**SUPPLEMENTARY MATERIAL: Identifying and exploring biohydrogenating rumen bacteria with emphasis on pathways including *trans*-10 intermediates**

Lore Dewanckele,\* Jeyamalar Jeyanathan,\* Bruno Vlaeminck,† Veerle Fievez‡

Laboratory for Animal Nutrition and Animal Product Quality (Lanupro), Department of Animal Sciences and Aquatic Ecology, Ghent University, Ghent, Belgium

\*Shared first authorship

†Present address: Research Group Marine Biology, Department of Biology, Ghent University, Ghent, Belgium.

‡Address correspondence to Veerle Fievez, Veerle.Fievez@UGent.be.

**Table S1** Influence of lactate on OD600 of different bacterial strains after 24 h of incubation with 40 µg/mL 18:2*n*-6 (Exp. 2)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Control | Lactate | SEM | *P*-value |
| *Acidaminococcus fermentans* VR4 | 0.15 | 0.06 | 0.015 | 0.003 |
| *Acidaminococcus intestini* ADV 255.99 | 0.24 | 0.08 | 0.050 | < 0.001 |
| *Bifidobacterium adolescentis* RU 424 | 0.47 | 0.28 | 0.131 | 0.340 |
| *Bifidobacterium pseudolongum* RU224 | 0.44 | 0.38 | 0.143 | 0.761 |
| *Butyrivibrio fibrisolvens* D1 | 1.17 | 1.17 | 0.094 | 0.986 |
| *Butyrivibrio proteoclasticus* P18 | 1.11 | 1.07 | 0.047 | 0.577 |
| *Lactobacillus ruminis* RF1 | 1.04 | 0.86 | 0.019 | 0.001 |
| *Lactobacillus ruminis* RF2 | 1.00 | 0.82 | 0.019 | < 0.001 |
| *Cutibacterium acnes* DSM 1897 | 1.17 | 0.67 | 0.285 | 0.281 |
| *Ruminococcus albus* 7 | 0.06 | < 0.01 | 0.002 | < 0.001 |
| *Streptococcus equinus* Pearl 11 | 1.42 | 1.18 | 0.052 | 0.028 |
| *Streptococcus gallolyticus* DSM 16831 | 1.13 | 0.82 | 0.051 | 0.013 |
| *Megasphaera elsdenii* B159 | 1.19 | 1.40 | 0.082 | 0.157 |
| *Megasphaera elsdenii* T81 | 1.03 | 1.22 | 0.133 | 0.380 |
| *Megasphaera elsdenii* LC1 | 0.86 | 1.37 | 0.128 | 0.048 |
| *Megasphaera elsdenii* 2602A | 1.52 | 1.58 | 0.021 | 0.082 |
| *Megasphaera elsdenii* 3016B | 1.35 | 1.65 | 0.072 | 0.039 |
| *Megasphaera elsdenii* 3218A | 1.23 | 1.57 | 0.044 | 0.005 |
| *Megasphaera elsdenii* 3436A | 1.24 | 1.63 | 0.030 | < 0.001 |
| *Megasphaera elsdenii* 4251 | 1.25 | 1.56 | 0.088 | 0.065 |
| *Megasphaera elsdenii* 4257 | 1.31 | 1.65 | 0.051 | 0.009 |
| *Megasphaera elsdenii* 4296 | 1.29 | 1.70 | 0.050 | 0.005 |
| *Megasphaera elsdenii* 4400 | 0.13 | 0.12 | 0.085 | 0.953 |
| *Megasphaera elsdenii* 5045 | 1.34 | 1.58 | 0.033 | 0.006 |
| *Megasphaera elsdenii* 5052B | 1.39 | 1.20 | 0.032 | 0.013 |
| *Megasphaera elsdenii* 5596 | 1.29 | 1.55 | 0.037 | 0.008 |
| *Selenomonas ruminantium* GA-192 | 0.97 | 1.21 | 0.074 | 0.084 |
| *Selenomonas ruminantium* PC 18 | 0.92 | 1.82 | 0.062 | < 0.001 |

**Table S2** Influence of lactate on volatile fatty acids produced by different bacterial strains after 24 h of incubation with 40 µg/mL 18:2*n*-6 (exp. 2)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Control | Lactate | SEM | *P*-value |
| Total VFA formed (µmol/tube)a | Main VFA productsb | Total VFA formed (µmol/tube)a | Main VFA productsb |
| *Acidaminococcus fermentans* VR4 | 54.8 | A, B | 54.0 | A, B | 5.289 | 0.920 |
| *Acidaminococcus intestini* ADV 255.99 | 57.3 | A, B | 71.7 | A, B | 7.161 | 0.093 |
| *Bifidobacterium adolescentis* RU 424 | 249.7 | A | 235.7 | A | 9.774 | 0.155 |
| *Bifidobacterium pseudolongum* RU224 | 212.9 | A | 199.7 | A | 23.02 | 0.437 |
| *Butyrivibrio fibrisolvens* D1 | 128.4 | B, A | 146.6 | B, A | 6.398 | 0.115 |
| *Butyrivibrio proteoclasticus* P18 | 168.6 | B, A | 158.1 | B, A | 6.695 | 0.315 |
| *Lactobacillus ruminis* RF1 | 22.7 | A, P | 19.2 | A, B | 12.97 | 0.478 |
| *Lactobacillus ruminis* RF2 | 12.7 | A, P | 27.2 | A | 4.485 | 0.072 |
| *Cutibacterium acnes* DSM 1897 | 118.5 | P, A | 107.0 | P, A | 38.88 | 0.844 |
| *Ruminococcus albus* 7 | 29.8 | A | 18.2 | A | 3.121 | 0.047 |
| *Streptococcus equinus* Pearl 11 | 19.5 | A | 15.0 | A | 2.240 | 0.228 |
| *Streptococcus gallolyticus* DSM 16831 | 12.6 | A | 22.7 | A | 5.726 | 0.281 |
| *Megasphaera elsdenii* B159 | 149.2 | B | 913.1 | P, A, B | 16.37 | < 0.001 |
| *Megasphaera elsdenii* T81 | 125.7 | B | 822.9 | P, A, B | 40.47 | < 0.001 |
| *Megasphaera elsdenii* LC1 | 122.9 | B, A | 905.3 | A, P, B | 35.35 | < 0.001 |
| *Megasphaera elsdenii* 2602A | 191.2 | B, P | 1103.6 | P, A, B | 61.68 | < 0.001 |
| *Megasphaera elsdenii* 3016B | 136.6 | B | 885.7 | P, A, B | 27.70 | < 0.001 |
| *Megasphaera elsdenii* 3218A | 136.0 | B | 856.2 | P, A, B | 37.37 | < 0.001 |
| *Megasphaera elsdenii* 3436A | 117.1 | B | 861.7 | P. A, B | 23.09 | < 0.001 |
| *Megasphaera elsdenii* 4251 | 125.7 | B | 857.0 | P, A, B | 35.99 | < 0.001 |
| *Megasphaera elsdenii* 4257 | 125.7 | B | 851.6 | P, A, B | 32.36 | < 0.001 |
| *Megasphaera elsdenii* 4296 | 121.6 | B | 870.6 | P, A, B | 40.50 | < 0.001 |
| *Megasphaera elsdenii* 4400 | 59.0 | A, P | 80.3 | A, P | 37.34 | 0.707 |
| *Megasphaera elsdenii* 5045 | 126.7 | B | 803.1 | P, A, B | 51.76 | < 0.001 |
| *Megasphaera elsdenii* 5052B | 64.3 | A | 66.2 | A | 6.744 | 0.846 |
| *Megasphaera elsdenii* 5596 | 125.5 | B | 858.0 | P, A, B | 34.20 | < 0.001 |
| *Selenomonas ruminantium* GA-192 | 81.4 | P, A | 194.2 | P, A | 17.49 | 0.010 |
| *Selenomonas ruminantium* PC 18 | 241.9 | P, A | 1181.7 | P, A | 108.8 | 0.004 |

a Measured fermentation products were acetate, propionate, isobutyrate, butyrate, isovalerate, valerate and caproate.

b Main VFA product. A, acetate; B, butyrate; P, propionate; in decreasing order of importance.

**Table S3** Net production (µmol per tube) of propionic acid and butyric acid by different biomass ratios of *Butyrivibrio fibrisolvens* D1 to *Cutibacterium acnes* DSM 1897 in the inoculum after 24 h of incubation under different growth conditionsa

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | *B. fibrisolvens* (%)/*C. acnes* (%) | SEM | *P*-value |
|  |  | 100/0 | 50/50 | 10/90 | 2/98 | 0.4/99.6 | 0/100 | Linear | Quadratic |
| Propionic acid | Control | 0.77 | 21.74 | 28.75 | 32.46 | 31.78 | 37.88 | 12.310 | < 0.001 | 0.832 |
| Low pH | 1.06\* | 10.15\* | 18.87\* | 23.16\* | 21.53\* | 21.27\* | < 0.001 | 0.698 |
| DHA | 1.24\* | 15.48\* | 25.49\* | 28.06\* | 26.97\* | 28.65\* | < 0.001 | 0.377 |
| Butyric acid | Control | 106.70 | 74.48 | 58.31 | 36.37 | 52.67 | 0.79 | 9.630 | 0.022 | 0.077 |
| Low pH | 8.69\* | 4.24\* | 1.82\* | 1.23\* | 0.69\* | 0.50 | 0.359 | 0.967 |
| DHA | 20.81\* | 26.10\* | 15.11\* | 4.91\* | 3.20\* | 0.69 | < 0.001 | 0.203 |

a Low pH, control medium with pH adjusted to 5.5; DHA (docosahexaenoic acid), control medium containing 40 µg/mL of 22:6*n*-3; All growth media contained 40 µg/mL of 18:2*n*-6.

\* Means differ (*P* < 0.05) from the control growth mediumwithin the same ratio.