Prebiotics mitigate in vitro sulfur-containing odour generation in caecal content of pigs

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Abstract

The objective of this study was to examine the effects and role of prebiotics, such as inulin, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GAS), to mitigate sulfur-containing odour gases, hydrogen sulfide (H₂S) and methyl mercaptan (CH₃SH) using pigs as in vitro study model. Inocula obtained from pigs were incubated at 39°C for 24 h using 550 mg sterilised substrate (caecal contents supplemented with or without 50 mg prebiotics). Production of total gas, H₂S and CH₃SH were determined. The results showed that total gas production for the caecal content of pigs was 57.3 mL, and that for H₂S and CH₃SH was 220.2 and 15.2 μL, respectively. The total gas production increased (P<0.05), whereas concentrations of H₂S and CH₃SH decreased (P<0.05) with supplementation of prebiotics. Among the prebiotics, inulin was the most effective in mitigating H₂S and CH₃SH productions, reducing the two malodorous gases by 14.7 and 19.8%, respectively. The reduction of the above two sulfur-containing gases was supported by lower sulfate-reducing bacteria population and higher sulfate radical concentrations in the prebiotics, particularly that of inulin supplementation group.

Introduction

Odour from livestock, including pig farming, is a nuisance to the neighborhood and is often the primary public compliant relating to livestock farming. Sources of odour generated in pig production facilities can be classified into sulfur-containing compounds, volatile fatty acids (VFAs), phenols and indoles, and ammonia and volatile amines (Le et al., 2005). Sulfur-containing compounds are the predominant ones because the concentration of hydrogen sulfide (H₂S) is only second to that of ammonia. Another predominant sulfur-containing compound is methyl mercaptan (CH₃SH) which is considered a key component which causes bad smell owing to its extremely low odour threshold (O’Neill and Phillips, 1992). In addition to the adverse effects on the air quality, H₂S has also been reported to cause chronic intestinal inflammation (Loubinoux et al., 2002a; Devkota et al., 2012). Therefore, reducing H₂S and CH₃SH, which represent sulfur-containing odour generated from the large intestine of pigs besides mitigating the environmental problems will also have a positive effect on the intestinal health of the pigs.

Gibson et al. (2004) defined that prebiotics as a group of compounds which are indigestible by the host but can easily be fermented by the intestinal flora and selectively stimulate the growth of bacteria that promote the gut health of the host. Inulin, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GAS) are some of the compounds which meet the criteria as prebiotics. Accordingly, we hypothesise that prebiotics may intervene with one or both of the malodorous compounds generating pathways mentioned above, thereby reducing the production of sulfur-containing odour. However, we do not know of any reports in the literature on the use of prebiotics to mitigate sulfur-containing malodorous compounds in large intestine of pigs. Thus, the objective of this study was to examine the potential and role of prebiotics such as inulin, FOS, and GAS in mitigating the emission of sulfur-containing odour in vitro using pigs as study model. We examined the fermentative degradation of sulfur-containing proteins and changes associated with SO₄²⁻ concentrations, thereby revealing the way prebiotics inhibited the production of sulfur-containing odour.

Materials and methods

Donor animals and preparation of inoculums and fermentative substrate

Six Duroc x Landrace x Yorkshire male pigs of 95±1 kg body weight were used as donor animals of the inoculums for this study. The animals were housed in individual pens and fed a corn-soybean basal diet (consisting of 69.735% corn, 20.5% soybean meal, 7.4% wheat bran, 0.9% limestone, 0.5% Ca(HPO₄), 0.4% salt, 0.065% lysine, 0.5%; premix with a calculated digestible energy of 13.39 MJ/kg and crude protein of 15.5%) at equal portions, twice daily at 08:00 and 16:00 h. Throughout the experimental period, the animals were managed according to the protocols approved by the Animal Experimental Committee of Guangdong Institute of Animal Science, China.

After 28 days of feeding, the pigs were slaughtered 2 h after morning feeding. The large intestinal contents of the 6 pigs (as replicates) were separately collected as inoculums for the study and were individually mixed thoroughly with sodium and ammonia bicarbonate buffer solution (35 g NaHCO₃ plus 4 g NH₄HCO₃ per L) in a 1:3 (W/V) ratio according to Ly et al. (1997). The 6 intestinal and buffer mixtures were individually squeezed through 4
layers of surgical gauze into 6 bottles and continuously bubbled with CO₂ at 39°C.

Caecal contents collected from another 6 pigs fed with similar corn-soybean basal diet were used as substrate for the *in vitro* fermentation. Freely obtained caecal contents from the 6 pigs were uniformly mixed, autoclaved at 120°C for 15 min, freeze dried, sieved through a 100-mesh, and stored pending for later use. The prebiotics used in the study were inulin (DP 2-6; Weide Biotechnology Co., Xining, China), FOS (DP 2-6; Weide Biotechnology Co.), GAS (DP 2-6; Quantum Hi-Tech Biological Co., Jiangmen, China).

**In vitro gas production**

*In vitro* gas production was performed as described by Menke and Steinigass (1988) and adapted for use in pigs by Ly et al. (1997). The treatments included a control (Control: 500 mg substrate), and three prebiotic groups (inulin, FOS and GAS: 500 mg substrate plus 50 mg of the respective prebiotic). The prebiotics to substrate used for the study were used following the recommendations of the manufacturer (20 kg of prebiotic per metabolic benefit of feed, or 2%) and the assumption that dry matter (DM) digestibility in the small intestine of pigs is 80% thus, 500 mg caecal DM contents (amount of substrate used for this study) would have come from 2500 mg feed (DM), and the 2% prebiotics supplementation of prebiotics would be approximately 50 mg. A blank group (only inulins with no substrate) was also included. Each treatment was replicated 6 times using the 6 inulins described previously. The above procedure was repeated for a second run. The fermentation inoculum (30 mL) prepared accordingly was added to a 100 mL-glass syringe (Häberle, Schwerte, Germany) containing a pre-weighed substrate and the prebiotics described previously. After removing the air from the head-space, the syringe was sealed with a sealing clip. After taking the initial reading of each syringe, they were placed in a 39°C incubator rotated at 60 rpm for 24 h. According to the change in the piston position, the volume of gas produced was recorded at 2 h intervals for 24 h. The two runs were carried out independently in two incubators.

**Sampling and analysis**

After 24 h, the fermentation was terminated and the final reading of each syringe was recorded. The gas accumulated in the head-space of each syringe was collected with a gas tight syringe (Hamilton, Reno, NV, USA) and immediately transferred into Teflon gas-bag. The fermentation solution was transferred to a 50 mL centrifuge tube kept in an ice-box to stop the fermentation process, and was later centrifuged at 2000g at 4°C for 5 min. The supernatant was separated, and transferred into another 50 mL centrifuge tube, sealed well, then stored at −80°C for VFAs concentration, SO₄²⁻ concentration, sulfate-reducing bacteria (SRB) quantity and methionine gamma-lyase (MGL) mRNA expression analysis. Gas chromatograph (GC-2010; Shimadzu, Kyoto, Japan) equipped with a flame photometric detector and a HP-Plot Q column (30 m×0.32 mm×0.25 µm) (Agilent Technologies, Santa Clara, CA, USA) was used to measure the concentrations of H₂S and CH₃SH. Temperatures of the injector oven and detector were 150 and 250°C, respectively. Temperatures of the column oven was initially kept at 105°C for 1 min, and then heated up to 180°C at a rate of 15°C/min and kept for 1 min. The gas flow rates for nitrogen (N₂), hydrogen (H₂) and air were 2, 40 and 60 mL/min, respectively. A standard gas (Foshan Ms Messer Gas Co., Foshan, China) solution containing known concentrations of H₂S and CH₃SH (balanced with nitrogen) was used to determine the peak time of the two gases. A standard non-linear regression model was established, based on the proportional relationship between the concentrations and the square root of the responding peak heights. A 50-µL gas sample was injected with a 250-µL syringe. The H₂S and CH₃SH concentrations were obtained by substituting the regression equation of the standard curve with the obtained peak heights. The production of the sulfur-containing odour was obtained by multiplying the measured sulfur-containing odour concentrations in the sample with the total gas production.

To determine the post-fermentation VFAs concentrations, 1 mL of the inoculum supernatant was transferred into Eppendorf tubes and 200 µL of 25% metaphosphoric acid was added to acidify the samples (within one week after sampling). After being centrifuged at 10,000×g at 4°C for 10 min, 1 µL of the supernatant was filtered through a 0.22-µm filter and was injected into the gas chromatograph equipped with a flame ionization detector and a DB-FFAP column (30 m×0.32 mm×0.25 µm) (Agilent Technologies) to determine VFAs molar concentrations. Temperatures of the injector oven and detector were 220 and 250°C, respectively. Temperatures of the column oven was initially kept at 60°C for 2 min, and then heated up to 150°C at a rate of 30°C/min and then kept for 2.5 min before being finally heated up to 250°C at a rate of 40°C/min. The gas flow rates for N₂, H₂ and air were 50, 40 and 400 mL/min, respectively. A standard solution containing known concentrations of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate was used to determine the peak time of these VFAs. A standard linear regression model was established, based on the relationship between the concentrations and the peak areas. The concentration of each VFA of samples was obtained by substituting the regression equation of the standard curve with the corresponding peak area of the VFAs.

A turbidimetric method was used to determine the concentration of SO₄²⁻ in the inoculum after 24-h fermentation. Accordingly, 5 mL of the inoculum supernatant (after being centrifuged at 10,000×g at 4°C for 10 min) was poured into a 100 mL beaker. The solution was diluted 10 folds, and 0.5 mL of concentrated hydrochloric acid (µ=1.19g/mL) was added to it. The resulting solution was stirred with a magnetic stirrer, and 2.5 mL of a stabilising solution (a filtered solution containing 40 g barium chloride, 300 mL distilled water, sodium chloride, 50 mL of glycerol, and 100 mL of 95% ethanol) added to it. This solution was stirred for 2 min and then transferred into a 1 cm colorimeter cell. The absorbance was measured at a wavelength of 620 nm. The concentration of SO₄²⁻ was obtained from a standard curve correlating the SO₄²⁻ concentration with the absorbance.

The functional gene (*aprA*) of SRB in the post-fermentation solution was quantified using real-time quantitative (q)-polymerase chain reaction (PCR), to determine the effect of prebiotics on SRB after a 24-h *in vitro* fermentation. The DNA was extracted from a 1.5 mL inoculum supernatant, using EZNA™ Stool DNA Kit (Omega, Norcross, GA, USA), according to the manufacturer’s instructions. The integrity of the DNA samples was examined by electrophoresis on a 1% agarose gel (stained with ethidium bromide). The DNA purity was determined by measuring the absorbance ratio of the sample at 260 and 280 nm, using an ultrafine ultraviolet (UV) spectrophotometer (for the sample, the OD260:OD280 ratio of DNA was approximately 1.8). The extracted DNA was stored at −20°C and was used as a PCR template for the real-time quantification performed on an ABI 7300 instrument (Thermo Fisher Scientific Inc., Waltham, MA, USA). The primers designed (Meyer and Kuever, 2007) to amplify *aprA* of the SRB are as follows: 5’-TGGCATATGATGATCCGGG-3’ (forward); 5’-GGGCGTACCGCTTCGGA-3’ (reverse). Final reaction mixtures contained 10 µL SYBR® Green Realtime PCR Master Mix (Toyobo Co., Osaka, Japan), 0.5 µL of each primer, 1 µL of DNA samples and 8 µL H₂O. The q-PCR cycle was set: initial denaturation step for 5 min at 95°C, followed by 35 cycles of denaturing for 15 s at 95°C, annealing for 30 s...
at 60°C, and elongation for 25 sec at 72°C, followed by a 10-min final extension step at 72°C. A standard curve for q-PCR was obtained by preparing 10-fold dilutions of plasmids (PMD-18T; Takara, Yokohama, Japan) containing 396-bp partial aprA gene fragment and it had a linear scope of detection ranged from 10^{-4} to 10^{9} target molecule numbers, with a slop of -3.396 (R^2=0.994) and amplification efficiency of 96.991%. The copy number of the target gene per milliliter of the sample was also determined.

The mRNA of megl guiding the synthesis of MGL was quantified using q-PCR with 16S rRNA as the reference gene, for determining the effect of prebiotics on the synthesis of MGL by the flora after a 24-h in vitro fermentation. Accordingly, 3 mL of the inoculum supernatant was centrifuged at 13,000×g under 4°C for 2 min; the supernatant was discarded, and the total RNA of the flora in the precipitate was extracted using the TRIzol method. The integrity of the RNA samples was examined through the gel electrophoresis of a 1% agarose gel (stained with ethidium bromide).

The mRNA purity was determined by measuring the absorbance ratio of the sample at 260 and 280 nm, using an ultrafine UV spectrophotometer (for the samples, the OD260:OD280 was considered as the experimental unit. Data were presented as means. Duncan’s multiple comparison was conducted whenever significant differences were observed. A difference with P<0.05 was considered statistically significant.

### Results

Total gas production for the control group was 57.3 mL, and that for H$_2$S and CH$_3$SH was 220.2 and 15.2 μL, respectively. Prebiotics supplementation increased (P<0.05) total gas production by approximately 10%. Among the prebiotics, only inulin and FOS significantly reduced (P<0.05) H$_2$S production, while all the three prebiotics significantly reduced CH$_3$SH production (P<0.05). Among the three prebiotics, inulin exhibited the highest reduction potential for H$_2$S and CH$_3$SH (Table 1).

Acetate and total VFAs productions increased with supplementation of inulin and FOS, while GAS had no effect on production of the above VFA. Although supplementation of the three prebiotics increased butyrate production (P<0.05), the effect of inulin on butyrate, as well as acetate and propionate production was most predominant. Supplementation of the three prebiotics reduced (P<0.05) valerate and isovalerate production by 44 and 26% compared to the control (0.77 vs 1.37 mmol/mL, 0.23 vs 0.31 mmol/mL) (Table 2).

### Table 1. Effects of prebiotics on 24 h in vitro total gas and sulfur-containing odour production.

| Treatments | Significance | SEM |
|------------|--------------|-----|
| Control    | Inulin       | FOS | GAS |
| Total gas, mL | 57.3^{+} | 62.3^{+} | 62.2^{+} | 62.8^{+} | *** | 0.47 |
| H$_2$S, μL  | 220.2^{+} | 187.8^{+} | 210.2^{+} | 214.9^{+} | *** | 2.39 |
| CH$_3$SH, μL | 3.84^{+} | 3.01^{d} | 3.38^{c} | 3.42^{b} | *** | 0.05 |
| CH$_3$SH/total, L/mL | 0.26^{c} | 0.19^{d} | 0.21^{c} | 0.22^{b} | *** | 0.02 |

FOS, fructo-oligo saccharides; GAS, galacto-oligo saccharides; H$_2$S, hydrogen sulfide; CH$_3$SH, methyl mercaptan. Data were means of 6 syringes of 2 runs (12 replicates per treatment). **Means within a row with different letter superscripts differ significantly (P<0.05). ***P<0.01.

### Table 2. Effects of prebiotics on 24 h in vitro concentrations and molar proportion of volatile fatty acids.

| Treatments | Significance | SEM |
|------------|--------------|-----|
| Control    | Inulin       | FOS | GAS |
| Concentration of VFAs, mmol/mL | Acetate | 16.68^{+} | 20.31^{c} | 17.87^{+} | 16.93^{+} | *** | 0.27 |
| Propionate | 10.64^{+} | 12.58^{c} | 11.44^{+} | 10.53^{+} | *** | 0.18 |
| Isovalerate | 0.31^{+} | 0.20^{a} | 0.23^{b} | 0.24^{d} | *** | 0.01 |
| Butyrate   | 3.82^{+} | 5.29^{d} | 4.98^{c} | 4.65^{b} | *** | 0.12 |
| Isovalerate | 0.95^{+} | 0.50^{d} | 0.64^{c} | 0.73^{b} | ** | 0.04 |
| Total VFAs | 33.01^{+} | 39.39^{b} | 35.51^{b} | 33.35^{b} | *** | 0.47 |

Molar proportion of VFAs, mmol/100 mmol

| Treatments | Significance | SEM |
|------------|--------------|-----|
| Control    | Inulin       | FOS | GAS |
| Concentration of VFAs, mmol/mL | Acetate | 50.45 | 51.49 | 50.35 | 50.79 | - | 0.23 |
| Propionate | 32.16 | 31.88 | 32.23 | 31.56 | - | 0.26 |
| Isovalerate | 0.65 | 0.67 | 0.61 | 0.62 | - | 0.02 |
| Butyrate   | 11.52^{+} | 13.42^{b} | 14.01^{b} | 13.96^{b} | * | 0.34 |
| Isovalerate | 0.95^{+} | 0.50^{a} | 0.64^{b} | 0.73^{b} | ** | 0.04 |
| Butyrate   | 4.16^{+} | 1.91^{b} | 2.18^{e} | 2.37^{d} | *** | 0.19 |

FOS, fructo-oligo saccharides; GAS, galacto-oligo saccharides; VFAs, volatile fatty acids. **Means within a row with different letter superscripts differ significantly (P<0.05). Data were means of 6 syringes of 2 runs (12 replicates per treatment). *P<0.05; **P<0.01; ***P<0.001.
Compared to the control, higher (P<0.05) concentrations of SO$_4^{2-}$ were detected in the inulin and FOS treatments. The increase was more pronounced for the inulin group with its SO$_4^{2-}$ concentrations (391.8 mg/L) approximately 34% higher than that of the control (291.2 mg/L). Supplementation of GAS had no effect on SO$_4^{2-}$ production and averaged 302.75 mg/L (Figure 1A).

Supplementation of FOS and particularly inulin reduced (P<0.05) aprA gene (a functional gene of SRB) copy numbers, indicating that the SRB number was reduced by supplementation of these two prebiotics, while GAS had no effect on aprA gene copy numbers (Figure 1B).

Bacteria synthesised MGL for the fermentative degradation and utilisation of sulfur-containing proteins. Supplementation of all the three prebiotics significantly (P<0.05) downregulated MGL mRNA expressions to 0.55-0.57 fold of the control. No significant differences were observed among the supplemented groups (Figure 2).

**Discussion**

The findings of the present study showed that supplementation of the three prebiotics increased (P<0.05) in vitro total gas production. Since total gas produced during in vitro fermentation reflects the activity of the microorganisms (Blümmel and Ørskov, 1993), results of the present study are thus as expected because supplementation of prebiotics (fermentable carbohydrates) provided additional source of energy for the microbes to ferment the substrate.

Among the prebiotics, only inulin and FOS reduced the production of H$_2$S, while supplementation of all the three prebiotics reduced CH$_3$SH production. In both cases, inulin was the most effective (Table 1). The reductions of H$_2$S and CH$_3$SH by prebiotic supplementation expressed as ratio of total gas production remained lower than those of the control group suggesting that they were absolute and not been diluted by the higher gas productions by prebiotic supplementation.

Sulfur-containing odours were mainly generated by the following two pathways (Figure 3): the fermentative degradation of sulfur-containing proteins (Tanaka *et al*., 1977; Nakayama *et al*., 1984) and the reduction of SO$_4^{2-}$ to H$_2$S (Gibson, 1990). Putting all data together, our results seem to suggest that the caecal contents collected from pigs (as substrate for this study) were low in carbo-
drates as evident by the increased total gas production when supplemented with prebiotics. If the above assumption is true, under carbohydrate-deficient condition, intestinal micro-flora will ferment proteins, peptides, amino acids as well as other nitrogenous compounds as energy sources. In comparison with carbohydrates, fermentation of nitrogenous compounds by the gastro-intestinal bacteria produces substances that are more obnoxious (Blaut and Clavel, 2007). For example, the fermentation of cysteine and methionine can produce sulfur-containing malodorous molecules such as H₂S and CH₃SH (Magee et al., 2000).

Gastro-intestinal bacteria are required to synthesise MGL for the fermentative degradation and utilisation of sulfur-containing proteins (Manukhov et al., 2005). The relative expressions of MGL mRNA for the prebiotic-supplemented groups were significantly lower than that for the control group (Figure 2), which suggests that bacterial zymolysis of the sulfur-containing proteins, to obtain energy, occurred relatively slowly for the energy-rich prebiotic-supplemented groups than that in the control group.

Valerate is a peptide metabolite obtained via deamination during fermentation (Le et al., 2005), and it has a strong correlation with odour production (O’Shea et al., 2010). Our results indicated that concentrations of valerate in the prebiotic-supplemented groups were significantly lower than those in the control, which further implies a lower rate of protein fermentation occurred in the prebiotic-supplemented groups. There were no significant differences in the relative expression of the MGL mRNA and the concentration of valerate among the inulin, FOS, and GAS, which indicates that there were no differences in the effects of the three prebiotics – solely as supplements of carbohydrates – on the pathways corresponding to the fermentation of sulfur-containing proteins (Pathway I in Figure 3). However, there were differences in the sulfur-containing odour yield among the prebiotic groups, due to their differential effects on another pathway (Pathway II in Figure 3) that produced sulfur-containing odour.

Sulfate reducing bacteria can use preliminarily degraded organic substances as electron donors to reduce sulfate to produce H₂S (Loubinoux et al., 2002b). Effects of prebiotics (fermentable carbohydrates) on SRB have been extensively studied, but the results were inconsistent. Rey et al. (2013) reported that diet with low fermentable carbohydrates is associated with increased levels of SRB. Lewis et al. (2005) reported that although H₂S concentration in the human faeces after consuming FOS decreased, the number of SRB remained unchanged. Conversely, Christophersen et al. (2013) reported that during in vitro human flora fermentation, the addition of xylo-oligosaccharides increased, whereas the addition of inulin decreased the number of SRB. As previously mentioned, results of this study showed that inulin is most effective in reducing H₂S production (Table 1) followed by FOS, while GAS had no significant effect on H₂S reduction. The above finding is supported by data of the functional gene (aprA) of the SRB (Figure 1B), which showed that supplement of inulin and FOS significantly decreased the number of SRB, while GAS had no effect on SRB. Similar data of the acetate, propionate, isobutyrate and butyrate concentrations (electron donors of SO₄²⁻ reduction) (Table 2), and the SO₄²⁻ concentration (Figure 1A) further support that inulin is more effective in decreasing the production of H₂S generated via the reduction of SO₄²⁻ than the other two prebiotics. The inulin-mediated inhibition of SRB may be achieved by the ability of inulin to selectively stimulate the growth of certain bacteria that compete with SRB for the organic substrates. During the fermentation process, polymeric organic substances (proteins, polysaccharides and fats) are first hydrolysed into mid-sized organic molecules (peptides, oligosaccharides, and long-chain fatty acid), and these are then hydrolysed into low molecular weight organic compounds (amino acids, monosaccharides, and short chain fatty acids).

Utilisation of the mid-sized and low molecular weight organic substances (Muyzer and Stams, 2008). In view of the significant accumulation of low molecular weight organic substances such as acetate, propionate, isobutyrate (Table 2), we hypothesise that the inhibition of SRB caused by inulin might occur during the competitive utilisation of the mid-sized organic molecules.

**Conclusions**

In conclusion, supplementation of inulin, FOS, or GAS reduced the fermentation of sulfur-containing proteins, and thus reduced the concentrations of sulfur-containing odour (proteins fermentation pathway). Among the three prebiotics, inulin was the most effective, followed by FOS and GAS in the reduction of sulfur-containing odour. The respective effectiveness of the prebiotics was further supported by their inhibitory effect on sulfate reduction pathway.

**References**

Blaut, M., Clavel, T., 2007. Metabolic diversity of the intestinal microbiota: implications for health and disease. J. Nutr. 137:751-755.

Blümml, M., Ørskov, E.R., 1993. Comparison of in vitro gas production and nylon bag degradability of roughages in predicting
feed intake in cattle. Anim. Feed Sci. Tech. 40:109-119.

Christoffersen, C.T., Petersen, A., Licht, T.R., Conlon, M.A., 2013. Xylo-oligosaccharides and inulin affect genotoxicity and bacterial populations differently in a human colonic simulator challenged with soy protein. Nutrients 5:3740-3756.

Dennman, S.E., McSweeney, C.S., 2006. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. FEMS Microbiol. Ecol. 58:572-582.

Devkota, S., Wang, Y., Musch, M.W., Leone, V., Fehlner-Peach, H., Nadimipalli, A., Antonopoulos, D.A., Jahri, B., Chang, E.B., 2012. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/−mice. Nature 487:104-108.

Forquin, M.P., Hebert, A., Roux, A., Aubert, J., Proux, C., Heilier, J.F., Landaud, S., Junot, C., Bonnarme, P., Martin-Verstraete, I., 2011. Global regulation of the response to sulfur availability in the cheese-related bacterium Brevibacterium aurantiacum. Appl. Environ. Microb. 77:1449-1459.

Gibson, G.R., 1990. Physiology and ecology of the sulphate reducing bacteria. J. Appl. Bacteriol. 69:769-797.

Gibson, G.R., Probert, H.M., Loo, J.V., Rastall, R.A., Roberfroid, M.B., 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr. Res. Rev. 17:259-275.

Le, P.D., Aarnink, A.J., Ogink, N.W., Becker, P.M., Verstegen, M.W., 2005. Odour from animal production facilities: its relationship to diet. Nutr. Res. Rev. 18:3-30.

Lewis, S., Brazier, J., Beard, D., Nazem, N., Proctor, D., 2005. Effects of metronidazole and oligofructose on faecal concentrations of sulphate-reducing bacteria and their activity in human volunteers. Scand. J. Gastroentero. 40:1296-1303.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:402-408.

Lomans, B.P., Pol, A., Op den Camp, H.J.M., 2002a. Microbial cycling of volatile organic sulfur compounds in anoxic environments. Water Sci. Technol. 45:55-60.

Lomans, B.P., van der Drift, C., Pol, A., Op den Camp, H.J.M., 2002b. Microbial cycling of volatile organic sulfur compounds. Cell. Mol. Life Sci. 59:575-588.

Loubinoux, J., Bronowicki, J.P., Pereira, I.A.C., Mougenel, J.L., Faou, A.E., 2002a. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. FEMS Microbiol. Ecol. 40:107-112.

Loubinoux, J., Valente, F.M.A., Pereira, I.A.C., Costa, A., Grimont, P.D., Le Faou, A.E., 2002b. Reclassification of the only species of the genus Desulfomonas, Desulfomonas pigra, as Desulfovibrio piger comb. nov. Int. J. Syst. Evol. Mic. 52:1306-1309.

Ly, J., Lai, N.V., Rodriguez, L., Preston, T.R., 1997. In vitro gas production and washing losses of tropical crop residues for ruminants and pigs. Available from: http://www.lrrd.org/lrrd9/4/ly941.htm

Magee, E.A., Richardson, C.J., Hughes, R., Cummings, J.H., 2000. Contribution of dietary protein to sulfide production in the large intestine: an in vitro and a controlled feeding study in humans. Am. J. Clin. Nutr. 72:1488-1494.

Manukhov, I.V., Mamaeva, D.V., Rastorguev, S.M., Faleev, N.G., Morozova, E.A., Demidkina, TV., Zavilgelsky, G.B., 2005. A gene encoding L-methionine γ-lyase is present in Enterobacteriaceae family genomes: identification and characterization of Citrobacter freundii L-methionine γ-lyase. J. Bacteriol. 187:3889-3893.

Menke, K.H., Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28:7-55.

Meyer, B., Kuever, J., 2007. Molecular analysis of the diversity of sulfate-reducing and sulfur-oxidizing prokaryotes in the environment, using aprA as functional marker gene. Appl. Environ. Microb. 73:7664-7679.

Muyzer, G., Stams, A.J., 2008. The ecology and biotechnology of sulphate-reducing bacteria. Nat. Rev. Microbiol. 6:441-454.

Nakayama, T., Esaki, N., Lee, W.J., Tanaka, I., Tanaka, H., Soda, K., 1984. Purification and properties of L-methionine γ-lyase from Aeromonas sp. Agr. Biol. Chem. Tokyo 48:2367-2369.

O'Neill, D.H., Phillips, V.R., 1992. A review of the control of odour nuisance from livestock buildings. Part 3. Properties of the odorous substances which have been identified in livestock wastes or in the air around them. J. Agr. Eng. Res. 53:23-30.

O'Shea, C.J., Lynch, M.B., Callan, J.J., O'Doherty, J.V., 2010. Dietary supplementation with chitosan at high and low crude protein concentrations promotes Enterobacteriaceae in the caecum and colon and increases manure odour emissions from finisher boars. Livest. Sci. 134:198-201.

Rey, F.E., Gonzalez, M.D., Cheng, J., Wu, M., Ahern, P.P., Gordon, J.I., 2013. Metabolic niche of a prominent sulfate-reducing human gut bacterium. P. Natl. Acad. Sci. USA 110:13582-13587.

Tanaka, H., Esaki, N., Soda, K., 1977. Properties of L-methionine γ-lyase from Pseudomonas ovalis. Biochemistry 16:100-106.