**Immunogenic inhibition of prominent ruminal bacteria as a means to reduce lipolysis and
biohydrogenation activity *in vitro*[†]**

**Holly D. Edwards^{1,2*}, Weilin L. Shelver³, Seongho Choi⁴, David J. Nisbet¹, Nathan A.
Krueger¹, Robin C. Anderson¹, and Stephen B. Smith²**

¹United States Department of Agriculture/Agricultural Research Service, Southern Plains
Agricultural Research Center, Food & Feed Safety Research Unit, College Station, TX 77845,

²Department of Animal Science, Texas A&M University, College Station, TX 77843, ³United

States Department of Agriculture/Agricultural Research Service, Biosciences Research

Laboratory, Fargo, ND, 58102, ⁴Department of Animal Science,

Chungbuk National University, Cheongju, 28644, Republic of Korea

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*Corresponding Author: Dr. Holly D. Edwards, tel +001-979-436-2300,

hdedwards23@gmail.com

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Abstract

Lipolysis and biohydrogenation in ruminal animals promote the accumulation of saturated fatty acids in their meat and milk. Antibodies were generated against key ruminal lipase contributors *Anaerovibrio lipolyticus*, *Butyrivibrio fibrisolvens*, *Propionibacterium avidum* and *acnes*. An anti-*Pseudomonas* lipase antibody was generated to determine if an antibody against a purified protein would be more effective. Each bacterium was cultured and assayed without or with increasing levels of each antibody. *Butyrivibrio fibrisolvens* H17C also participates in biohydrogenation and therefore the antibody was tested to determine if it could effectively reduce biohydrogenation. *Butyrivibrio fibrisolvens* was assayed without and with the anti-*B. fibrisolvens* antibody and linoleic or α -linolenic acid. All antibodies were effective at reducing lipolysis with the anti-*Pseudomonas* lipase averaging a 78% reduction. The anti- *B. fibrisolvens* showed a tendency for a reduction ($P = 0.0713$) in biohydrogenation products of α -linolenic acid. Results demonstrate that lipolysis and biohydrogenation can be immunologically inhibited *in vitro*.

1. Introduction

Increasing the content of unsaturated fatty acids in the human diet is thought to invoke health benefits. The 2015-2020 USDA dietary guidelines suggest that a healthy diet should contain less than 10 percent of calories from saturated fatty acids, and *trans*-fatty acid consumption should be as low as possible ("Dietary guidelines for Americans 2015-2020, Eighth Edition,"). The quality of dietary lipids for human consumption is related to the development of insulin resistance and metabolic syndromes, such as blood lipid disorders, hypertension, propensity for thrombus formation, abdominal obesity, and Type II diabetes mellitus (DeFronzo & Ferrannini, 1991). Summers, Fielding, Bradshaw, Ilic, Beysen, Clark, et al. (2002) demonstrated that insulin sensitivity increased and plasma LDL cholesterol concentrations and abdominal subcutaneous fat mass decreased in human subjects fed a diet rich in polyunsaturated fatty acids as compared to a diet high in saturated fatty acids.

Resurreccion (2003) suggested that U.S. consumers find healthiness to be the most important quality trait of beef. Food products derived from cattle, relative to plants, contain a high concentration of saturated and *tran*-fats, a consequence of microbial lipolysis and biohydrogenation. Ruminal biohydrogenation is one of the few natural sources of *trans*-fatty acids. In order for biohydrogenation in the rumen to occur, free fatty acids must first be hydrolyzed from their triacylglycerol precursors (Harfoot & Hazlewood, 1997) via extracellular lipolysis. Several microorganisms have been recognized as contributors to ruminal lipolysis and biohydrogenation activity. The present study focuses on the extracellular, lipase-producing bacteria *Anaerovibrio lipolyticus* 5s, *Butyrivibrio fibrisolvens* H17C, *Propionibacterium avidum* and *P. acnes*.

In addition to contributing to lipolysis (Geoffrey Hazlewood & Dawson, 1979), *Butyrivibrio fibrisolvens* participates in the biohydrogenation pathway (Polan, McNeill, & Tove, 1964; Wallace, Chaudhary, McKain, McEwan, Richardson, Vercoe, et al., 2006) by facilitating the initial isomerization reaction for both linoleic and α -linolenic acid (Polan, McNeill, & Tove, 1964), producing CLA (conjugated linoleic acid) isomers (Bessa, Santos-Silva, Ribeiro, & Portugal, 2000; Destailats, Trottier, Galvez, & Angers, 2005). The present study examines antibodies created against *Anaerovibrio lipolyticus* 5s, *Butyrivibrio fibrisolvens* H17C, *Propionibacterium avidum* and *P. acnes* lipases and their ability to inhibit lipolysis; and in the case of *B. fibrisolvens* H17C, biohydrogenation. As lipolysis is an extracellular process we hypothesized that the antibodies would be most effective at inhibiting lipolysis rather than biohydrogenation, which is an intracellular process. An antibody generated against a purified *Pseudomonas* lipase was also included in this study to determine if extracellular lipolytic activity could be inhibited to a greater degree by using an antibody with more specificity for the lipase itself compared to an antibody against whole cell preparations. We hypothesized that by immunizing ruminants against bacteria and/or their purified lipases or the initial stages of biohydrogenation, saturated and *trans*-fatty acids would be significantly reduced.

2. Materials and Methods

2.1. Microbial preparation and handling procedures

All chemicals were purchased from Sigma-Aldrich unless otherwise noted. Pure cultures of *A. lipolyticus* 5s and *B. fibrisolvens* H17C were obtained from Dr. Jay Yankee, Agriculture-Agri Food Canada. Strains of *P. avidum* and *P. acnes* were previously isolated from the rumen of

46 a pastured cow (Krueger, Anderson, Callaway, Edrington, & Nisbet, 2008). For long-term
 47 preservation of pure cultures, bacteria were stored in 20% anaerobic glycerol at -80°C. Upon
 48 removal from storage, each bacterial species was revived via two consecutive 24 to 48 h cultures
 49 in 10 ml standard anaerobic broth supplemented with 2% pre-sterilized (via autoclave) olive oil.
 50 Standard anaerobic broth medium contained (per liter): 292 mg K_2HPO_4 , 292 mg $KHPO_4$, 480
 51 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4 \cdot 7H_2O$, 64 mg $CaCl_2 \cdot 2H_2O$, 4,000 mg Na_2CO_3 , 600
 52 mg cysteine-hydrochloride, 10 g trypticase and 2.5 g yeast extract (BBL Microbiology Systems,
 53 Cockeysville, MD), branched-chain fatty acids (1 mmol each of isobutyrate, isovalerate, and 2-
 54 methylbutyrate), 2 µg hemin, vitamin mix (20 mg each thiamine, pantothenate, nicotinamide,
 55 pyridoxine HCL, riboflavin, 1 mg *p*-aminobenzoic acid, 0.5 mg biotin, 0.5 mg folic acid, 0.2 mg
 56 vitamin B-12, and 0.5 mg lipoic acid), 0.1 g D-glucose and trace minerals (Cotta & Russell,
 57 1982). Medium was boiled to displace dissolved oxygen and then cooled on ice while being
 58 saturated with a continuous flow of O₂-free CO₂ and then distributed to 18 x 150 mm crimp top
 59 tubes (10 ml) or to 100 ml crimp top glass walled vials (80 ml) containing 4 ml of olive oil using
 60 the anaerobic Hungate technique and capped (Bryant, 1972). Tubes and vials were immediately
 61 autoclaved and cooled to room temperature before use. Pre-sterilized olive oil was supplemented
 62 as a nutrient source to the 18 x 150 tubes using a syringe and needle. Upon inoculation (0.2 ml
 63 pure culture cells in 20% glycerol), tubes and vials were incubated (39°C) with agitation (35
 64 rpm) in an Innova™ Model 4000 Shaking Incubator (New Brunswick Scientific Co., Inc.,
 65 Edison, NJ). Cells for lipolytic assay were grown in the vials containing 80 ml standard medium
 66 until late log phase, determined via measurement of absorbance at 620 wavelength in a
 67 Spectronic 20 Spectrophotometer (Thermo Spectronic, Rochester, NY) to be 0.4 to 0.5 for *B.*
 68 *fibrisolvens H17C, 1.2 to 1.3 for *P. avidum*, and 1.0 to 1.2 for *A. lipolyticus* and *P. acnes*.*

2.2. Antibody preparation

Anaerovibrio lipolyticus 5s, *B. fibrisolvens* H17C, *P. avidum*, and *P. acnes* cells were harvested from 20 ml cultures and washed two consecutive times with 0.1 M sodium phosphate buffered saline (PBS) at pH 7.0. Following the final wash, bacterial cells and the purified *Pseudomonas* lipase, which was purchased from Sigma Aldrich (St. Louis, MO), were suspended with 1 ml of PBS and 1 ml of Adjuvant Titer Gold Max purchased from Sigma Aldrich (Milwaukee, WI). Three chickens were injected for each bacteria/lipase at 6 different locations at 3-wk intervals for 3 separate injections. After the first injection, normal saline was used in place of the adjuvant. One week following the final injection, eggs were harvested and antibodies were extracted from yolks using an Eggcellent IGY purification kit purchased from Pierce (Rockford, IL). Lowry protein assays were performed on the extracted material to determine antibody concentration. Chickens were cared for according to procedures approved by the Southern Plains Agricultural Research Center Animal Care and Use Committee (College Station, TX; protocol #2010005).

2.3. Determination of antibody effectiveness at reducing lipolytic activity against pure cultures of ruminal bacteria

Lipase assays were conducted in a 4 x 5 x 5 factorial arrangement to test anti- *A. lipolyticus* 5s, *-B. fibrisolvens* H17C, *-P. avidum*, *-P. acnes*, and *-Pseudomonas* lipase antibodies against the lipolytic activity of *A. lipolyticus* 5s, *B. fibrisolvens* H17C, *P. avidum*, and *P. acnes* at five antibody doses (0, 0.2, 1.0, 2.5, and 5.0 mg). Bacterial cells (4 ml) obtained from cultures of each bacterium grown to late log phase in 80 ml of standard anaerobic medium supplemented

with 4 ml olive oil were transferred anaerobically under a continuous stream of O₂-free CO₂ into 18 x 150 mm straight wall assay tubes pre-loaded with 21 g glass beads (which served as a solid support matrix), 0.3 ml olive oil, and 2 ml anaerobic buffer. The anaerobic buffer was prepared in the same manner as the media but only contained minerals and cysteine-hydrochloride. Each antibody dose was administered to separate assay tubes containing cells of each bacterium in triplicate. Assay tubes were closed with rubber stoppers and incubated in an incubator shaker at 39°C and 35 rpm, and reactions were stopped at 12 h by uncapping and freezing the tubes. Total free fatty acids were extracted and concentrations determined colorimetrically according to methods described by Kwon and Rhee (1986).

2.4. Determination of anti-*B. fibrisolvens* antibody against biohydrogenation activity of *B.*

fibrisolvens

Assays were conducted to specifically test the anti-*B. fibrisolvens* HI7C antibody against the biohydrogenation activity of *B. fibrisolvens* HI7C. Cells (4 ml) of *B. fibrisolvens* HI7C grown to late log phase in 80 ml of standard anaerobic medium supplemented with 4 ml olive oil were transferred anaerobically into assay tubes pre-loaded with 21 g glass beads and 2 ml anaerobic buffer as described above. Tubes in triplicate also contained either 6 mg of linoleic or 6 mg of α -linolenic acid, obtained from Nu Check Prep, Elysian MN, and 0 or 5.0 mg of anti-*B. fibrisolvens* HI7C antibody. Cultures were incubated for 1 or 3 h at 39°C and 35 rpm. Reactions were stopped at 1 and 3 h by uncapping and freezing the tubes. To determine the extent of biohydrogenation, total lipids were extracted from tubes using the method by Folch, Lees, & Stanley (1957). After methylation (Morrison & Smith, 1964), the fatty acid methyl esters (FAME) were analyzed using a Varian gas chromatograph as described by Sturdivant, Lunt,

Smith, & Smith (1992). Separation of FAME was accomplished on a fused silica capillary column CPSil88 [100.0 m × 0.25 mm (i.d.)]. Helium was used as the carrier gas. After 32 min at 180°C, the oven temperature was increased at a rate of 20°C/min to 225°C and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270 and 300°C, respectively. Biohydrogenation was calculated according to the appearance of CLA isomers and expressed as pmol/ml based on the molecular weight of CLA.

2.5. Statistical analysis

The effects of each antibody on lipolytic activity were analyzed within each antibody against each bacterial species by a one-way analysis of variance (ANOVA) using Statistix v.9.0, Analytical Software (Tallahassee, FL) with a least significant difference (LSD) separation of means ($P < 0.05$). The model included the main effects of increasing antibody dose; $Y_i = \mu + \text{antibody dose}_i + \varepsilon_i$, where Y_i is the dependent variable, μ is the overall mean, antibody dose is the fixed effect of the experimental treatments ($i = 1$ to 4), and ε_i is the error term. A polynomial contrast appropriate for non-equally spaced treatments was performed to determine if increasing doses of antibody resulted in a linear or quadratic trend in percentage reduction of lipolytic activity.

Differences in biohydrogenation were tested for each free fatty acid using two-way ANOVA with a LSD separation of means ($P < 0.05$). The model included the main effects of increasing incubation points, with or without antibody, and their interaction: $Y_{ij} = \mu + \text{incubation}_i + \text{antibody}_j + (\text{incubation} \times \text{antibody})_{ij} + \varepsilon_{ij}$, where Y_{ij} is the dependent variable, μ is the overall mean, incubation period and antibody treatments are the fixed effects of the experimental treatments ($i = 1$ to 2; $j = 1$ to 2), incubation x antibody is the interaction, and ε_{ij} is the error term.

3. Results

3.1. Antibody inhibition of lipolytic activity in pure cultures of ruminal bacteria

Increasing amounts of each antibody were tested to determine the optimal antibody concentration for inhibiting lipolysis in each bacterial species (Table 1). The anti-*Pseudomonas* lipase antibody caused the greatest percentage inhibition of lipolytic activity, eliciting from 56% (*P. acnes*) to 96% (*B. fibrisolvens* H17C) inhibition of lipolysis. The anti-*Pseudomonas* lipase antibody caused a linear increase ($P < 0.05$) in the percentage reduction of lipolysis for *A. lipolyticus* ($P = 0.02$), *P. avidum* ($P = 0.05$), and *P. acnes* ($P < 0.001$). There was no dose effect of anti-*Pseudomonas* lipase antibody against lipolytic activity for *B. fibrisolvens* H17C ($P \geq 0.12$). The anti-*P. avidum* antibody also displayed a linear increase ($P \leq 0.03$) in lipolytic inhibition against all bacteria except *A. lipolyticus* 5s.

The anti-*A. lipolyticus* 5s and anti-*P. acnes* antibodies exhibited less cross reactivity than the other antibodies tested in inhibiting lipolysis across bacterial species (Table 1). Both antibodies exhibited a linear dose effect ($P = 0.01$) against *P. avidum*. The anti-*B. fibrisolvens* H17C antibody displayed linear effects for the percentage reduction of lipolytic activity for *P. acnes* ($P = 0.03$) and *A. lipolyticus* 5s ($P = 0.05$). The anti-*B. fibrisolvens* antibody elicited the only quadratic effect ($P = 0.02$), with the lowest and the higher doses causing the greatest reductions in lipolysis for *A. lipolyticus* 5s. The anti-*P. acnes* antibody was ineffective when administered against *A. lipolyticus* 5s cultures regardless of dose.

3.2. Effectiveness of anti-*B. fibrisolvens* HI7C antibody against biohydrogenation activity of pure cultures of *B. fibrisolvens* HI7C

The effectiveness of the anti-*B. fibrisolvens* HI7C antibody against itself in pure cultures in reducing CLA isomers of linoleic and α -linolenic acid is shown in Table 2. Biohydrogenation products were quantified by summing CLA isomers produced from linoleic or α -linolenic acid by gas chromatography. *Butyrivibrio fibrisolvens* HI7C cultures treated with the anti-*B. fibrisolvens* HI7C antibody showed a tendency for a significant reduction ($P = 0.0713$) in the production of CLA isomers from α -linolenic acid compared to the untreated control cultures after 1 h of incubation. There was neither a significant effect ($P = 0.8711$) of incubation time on biohydrogenation of α -linolenic acid nor a significant ($P = 0.8758$) incubation time x antibody interaction. By comparison, *B. fibrisolvens* HI7C cultures treated with the anti-*B. fibrisolvens* HI7C antibody did not show a significant reduction ($P = 0.1516$) in production of CLA isomers from linoleic acid when compared to the controls incubated likewise but without antibody treatment. However, for linoleic acid there was a significant ($P < 0.001$) increase in the accumulation of biohydrogenation products during the longer 3 h incubation time. There was no significant ($P = 0.1637$) antibody x time interaction for linoleic acid.

Figure 1 shows a visual comparison of the chromatographs between anti-*B. fibrisolvens* HI7C antibody treated and control cultures of *B. fibrisolvens* HI7C incubated with 6 mg α -linolenic acid, following 1 h incubation. The antibody-treated cultures contained less CLA isomers than the untreated cultures.

4. Discussion

Lipase-expressing bacteria found in the rumen play a major role in altering the composition of lipids that enter the rumen. Different technologies can be used to protect the essential dietary unsaturated fatty acids of the n-6 and n-3 family. There are currently several technologies that have been developed to protect lipids from biohydrogenation, such as the use of calcium salts or encapsulation of fat in a matrix of aldehyde-treated protein (de Veth, Gulati, Luchini, & Bauman, 2005; Harvatine & Allen, 2006; Jenkins & Bridges, 2007; Perfield II, Lock, Pfeiffer, & Bauman, 2004). These techniques have shown varying degrees of success, but they have presented some limitations. The technology reported in the current study focuses on a two-fold approach to protect unsaturated fatty acids in the rumen by first preventing lipase-producing bacteria from creating a free carboxyl group via lipolysis, a requirement for biohydrogenation, and secondly disrupting biohydrogenation at its initial stages.

Extracellular lipases expressed by bacteria catalyze the hydrolysis (lipolysis) of mono-, di- and triacylglycerols to free fatty acids and glycerol (Aravindan, Anbumathi, & Viruthagiri, 2007; Pandey, Benjamin, Soccol, Nigam, Krieger, & Soccol, 1999). There are several subspecies of microorganisms found in the rumen that are capable of hydrolyzing triacylglycerols. The current study focused on two organisms that have previously shown the greatest lipolytic activity *in vitro*, *P. acnes* and *P. avidum*. *Propionibacterium* have been known to be present in the rumen between 10^4 and 10^6 CFU/ml of rumen fluid (Zimmer, 1999). *Butyrivibrio fibrisolvens* H17C and *A. lipolyticus* 5S were also investigated in this study due to previous findings implicating them as major contributors to lipolysis (Henderson, 1971; Henderson & Hodgkiss, 1973a, 1973b; Polan, McNeill, & Tove, 1964; Prins, Lankhorst, van der Meer, & Van Nevel, 1975). The lipases produced from these bacteria are predominantly produced during log phase of growth (GP

Hazlewood, Clark, & RMC, 1980; Henderson, 1971; Henderson & Hodgkiss, 1973a; Pablo, Hammons, Bradley, & Fulton, 1974). Thus, for the present study, all bacteria were cultured to late log phase before beginning the incubation assays so lipase production was assumed to be at its peak.

The introduction of antibodies created against specific ruminal bacteria or their lipases displayed significant reductions in lipolytic activity for each bacterium in pure culture. This was to be expected because the lipase for these organisms is extracellular, allowing for optimal interaction between the lipase and the antibody. However, we also documented that antibodies were functional at reducing lipolytic activity against cultures for which they were not specifically generated against. These bacteria, while all lipase producers, are somewhat different in functionality. *Butyrivibrio* species have been found to contribute mainly but not exclusively to the hydrolysis of galactolipids and phospholipids (Lourenço, Ramos-Morales, & Wallace, 2010), while *Anaerovibrio lipolyticus* has the ability to hydrolyze triacylglycerols and other esterified fatty acids (Henderson, 1971). *Anaerovibrio lipolyticus* is limited in its capacity to ferment carbohydrates, using only glycerol, fructose, and ribose which are fermented to acetic, propionic, and succinic acid (Henderson, 1975). In spite of these differences in functionality, our results indicate that the lipases expressed by these bacteria may be genetically conserved.

The inclusion of the anti-*Pseudomonas* lipase in this study was to determine if using a purified lipase antibody would exhibit greater specificity in inhibiting lipolysis in comparison to an antibody generated from a whole cell preparation. Results showed that the anti-*Pseudomonas* antibody was more sensitive but exhibited considerable cross-reactivity, and was the most effective at reducing lipolytic activity for all of the bacteria, averaging an almost 78% reduction in activity at the highest dose.

Unlike lipolysis, the process of biohydrogenation takes place intracellularly (Chalupa & Kutches, 1968) and thus we hypothesized that the antibodies would not be as effective at reducing biohydrogenation as they would be against lipolytic activity. For this study, free fatty acid substrates were used so biohydrogenation rates were not confounded by the rates of lipolysis. Results showed that the anti-*B. fibrisolvens* H17C antibody was marginally effective at reducing the CLA isomers of α -linolenic acid in pure cultures of *B. fibrisolvens* H17C but not at reducing the hydrogenation of linoleic acid. Overall, results indicated that the formation of biohydrogenation products was higher for α -linolenic acid than for linoleic acid in the absence of antibodies. These results are consistent with Edwards, Anderson, Taylor, Miller, Hardin, Nisbet, et al. (2013) who found that linoleic-rich corn oil was somewhat resistant to lipolysis in the presence of *B. fibrisolvens* H17C. Paillard, McKain, Chaudhary, Walker, Pizette, Koppova, et al. (2006) also reported that some ruminal species of *B. fibrisolvens* were inhibited by low levels of linoleic acid, including species *B. fibriosolvens* H17C. Similarly, Choi & Song (2005) supplemented oils rich in either linoleic or α -linolenic acid into mixed ruminal cultures *in vitro* and found that production of CLA isomers had a tendency to be higher in cultures that were supplemented with α -linolenic acid over linoleic acid, indicating higher rates of isomerization and biohydrogenation in the presence of α -linolenic acid. Analysis of FAME produced during culture indicated the efficacy of the *B. fibrisolvens* H17C antibody in reducing biohydrogenation.

Maia, Chaudhary, Bestwick, Richardson, McKain, Larson, et al. (2010) investigated the mechanisms by which polyunsaturated fatty acids affect the growth of *B. fibrisolvens*, and reported that linoleic and α -linolenic acid lengthened the lag phase of *B. fibrisolvens*, with α -linolenic acid having the greatest effect. However, growth occurred only when polyunsaturated fatty acids had been converted to *trans*-vaccenic acid. Maia, Chaudhary, Bestwick, Richardson,

McKain, Larson et al. (2010) concluded that lipolysis and biohydrogenation are required to enable *B. fibrisolvens* to survive the bacteriostatic effects of polyunsaturated fatty acids. The observation of a shorter log phase for *B. fibrisolvens* H17C in the presence of linoleic acid may explain the decrease in biohydrogenation products as compared to α -linolenic acid. A shorter lag phase may not allow the antibody sufficient opportunity to disrupt biohydrogenation by *B. fibrisolvens* H17C.

5. Conclusion

Results from the present study show that antibodies generated against prominent ruminal bacteria that contribute to lipolysis and biohydrogenation are effective at reducing the saturation of fatty acids. Additionally, each antibody displayed cross reactivity against other pure cultures, suggesting genetic similarity among the lipases secreted by these bacteria. Therefore, each individual antibody may be effective against a variety of lipase-producing bacteria, especially an antibody preparation against a purified lipase. Further research is warranted to determine if it is possible to immunize ruminants against lipolytic bacteria to increase the escape of unsaturated fatty acids from the rumen for absorption and assimilation.

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Table 1. Effect of antibody dose on pure cultures of ruminal lipase producing bacteria,

		Percent reduction in lipolytic activity					<i>P</i> -values		
Bacteria	Antibody	Antibody dose				SEM	Dose	Linear	Quadratic
		0.2 mg	1.0 mg	2.5 mg	5.0 mg				
<i>A. lipolyticus</i> 5s	<i>A. lipolyticus</i> 5s [†]	0.07	0.00	0.00	18.02	9.01	0.44	0.21	0.34
	<i>P. acnes</i> [†]	0.00	0.00	0.00	0.00	---	---	---	---
	<i>P. avidum</i> [‡]	40.18	30.02	63.72	63.07	22.60	0.29	0.25	0.77
	<i>B. fibrisolvens</i> H17C [‡]	60.52	8.39	50.70	53.31	11.77	0.05	0.84	0.02
	<i>Pseudomonas</i> lipase [‡]	34.33	33.76	76.36	76.46	14.05	0.09	0.02	0.98
<i>B. fibrisolvens</i> H17C	<i>A. lipolyticus</i> 5s [†]	33.93	39.89	31.94	41.87	16.97	0.97	0.83	0.90
	<i>P. acnes</i> [†]	36.32	56.32	64.60	47.65	13.26	0.51	0.49	0.82
	<i>P. avidum</i> [‡]	30.23 ^b	50.68 ^b	52.86 ^{ab}	92.12 ^a	12.58	0.04	0.01	0.47
	<i>B. fibrisolvens</i> H17C [‡]	47.50	47.94	86.87	74.50	13.81	0.18	0.08	0.65
	<i>Pseudomonas</i> lipase [‡]	66.64	54.14	77.52	96.03	14.63	0.29	0.12	0.32
<i>P. avidum</i>	<i>A. lipolyticus</i> 5S [†]	13.85 ^b	41.88 ^a	54.03 ^a	63.18 ^a	8.28	0.01	0.001	0.28
	<i>P. acnes</i> [†]	39.57 ^c	57.51 ^{bc}	52.47 ^{ab}	69.66 ^a	4.50	0.01	0.003	0.93
	<i>P. avidum</i> [‡]	6.71	14.12	31.95	59.00	14.64	0.13	0.02	0.52
	<i>B. fibrisolvens</i> H17C [‡]	21.54	21.33	20.59	48.69	14.98	0.50	0.26	0.37
	<i>Pseudomonas</i> lipase [‡]	33.81	36.43	37.72	81.35	14.10	0.12	0.05	0.18
<i>P. acnes</i>	<i>A. lipolyticus</i> 5s [†]	7.47	15.36	14.59	26.49	9.88	0.61	0.23	0.84
	<i>P. acnes</i> [†]	14.61	2.64	0.00	38.67	8.91	0.05	0.11	0.02
	<i>P. avidum</i> [‡]	0.56 ^b	9.59 ^b	23.72 ^{ab}	50.63 ^a	9.18	0.02	0.004	0.35
	<i>B. fibrisolvens</i> H17C [‡]	20.22	16.63	26.10	48.01	9.70	0.09	0.03	0.17
	<i>Pseudomonas</i> lipase [‡]	4.76 ^b	7.10 ^b	19.92 ^b	55.90 ^a	7.88	0.01	0.001	0.06

^{abc} Means within rows with unlike superscripts differ. Data are least square means as a percent of control lipolytic activity.

[†]Control values for *A. lipolyticus* 5s, *B. fibrisolvens* H17C, *P. avidum*, and *P. acnes* were 962, 3,361, 15,771, and 2,902 µmol/mL, respectively.

[‡]Control values for *A. lipolyticus* 5s, *B. fibrisolvens* H17C, *P. avidum*, and *P. acnes* were 4,008, 2,616, 9,105, and 4,542 µmol/mL, respectively.

Table 2. Effect of anti-*B. fibrisolvens* H17C antibody treatment on accumulation of biohydrogenation products, quantified by summing the conjugated linoleic acid (CLA) isomers, following 1 and 3 h incubation of *B. fibrisolvens* H17C with added linoleic or α -linolenic acid. CLA isomers produced from linoleic or α -linolenic acid.

Antibody dose/tube	pmol CLA Isomer Formation ml ^{-1†}				SEM	Main treatment effects		
	Incubation Time (h)					Antibody	Incubation	Interaction
	1		3					
Linoleic acid	0.0 mg	5.0 mg	0.0 mg	5.0 mg	41.164	0.152	< 0.001	0.164
α -Linolenic acid	1161.3	1080.8	639.9	638.4	126.68	0.071	0.871	0.876

†Data are least square means of the sum of conjugated linoleic acid (CLA) isomers.

Figure(s) 1

A

B

Figure 1. Gas chromatographs depicting the fatty acid composition of pure cultures of *B. fibrisolvens* H17C incubated with 6 mg α -linolenic acid, without (A) and with (B) anti-*B. fibrisolvens* H17C antibody, following 1 h of incubation. Cultures that did not contain the anti-*B. fibrisolvens* H17C antibody demonstrated extensive biohydrogenation compared to tubes that contained the anti-*B. fibrisolvens* H17C antibody, as indicated by the increase of peaks for CLA isomers (RT approximately 45.0-55.0 min).