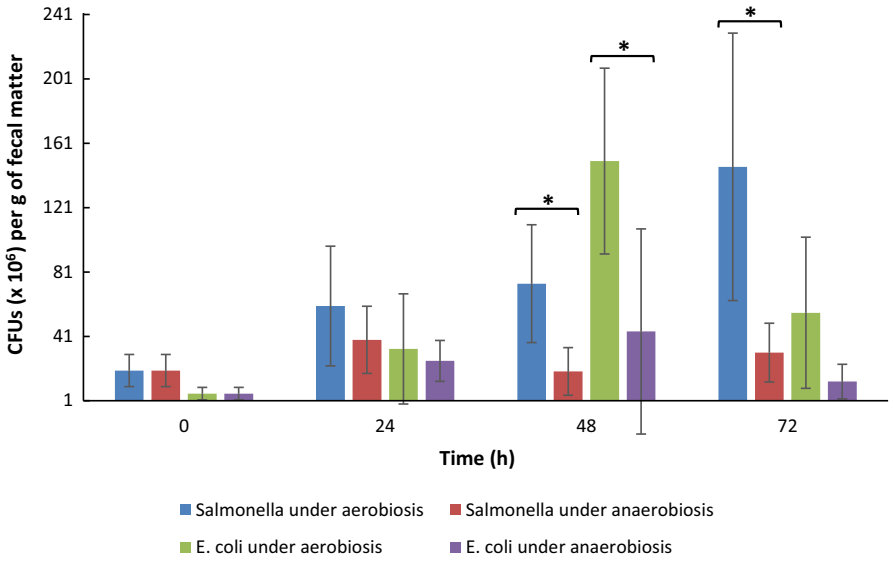

Fig. 2. Growth of *Salmonella* Infantis and endogenous *E. coli* in chicken faecal matter, under aerobic and anaerobic conditions. Typical *Salmonella* colonies were counted in XLD and XLD with NIT (12 mg L^{-1}) (we took advantage of the *Salmonella* strain's resistance to nitrofurantoin to facilitate *Salmonella* colony count), and *E. coli* was counted in 3M™ Petrifilm *E. coli*/Coliform Count Plates. Data shown are means ± SD. Asterisks indicate a statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic growth. The number of Petri dishes counted (replicate counts) is represented by *n*. For *Salmonella* 0 h, 72 h of aerobiosis and 48 h of anaerobiosis $n = 14$; for 24 h of aerobiosis $n = 15$; for 48 h of aerobiosis $n = 6$; for 24 h of anaerobiosis $n = 16$; and for 72 h of anaerobiosis $n = 10$. For *E. coli* 0 h, 24 h of anaerobiosis and 72 h of anaerobiosis $n = 6$; for 24 h of aerobiosis $n = 7$, for 48 and 72 h of aerobiosis $n = 4$; and for 48 h of anaerobiosis $n = 8$. These experiments were performed twice and correspond to the first trial.

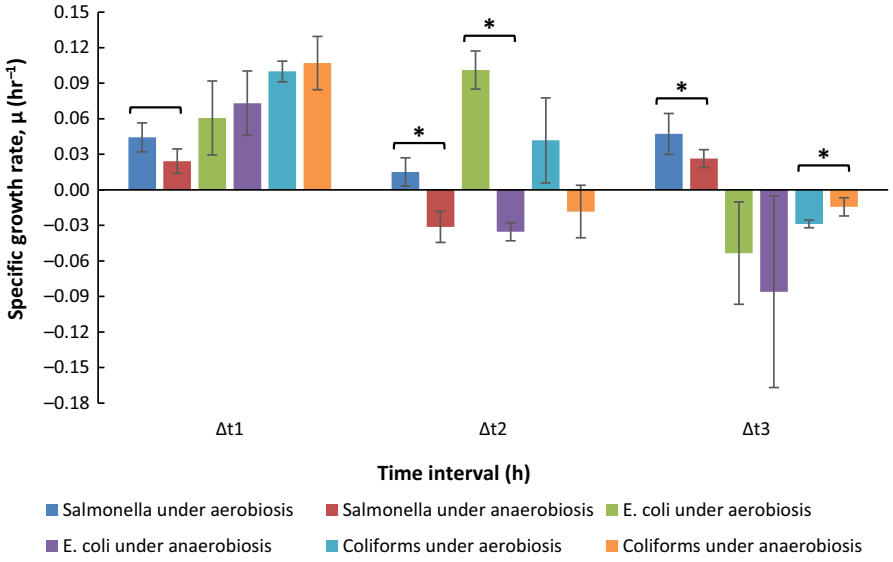

Fig. 3. Specific growth rate for *Salmonella* Infantis, endogenous *E. coli* and total coliforms, under aerobic and anaerobic conditions. Specific growth rate, μ, was calculated with the formula: $\mu = 2.3\log(N/No)/\Delta t$, where *N* is the final population after a time interval of incubation, Δt, and *No* is the initial population. The incubation times were t1 = 0 h, t2 = 24 h, t3 = 48 h and t4 = 72 h. And the intervals were Δt1 = t2–t1, Δt2 = t3–t2 and Δt3 = t4–t3. Data shown are means ± SD. Asterisks indicate a statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic conditions. The number of Petri dishes counted is represented by *n*. For *Salmonella* Δt1 aerobiosis and anaerobiosis, and Δt2 anaerobiosis $n = 14$; for Δt2 and Δt3 aerobiosis $n = 6$; and for Δt3 anaerobiosis $n = 8$. For *E. coli* Δt1 aerobiosis and anaerobiosis, Δt2 anaerobiosis and Δt3 anaerobiosis $n = 6$; and for Δt2 and Δt3 aerobiosis $n = 4$. For total coliforms Δt1, Δt2 and Δt3 aerobiosis, and for Δt1 anaerobiosis $n = 4$; and for Δt2 and Δt3 anaerobiosis $n = 3$. These data correspond to the first trial.

detected on day 7 and reached a peak on day 9 (1.8×10^8 cells per g of faecal matter) (Fig. S7), which coincided with a reduction in the number of lactose-fermenting bacteria colonies. On days 10 to 12, *Salmonella* growth was not detected, but lactose fermenters kept on growing, and glucuronidase reaction indicated

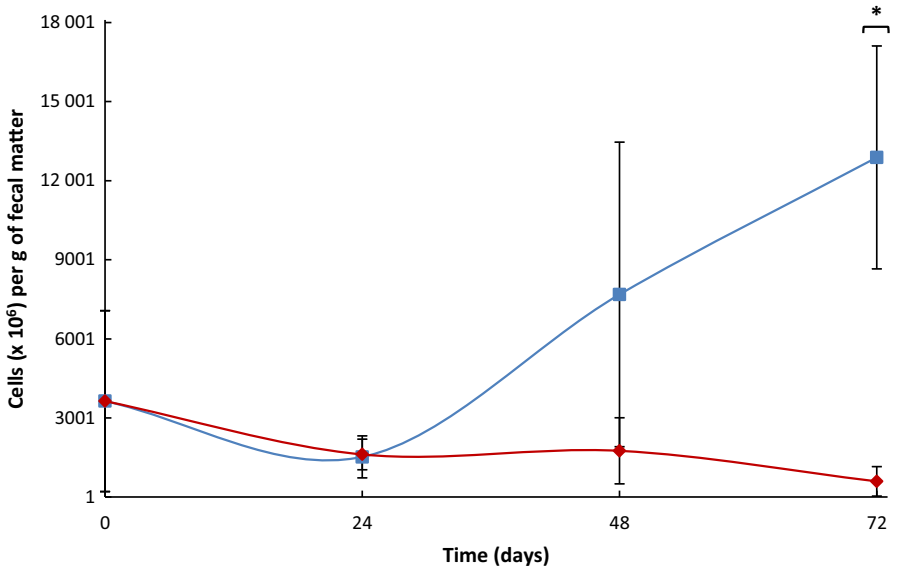

Fig. 4. Growth curves of *Salmonella* serovars: Infantis, Heidelberg, Brandenburg and Stanley. Curves were obtained by 3M™ Molecular Detection Assay 2 – Salmonella (MDA2SAL). The blue line corresponds to the growth under aerobic conditions and the red one, to the growth under anaerobic conditions. Data shown are means ± SD. Asterisk indicates a statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic growth. The number of independent readings is represented by *n*; for all data points $n = 4$. The experiment was performed once and corresponds to the first trial.

that 94% of them were *E. coli*. We suspect that the massive growth of lactose-fermenting bacteria was due to a different diet used in chickens during the second trial (Shang *et al*., 2018). In anaerobiosis, we observed no growth of *Salmonella* or lactose-fermenting bacteria from days 1 to 12.

Growth rates of *Salmonella* and *E. coli,* at different incubation time intervals, suggested a negative correlation which may indicate antagonism between these two bacterial genera (Fig. 3). We posit that *E. coli*'s initial massive replication may limit the availability of oxygen for *Salmonella* growth; once *E. coli* growth begins to decrease, *Salmonella* grows faster. Competition between these two bacterial genera has been described previously in the gut (Barrow *et al*., 2015; Velazquez *et al*., 2019) and in ready-to-eat and fresh foods, to such an extent that some authors consider that *E. coli* may not be a good indicator of *Salmonella* (Gómez-Aldapa *et al*., 2013).

To investigate whether there was antagonism between *Salmonella* and *E. coli* in faeces, we inoculated equal concentrations (10^9 cells) of *S.*Infantis and an *E. coli* (isolated from chicken) in 10 g of sterile chicken faecal matter; inoculated samples were incubated in aerobiosis and anaerobiosis for 6 days. We observed that aerobic *E. coli* growth from day 3th to 6th was significantly higher than *Salmonella*'s (*P* values days 3 to 6 were: 1.20×10^{-5}, 1.86×10^{-2}, 1.54×10^{-6} and 5.09×10^{-5}, respectively) (Fig. S8), which suggests some level of competition between these two bacteria. This finding is in agreement with previous reports (Shang *et al*., 2018). There were two differences between the results of the experiments in fresh faecal matter and sterilized faecal matter: (i) the interference of *E. coli* growth occurred later in sterile faecal matter (Fig. 2, Fig. S8); and (ii) there was no difference between growth under aerobic or anaerobic conditions, except for *Salmonella* on day 5 (Fig. S9). These differences may be due to physical and chemical modifications of the faecal matter by heat sterilization; autoclaved faecal matter was drier and harder probably due to dehydration and starch gelatinization (Weurding *et al*., 2001). Additionally, lower water activity may protect *Salmonella* (Santos *et al*., 2005).

To ascertain whether the aerobic or anaerobic environments are determining factors in the growth of *Salmonella* and *E. coli* in chicken faecal matter, we inoculated fresh faecal matter with *Lactobacillus reuteri* strain *LrRR* (López *et al*., 2019), an anaerobic bacterium (Kandler *et al*., 1980; Ianniello *et al*., 2015), and our results showed that the growth of LrRR was significantly higher in anaerobiosis on days 2 and 3 ($P = 4.48 \times 10^{-3}$ and 6.86×10^{-5}, respectively) (Fig. S10), which is an additional evidence that the presence or absence of oxygen in the environment is a factor that determines the differential growth of *Salmonella* and *E. coli* in fresh chicken faeces. On day 6, we observed that LrRR growth in aerobiosis and anaerobiosis produced the same numbers of colonies; we speculate that aerotolerant mutant bacteria may have been selected during the incubation period, a phenomenon described previously in *Lactobacillus* (Ianniello *et al*., 2015).

Our results indicate that *Salmonella* and other *Enterobacteriaceae* multiply massively and aerobically in fresh chicken faecal matter; in fact, faecal matter incubated under aerobic conditions has more *Salmonella* (on average 10 times more) than freshly released faeces. Our results show clear evidence that the faecal matter is a transient but very important component of the *Enterobacteriaceae* life cycle, where enterobacterial population expands (Russell and Jarvis, 2001; Vasco *et al*., 2015; Barrera *et al*., 2018) increasing the chances of reaching other hosts.

Previous studies have shown that *E. coli* has a negative growth rate outside the host, with a short half-life (1 day in water, 1.5 days in sediment and 3 days in soil) (Winfield and Groisman, 2003); however, we have found that as long as it remains in faecal matter, *E. coli* continues to grow up to 12 days after being excreted in the environment (intermediate habitat) (Barrera *et al*., 2018). Also, it has been estimated that the doubling time of *E. coli* in its primary habitat (the intestine of warm-blooded animals) is 2 days (Winfield and Groisman, 2003), and our results indicate that its doubling time in the intermediate habitat during the first two days is less than 24 h (Fig. 2, Fig. S1). Our findings disagree with the notion that these bacteria enter a viable but not culturable status when excreted from the host (Winfield and Groisman, 2003). Additional studies are needed due to the relevance of this issue in public health.

Microbiologists have struggled to explain why bacteria adapted to the anaerobic intestinal milieu possess energetically costly machinery to use oxygen (Govantes *et al*., 2000). Further, it has been shown that aerobic respiration is not important for *Salmonella* intestinal colonization (Barrow *et al*., 2015). We hypothesize that the reason for this apparent evolutionary mystery may be related to the enterobacterial ability to grow in faecal matter under aerobic conditions. *Enterobacteriaceae* are facultative anaerobe which can synthesize ATP by different enzymatic pathways, depending on the external concentration of O_2 and the redox changes in the environment. When O_2 is available, the bacteria obtain energy by aerobic respiration, with O_2 being the final acceptor of electrons. In shortage of O_2, these bacteria generate ATP by one of the following mechanisms: (i) synthesis of terminal oxidases that allow the bacteria to take advantage of traces of O_2; (ii) use of other inorganic molecules (such as NO_3^- and $S_4O_6^{2-}$) as final electron acceptors (Yamamoto and Droffner, 1985; Bueno *et al*., 2012; Rivera *et al*., 2013); and (iii) use of organic compounds as donors and acceptors (Madigan *et al*., 2012). However, aerobic respiration produces much better performance in terms of ATP molecules per substrate molecule (Madigan *et al*., 2012).

Salmonella is responsible for hospitalizations and deaths worldwide (Omer *et al*., 2018; EFSA and ECDC, 2019) due to outbreaks associated not only with animal products but also with vegetables (Gunel *et al*., 2015; Omer *et al*., 2018). The presence of *Salmonella* in produce is associated with unintended environmental faecal contamination and the use of untreated manure as fertilizer (Fletcher *et al*., 2013). Our results have critical implications in waste management, contribute to select more efficient ways of treating manure through composting (Singh *et al*., 2012; Román *et al*., 2015) and suggest the need of anaerobic treatments for animal waste.

The loose consistency of avian faeces allows the entry of air, and this phenomenon may contribute to the proficiency of these animals to spread *Salmonella*. Similarly, loose stools caused by *Salmonella* infection may favour the growth of this bacterium in faecal matter from animals with different faecal texture.

The inconsistencies found in this study are probably due to the complex composition of faecal matter (food substrates and microbiota). Another limitation was the abundant growth of accompanying bacteria (lactose fermenters) that made it difficult the detection of *Salmonella* in XLD.

This type of studies is important because it helps to understand better the physiology of *Salmonella* and other members of the *Enterobacteriaceae* family. We addressed a neglected but crucial characteristic of *Salmonella* life cycle which may have an impact in public health.

Acknowledgements

We want to thank Alejandro Torres for providing us with *Salmonella*-free chickens for carrying out these experiments. We thank Karen Pacheco and Brenda Tito for their help in the laboratory work. And, in a special way, we thank Dr. Harald Brussow, Editor-in-Chief of *Microbial Biotechnology*, for his valuable suggestions. This work was funded by the Universidad San Francisco de Quito USFQ Postgraduate Funds.

Conflict of interest

The authors declare no conflict of interest.

References

Barrera, S., Cardenas, P., Graham, J.P., and Trueba, G. (2018) Changes in dominant *Escherichia coli* and antimicrobial resistance after 24 hr in fecal matter. *MicrobiologyOpen* **8:** e00643.

Barrow, P.A., Berchieri, A., de Freitas Neto, O.C., and Lovell, M. (2015) The contribution of aerobic and

anaerobic respiration to intestinal colonization and virulence for *Salmonella typhimurium* in the chicken. *Avian Pathol* **44:** 401–407.

Bueno, E., Mesa, S., Bedmar, E., Richardson, D.J., and Delgado, M.J. (2012) Bacterial adaptation of respiration from oxic to microoxic conditions: redox control. *Antioxid Redox Signal* **16:** 4051.

Callejón, R.M., Rodríguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M.C., and Troncoso, A.M. (2015) Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathog Dis* **12:** 32–38.

EFSA and ECDC (European Food Safety Authority and European Centre, & Control) (2019). The European Union One Health 2018 Zoonoses Report. *EFSA Journal*, **17:** 5926.

Fletcher, J., Leach, J.E., Eversole, K., and Tauxe, R. (2013) Human pathogens on plants: designing a multidisciplinary strategy for research. *Phytopathology Perspectives* **103:** 306–315.

Gómez-Aldapa, C., Rangel-Vargas, E., and Castro-Rosas, J. (2013) Frequency and correlation of some enteric indicator bacteria and Salmonella in ready-to-eat raw vegetable salads from Mexican Restaurants. *J Food Sci* **78:** 1201–1207.

Govantes, F., Albrecht, J.A., and Gunsalus, R.P. (2000) Oxygen regulation of the *Escherichia coli* cytochrome d oxidase (cydAB) operon: roles of multiple promoters and the Fnr-1 and Fnr-2 binding sites. *Mol Microbiol* **37:** 1456–1469.

Gunel, E., Polat Kilic, G., Bulut, E., Durul, B., Acar, S., Alpas, H., and Soyer, Y. (2015) Salmonella surveillance on fresh produce in retail in Turkey. *Int J Food Microbiol* **199:** 72–77.

Ianniello, R.G., Zheng, J., Zotta, T., and Ricciardi, A. (2015) Biochemical analysis of respiratory metabolism in the heterofermentative *Lactobacillus spicheri* and *Lactobacillus reuteri*. *J Appl Microbiol* **119:** 763–775.

Kandler, O., Stetter, K., and Kohl, R. (1980) Lactobacillus reuteri. *Zbl Bakt Hyg I Abt Orig* **269:** 264–269.

Khalid, S., Shahid, M., Natasha, I.B., Sarwar, T., Haidair, A., and Niazi, N. (2018) A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. *Int J Environ Res Public Health* **15:** 895.

López, L., Calderón, D., Cardenas, P., Prado, M.B., Valle, C., and Trueba, G. (2019) Evolutionary changes of an intestinal *Lactobacillus reuteri* during probiotic manufacture. *MicrobiologyOpen* **9:** e972.

Madigan, M., Martinko, J., Stahl, D., Clark, D. & (2012) Chapter 4. Nutrition, Culture, and Metabolism of Microorganisms. In *Brock. Biology of Microorganisms*, 13th edn. Espinoza, D., Cook, K., Cutt, S., Hutchinson, E., Marcus, E., and Cogan, D. (eds). San Francisco, CA: Pearson Education, pp. 98–107.

Mandrell, R.E. (2009) Enteric human pathogens associated with fresh produce: sources, transport, and ecology. In *Microbial Safety of Fresh Products*. Fan, X., Niemira, B.A., Doona, C.J., Feeherry, F.E., and Gravani, R.B. (eds). Ames, IA: Blackwell Publishing and the Institute of Food Technologists, pp. 5–41.

Medrano-Félix, J.A., Castro-del Campo, N., de Peraza Garay, F.J., Martínez-Rodríguez, C.I., and Chaidez, C. (2017) Carbon source utilization-based metabolic activity of Salmonella Oranienburg and Salmonella Saintpaul in river water. *Water and Environment Journal* **32:** 118–124.

Omer, M., Álvarez-Ordoñez, A., Prieto, M., Skjerve, E., Asehun, T., and Alvseike, A. (2018). A systematic review of bacterial foodborne outbreaks related to red meat and meat products. *Foodborne Pathog Dis*, **15:** 598–611.

Rivera, F., Winter, S.E., Lopez, C.A., Xavier, M.N., Winter, M.G., Nuccio, S., *et al*. (2013) Salmonella uses energy taxis to benefit from intestinal inflammation. *PLoS Pathog* **9:** 1–13.

Román, P., Martínez, M.M., and Pantoja, A. (2015) Chapter 3. Theoretical foundations of composting. In *Farmer's Compost Handbook. Experiences in Latin America*. Santiago, Chile: Food and Agriculture Organization of the United Nations Regional Office for Latin America and the Caribbean, pp. 11–21. http://www.fao.org/3/a-i3388e.pdf.

Russell, J.B., and Jarvis, G.N. (2001) Practical mechanisms for interrupting the oral-fecal life cycle of Escherichia coli. *J Mol Microbiol Biotechnol* **3:** 265–272.

Santos, F.B.O., Li, X., Payne, J.B., and Sheldon, B.W. (2005) Estimation of most probable number salmonella populations on commercial North Carolina Turkey Farms. *J Appl Poult Res* **14:** 700–708.

Shang, Y., Kumar, S., Oakley, B., and Kim, W.K. (2018) Chicken gut microbiota: importance and detection technology. *Front Vet Sci* **5:** 254.

Sharma, M., Millner, P.D., Hashem, F., Vinyard, B.T., East, C.L., Handy, E.T., *et al*. (2019) Survival of *Escherichia coli* in manure-amended soils is affected by spatiotemporal, agricultural, and weather factors in the Mid-Atlantic United States. *Appl Environ Microbiol* **85:** 1–23.

Singh, R., Kim, J., and Jiang, X. (2012) Heat inactivation of Salmonella spp. in fresh poultry compost by simulating early phase of composting process. *J ApplMicrobiol* **112:** 927–935.

Szogi, A.A., Vanotti, M.B., and Ro, K.S. (2015) Methods for treatment of animal manures to reduce nutrient pollution prior to soil application. *Curr Pollut Rep* **1:** 47–56.

Tiquia, S.M., Tam, N.F.Y., and Hodgkiss, I.J. (1998) Salmonella elimination during composting of spent pig litter. *Biores Technol* **63:** 193–196.

Vasco, G., Spindel, T., Carrera, S., Grigg, A., and Trueba, G. (2015) The role of aerobic respiration in the life cycle of *Escherichia coli*: public health implications. *Avances Cienc Ingenier* **7:** B7–B9.

Velazquez, E.M., Nguyen, H., Heasley, K.T., Saechao, C.H., Gil, L.M., Rogers, A.W.L., *et al*. (2019) Endogenous Enterobacteriaceae underlie variation in susceptibility to Salmonella infection. *Nature Microbiology* **4:** 1057–1064.

Weurding, R.E., Veldman, A., Veen, W.A.G., Van Der Aar, P.J., and Verstegen, M.W.A. (2001) Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs 1. *J Nutr* **131:** 2329–2335.

Winfield, M.D., and Groisman, E.A. (2003) Role of nonhost environments in the lifestyles of Salmonella and *Escherichia coli*. *Appl Environ Microbiol* **69:** 3687–3694.

Yamamoto, N., and Droffner, M.L. (1985) Mechanisms determining aerobic or anaerobic growth in the facultative

anaerobe *Salmonella typhimurium*. *Proc Natl Acad Sci USA* **82:** 2077–2081.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Data points of the growth of *Salmonella* Infantis inoculated in chicken fecal matter, under aerobic conditions. Typical *Salmonella* colonies were counted in XLD and XLD with NIT (12 mg l^{-1}). The number of Petri dishes counted is represented by *n*; for 0 and 72 h $n = 14$, for 24 h $n = 15$, and for 48 h $n = 6$. The experiment was performed twice and correspond to the first trial.

Fig. S2. Data points of the growth of *Salmonella* Infantis inoculated in chicken fecal matter, under anaerobic conditions. Typical *Salmonella* colonies were counted in XLD and XLD with NIT (12 mg l^{-1}). The number of Petri dishes counted is represented by *n*; for 0 and 48 h $n = 14$, for 24 h $n = 16$, and for 72 h $n = 10$. The experiment was performed twice and correspond to the first trial.

Fig. S3. Data points of the growth of endogenous *E. coli* in chicken fecal matter, under aerobic conditions. *E. coli* was counted in 3M™ Petrifilm *E. coli*/Coliform Count Plates. The number of Petri dishes counted is represented by *n*; for 0 h $n = 6$, for 24 h $n = 7$, for 48 and 72 h $n = 4$. The experiment was performed twice and correspond to the first trial.

Fig. S4. Data points of the growth of endogenous *E. coli* in chicken fecal matter, under anaerobic conditions. *E. coli* was counted in 3M™ Petrifilm *E. coli*/Coliform Count Plates. The number of Petri dishes counted is represented by *n*; for 0, 24 and 72 h $n = 6$, for 48 h $n = 8$. The experiment was performed twice and correspond to the first trial.

Fig. S5. Growth of endogenous total coliforms in chicken fecal matter, under aerobic and anaerobic conditions. The number of total coliforms corresponded to the sum of the red and blue colonies with gas in 3M™ Petrifilm *E. coli*/Coliform Count Plates incubated 24 and 48 h. Data shown are means ± SD. Asterisk indicates statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic growth. The number of Petri dishes counted is represented by *n*; for 0 h $n = 8$; for 24 h aerobiosis and anaerobiosis, 48 h aerobiosis and 72 h anaerobiosis $n = 4$; for 72 h aerobiosis $n = 6$; and for 48 h anaerobiosis $n = 3$. The experiment was performed twice and correspond to the first trial.

Fig. S6. Individual growth curves of *Salmonella* serovars. These curves were obtained by 3M™ Molecular Detection Assay 2 - Salmonella (MDA2SAL). The blue lines correspond to the growth under aerobic conditions and red ones, under anaerobic conditions. The number of independent readings is represented by *n*; for all data points $n = 1$. The experiment was performed once and correspond to the first trial.

Fig. S7. Data points of the growth of *Salmonella* Infantis inoculated in chicken fecal matter, under aerobic conditions, days 0 to 12. Typical *Salmonella* colonies were counted in XLD and XLD with NIT (12 mg l^{-1}). This graph considers the results of the first trial (2 repetitions) and the second trial (1 repetition). The number of Petri dishes counted is represented by *n*. For 0 days $n = 17$, for 1 day $n = 16$, for 2 and 9 days $n = 6$, for 3 days $n = 14$, for 7 days $n = 1$ and for 8 days $n = 2$.

Fig. S8. Growth curves of *Salmonella* Infantis and *E. coli* inoculated in sterile fecal matter, under aerobic conditions. Colonies were counted in MKL. Data shown are means ± SD. Asterisks indicate statistically significant difference (*t*-test, $P < 0.05$) between the number of *Salmonella* and *E. coli*. The number of Petri dishes counted is represented by *n*. For *Salmonella* $n = 4$, except on days 1 ($n = 3$) and 2 ($n = 2$). For *E. coli* $n = 4$, except on day 1 ($n = 3$). The experiment was performed once.

Fig. S9. Growth curves of *Salmonella* Infantis and *E. coli* inoculated in sterile fecal matter, under aerobic and anaerobic conditions. Colonies were counted in MKL. Data shown are means ± SD. Asterisk indicates statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic growth. The number of Petri dishes counted is represented by *n*. For *Salmonella* $n = 4$, except on day 1 aerobiosis and anaerobiosis, and day 3 anaerobiosis ($n = 3$), day 2 aerobiosis and anaerobiosis ($n = 2$) and day 6 anaerobiosis ($n = 8$). For *E. coli* $n = 4$, except on day 1 aerobiosis and anaerobiosis ($n = 3$), day 2 anaerobiosis ($n = 2$) and day 6 anaerobiosis ($n = 8$). The experiment was performed once.

Fig. S10. Growth curves of *Lactobacillus reuteri* rifampicin resistant in chicken fecal matter, under aerobic and anaerobic conditions. Colonies were counted in MRS agar + Rifampicin (100 μg ml^{-1}). The brown line corresponds to the growth under aerobiosis and the blue one, under anaerobiosis. Data shown are means ± SD. Asterisks indicate statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic growth. The number of Petri dishes counted is represented by *n*. For the data points $n = 4$, except for day 3 aerobiosis ($n = 3$) and day 6 ($n = 6$). The experiment was performed once.

Data S1: Experimental Procedures.

