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Minimal Carbon Requirements for Potential Colonizers of Other Planets

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Minimal Carbon Requirements for Potential Colonizers of Other Planets

An Honors Thesis submitted in partial fulfillment of the

requirements for Honors Studies in Biological Sciences

By

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Introduction

The NASA Office of Planetary Protection promotes the responsible scientific exploration of other planets. Regulations have been put in place to discourage interplanetary mission practices that would lead to the contamination of Earth-originating microbial life on other planets. Interplanetary contamination jeopardizes the potential to obtain reliable scientific evidence for extraterrestrial life. In order to combat this issue, the characteristics of theoretical planetary colonizers (particularly Mars in the case of this project) are studied. Earth-originating organisms arriving on Mars via spacecraft could give false positive biosignatures for extraterrestrials. Due to the conditions of the Martian environment, there are many potential candidates that could successfully colonize Mars.

The Martian environmental circumstances may seem like they are extreme, but there are extremophiles potentially well suited to the environment that Mars would provide them. In order to understand why this is, context regarding the formation process for planets themselves is needed. Planets are formed through supernova events. Dust and gases accumulate together while orbiting a new star and gradually get larger until they form large celestial bodies. During this process, many minerals can be trapped within the celestial body, and this may include sulfur and carbon among other elements. It is not just the Martian environment that is conducive to extremophile growth. Many moons and other exoplanets, even those outside of the habitual zone, can provide the right combination of environmental factors that would be conducive to psychrophilic or thermophilic growth, the Saturn moon Titan being a prime example of such a celestial body. There is also a level of uncertainty pertaining to the environmental content of these yet-to-be explored celestial bodies, just as there is with Mars. The exact carbon sources available on Mars are not yet known, so it remains to be seen which species of bacteria can conceivably grow there. However, sulfate, a prominent substance for terrestrial life, has been observed near the surface of Mars (Farquhar et. al, 2007), and that would certainly support the notion that microbial extremophile life could be viable. In particular, this evidence points to the potential for sulfate reducing microbial life viability on mars. Among the aforementioned potential colonizers of Mars are *Desulfovibrio arcticus* and *Desulfotalea psychrophila.*

Desulfovibrio arcticus is a psychrotolerant sulfate reducing bacterium initially discovered in permafrost near the Barents Sea. *D. arcticus*, as a sulfate reducer, reduces sulfate (SO_4^{2+}) to sulfide (S^{2-}) as sulfate is the final electron acceptor in the electron transport chain. Metabolism is indicated by the presence of a black precipitate depicted in figure 1, which is ferric sulfide, the aforementioned product of sulfate reduction. It can grow in temperatures between -2°C and 28°C, with the optimum temperature found to be 24°C (Pecheritsyna et al, 2012). It is able to use acetate, formate, ethanol, lactate, pyruvate and choline among other carbon sources as electron donors, opting to utilize sulfate, sulfite, thiosulfate, elemental sulfur, DMSO and Fe^{3+} as electron acceptors (Pecheritsyna et al, 2012).

Figure 1. *D. arcticus* stock with ferric sulfide present

Desulfotalea psychrophila is a psychrophilic sulfate reducing proteobacterium able to grow *in situ* below 0° C (Rabus et al, 2004). It was initially discovered in sediments from the frigid Arctic. Although the optimum growth temperature has been found to be between 10°C and 14°C, it has been found to be able to grow in temperatures as low as -1.8°C (Knoblauch, et al, 1999) Like *D. arcticus*, *D. psychrophila* completely reduces sulfate (SO_4^{2+}) to sulfide (S^{2-}) as sulfate is the final electron acceptor in the electron transport chain. Metabolism is indicated by the presence of a black flake precipitate depicted in figure 1, which is ferric sulfide, the aforementioned product of sulfate reduction. *D. psychrophila* has also been shown to be able to reduce other sulfates and sulfites to sulfide, such as thiosulfate and thiosulfite (Knoblauch et al, 2009). This organism is able to take up a variety of carbon sources through fermentation and respiratory metabolism pathways, including amino acids, alcohols, and carboxylic acids. (Knoblauch et al, 2009).

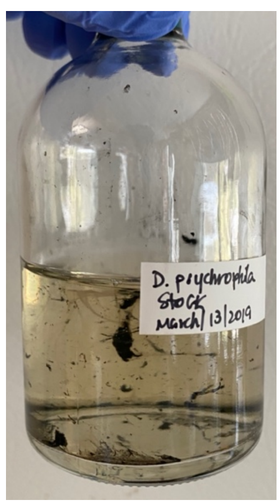


Figure 2. *D. psychrophila* stock with ferric sulfide present

Since it is not currently known what carbon sources are present on Mars, the bacteria were inoculated into several concentrations of different carbon sources. These carbon sources served as hypothetical scenarios of available resources on Mars. The bacterial species were evaluated on their ability to grow in the simulated conditions through quantitative polymerase chain reaction (qPCR) gene expression of the dsrAB gene. A full statistical analysis was conducted, testing to see if there is any growth that is statistically significant.

The dsrAB gene codes for dissimulating sulfite reductase. This is the final enzyme in the electron transport chains of several sulfate reducing microbes, including *D. arcticus* and *D. psychrophila*. The specific function of this enzyme is to catalyze the reduction of sulfite to sulfide during anaerobic respiration or act in the reverse during sulfur oxidation (Müller et al, 2014). The overall pathway for sulfate reduction is presented in figure 3, with specific emphasis on dsrAB activity reducing sulfite to sulfide (Santos et al, 2015).

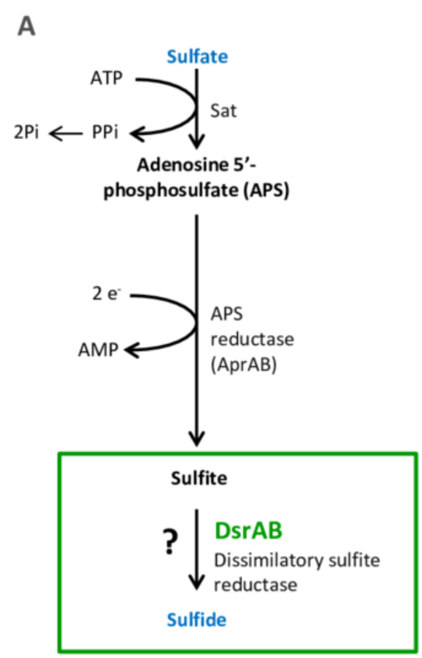


Figure 3. General Sulfate Reduction Pathway. Edited from Santos et al, 2015.

The carbon sources that were tested in this project were sodium acetate, glycerol, isobutyric acid, and dextrose. That is, a two-carbon structure, a three-carbon structure, a four-carbon structure, and a six-carbon structure. This was designed with the expressed purpose of testing how each organism reacts to the increasing complexity of the carbon sources. To test these carbon sources, modified versions of DSMZ 141 and DSMZ 1040 (DSMZ, 2017) were prepared without carbon sources. These carbon sources were then inoculated with the organisms to test them individually. As the unmodified DSMZ 141 and DSMZ 1040 media contain carbon sources that are much more complex than the ones tested in this experiment, the expectation was that all tested carbon source will show positive results in terms of metabolism. Through the qPCR technique, the goal is to measure the metabolism of the organism by measuring the expression of dsrAB. The qPCR analysis involves an understanding of how C_q values are read. C_q values and concentration have an inverse relationship. Increasing C_q values indicate decreasing concentration. As dsrAB is the final enzyme in the sulfate reduction pathway of both organisms, it should be expressed at a greater rate when inoculated with carbon sources it is able to metabolize, meaning that the C_q values should also be lower.

Materials and Methods

Preparing the DSMZ 141 Modified Medium

The medium that the *D. arcticus* cultures were inoculated in was a modified version of DSMZ 141 medium (DSMZ, 2017). It follows the procedure that was listed in the DSMZ guideline, but it was modified to exclude all carbon sources as an initial condition of the experiment. Sodium acetate, yeast extract, trypticase peptone, Wolin's vitamin solution, and cysteine were not included in the preparation. In addition, nitrilotriacetic acid was not included in the preparation for the modified Wolin's mineral solution. Sodium dithionate was used to replace cysteine in order to serve as an electron sink meant to mitigate the effects of oxygen in the event that any was present in the medium after sparging. The medium was composed of 0.34 g of KCl, 4 g of $MgCl_2$ x 6 H_2O, 3.45 g of $MgSO_4$ x $7H_2O$, 0.25 g of NH_4Cl, 0.14 g of $CaCl_2$ x 2 H_2O, 0.14 g of K_2HPO_4, and 18 g of NaCl. 10 mL of the Wolin's trace mineral solution was added. 2 mL of $Fe(NH_4)_2(SO_4)_2$ x 6 H_2O was added (0.1% weight by volume ratio). 0.5 mL of sodium resazurin solution was added (0.1% weight by volume ratio) as an indicator for the presence of oxygen. 0.5 g of Na_2S x 9 H_2O was added. Finally, 1000 mL of distilled H_2O was added per the preparation instructions.

In order to purge the oxygen from the solution and make it anoxic, it was sparged with an 80% H_2 and 20% CO_2 gas mixture for 45 minutes using a Pasteur pipette under low heat. At this time, 5 g of $NaHCO_3$ was added in order to normalize the pH of the solution to 7. It was then sealed and autoclaved for an hour at 121°C for the purpose of sterilization.

Preparing the DSMZ 1040 Modified Medium

The medium that the *D. psychrophila* cultures were inoculated in was a modified version of the DSMZ 1040 medium (DSMZ, 2017). It follows the procedure that was listed in the DSMZ guideline, but it was modified to exclude all carbon sources in order to perform the experiment. Yeast extract, Wolin's vitamin solution, and Na-DL-lactate were not included in the preparation. Sodium dithionate was used to replace cysteine in order to serve as an electron sink meant to mitigate the effects of oxygen in the event that any was present in the medium after sparging. The medium was composed of 35 g of SIGMA Sea Salts, 1 mL of Wolfe's mineral elixir, 0.5 mL of sodium resazurin solution (0.1% weight by volume ratio) as an indicator for the presence of oxygen, 1 g of Na_2CO_3, and 0.3 g of $Na_2S \times 9 H_2O$ was added. Finally, 1000 mL of distilled H_2O was added per the preparation instructions.

In order to purge the oxygen from the solution and make it anoxic, it was sparged with an 80% H_2 and 20% CO_2 gas mixture for 45 minutes using a Pasteur pipette under low heat. At this time, 5 g of $NaHCO_3$ was added in order to normalize the pH of the solution to 7. It was then sealed and autoclaved for an hour at 121°C for the purpose of sterilization.

Preparation of Vials and Serving of Media

Autoclaved 25 mL vials were prepared with four carbon sources: dextrose, glycerol, sodium acetate, and isobutyric acid. For each carbon source, five vials were filled with increasing percentages of each carbon source. Ten more vials were filled with the exact same increasing percentages, totaling fifteen vials and three sets of replicates. Finally, two vials were set aside for a positive and negative control. In total, seventeen vials were used per carbon source per organism tested. Table 1 details the vial arrangement for the benefit of the reader.

Table 1. Vial layout per Carbon Source

Replicate	Percentages
-	Positive Control (2.0%)
1	0.5%
1	1.0%
1	2.0%
1	3.0%
1	4.0%
2	0.5%
2	1.0%
2	2.0%
2	3.0%
2	4.0%
3	0.5%
3	1.0%
3	2.0%
3	3.0%
3	4.0%
-	Negative Control (2.0%)

Upon distributing the correct amount of each carbon source into the beakers, both the beakers and the media were moved into an anaerobic hood. A total of 136 25 mL vials, 136 pre-sterilized rubber stoppers, and 136 metal clamp rings were moved into the hood. Using an electronic pipette tool 15 mL of the appropriate medium was served into each vial. Samples 2-69 were served with DSMZ 1040 in order to grow D. psychrophila, and Samples 70-137 were served with DSMZ 141 to grow D. arcticus. After serving the media, each vial received 17 mg of sodium dithionate. At this point, the vials were sealed with a rubber stopper and metal ring. In order to counter the effects of the carbon sources on the pH, every sample was then manually adjusted using a standardized amount of HCl and NaOH. Every sodium acetate sample was adjusted with 0.3 mL of a 10% HCl solution and 0.06 of 10% NaOH solution. Every dextrose sample as adjusted with 0.3 mL of 10% HCl and 0.05 of 10% NaOH. Every glycerol sample was adjusted with 0.4 mL of 10% HCl and 0.1 of 10% NaOH. Every isobutyric acid sample was adjusted with 0.24 mL of 10% NaOH only. Upon completion of this step, the vials were ready for inoculation. Samples 2-69 were inoculated with *D. psychrophila*, while samples 69-137 were inoculated with *D. arcticus.* For both, the process was the same. Using either a stock *D. psychrophila* or stock *D. arcticus* culture, 5 mL was inserted into each beaker using a sterile needle and syringe.

After successful inoculation of both species, the beakers were removed from the anaerobic hood and stored in their most favorable temperature condition for growth. The 68 *D. psychrophila* samples were stored in a 4°C refrigerator for 5 weeks while the 68 D. *arcticus* samples were stored in a water bath held at 40°C for 5 weeks. Periodically, both were checked for sulfate reduction, as indicated by the presence of a black precipitate in ferric sulfide, and oxygen contamination, as indicated by a pink color courtesy of a reaction between oxygen and sodium resazurin.

RNA Extraction

After five weeks in incubation, the cultures were marked for RNA extraction. The cultures were moved into the anaerobic hood where 1.5 mL of each sample was extracted and put into labelled 2 mL collection tubes. The extraction procedure that was used was the TRIzol reagent protocol (Thermo Fisher Scientific, Waltham, MA). Modifications were made to increase the likelihood of RNA precipitation. At any time where the protocol called for centrifugation, the samples were centrifuged at 17,000 rcf for 30 minutes instead of 12,000 rcf for 15 minutes. Before the RNA was isolated, 500 µL of cold isopropanol was added to the samples before they were left in a -20°C freezer overnight (10 hours). After RNA was isolated, the pellet was resuspended in 50 µL of RNase-free water before being incubated in a heat block for 15 minutes at 55°C.

RNA Purification

Upon the completion of the extraction of RNA from the samples, the next step was to undergo purification of the RNA. Some of the resuspended samples of RNA were cleaned using the protocol and materials from the Qiagen (Hilden, Germany) RNeasy Mini Kit, while others were cleaned using the protocol and materials from the Zymo (Irvine, CA) Clean and Concentrator kit as the materials from the Qiagen kit had been exhausted. One modification was made whereby the samples were incubated in a heat block at 55°C for 10 minutes before final elution using RNase-free water. The concentrations of the samples were then measured using the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

RNA Concentration

The Nanodrop indicated that the RNA concentrations were lower than expected. The purified RNA was then concentrated using a Speed Vacuum Concentrator (Thermo Fisher Scientific, Waltham, MA). All samples were loaded into the speed vacuum centrifuge, lids open, and the heat and vacuum were activated while the centrifuge spun. After nearly drying out the samples, the samples were resuspended in 20 µL of RNase-free water and their concentrations were individually rechecked using the Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA).

DNA Extraction

After five weeks in incubation, the samples were marked for DNA extraction. The DNA extracted from the cells was used to create the curves upon which C_q values could be compared and standardized. This procedure was repeated for each organism tested. Five samples which demonstrated growth were selected and 1.8 mL were withdrawn from their vials and moved to 2 mL collection tubes. The extraction protocol and materials that were used were from the Mo Bio (Carlsbad, CA) DNA extraction kit. Several modifications were made to increase the yield of DNA. After the samples underwent a bead-beating vortex procedure, 4 µL of proteinase K was added to each Microbead Tube. The tubes were then incubated at 55°C for 30 minutes in a heat block. Upon the completion of the incubation, the samples were then allowed to cool at room temperature for 10 minutes. Then, 4 µL of RNase A was added to each tube. The tubes were then incubated at 37°C in an oven. The procedure is then followed again until after solution MD5 is added. At this point, the samples were incubated for 10 minutes at 55°C in the heat block to increase the likelihood of DNA elution through the spin filter membrane.

Concentration of DNA

The concentrations of the extracted DNA were lower than expected, so the samples were then concentrated using a Speed Vacuum Concentrator (Thermo Fisher Scientific, Waltham, MA). As the samples are from the same microbe and therefore the genome is the same, all samples were combined into one tube. It was loaded into the speed vacuum centrifuge, lid open, and the heat and vacuum were activated while the centrifuge spun. After running for 30 minutes, the concentration was rechecked using the Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA).

cDNA Synthesis

In order to analyze the samples via qPCR, the samples must first be converted from mRNA transcripts to cDNA. The cDNA solution was prepared using the protocol and materials from the Applied Biosystems (Foster City, CA) High Capacity Reverse Transcription Kit. The thermocycler program used to actually synthesize the cDNA from the preparation called for 25°C for 10 minutes before transitioning to 37°C for 120 minutes before transitioning to 85°C for 5 minutes before finally being held at 4°C. The samples were then immediately stored in a freezer at -20°C to keep cDNA stable until needed for qPCR.

Quantitative Polymerase Chain Reaction

As the goal for the experimental design is to test the expression of the dsrAB gene, qPCR was chosen as the method by which this would be accomplished. First, a five-level dilution of the genomic DNA was prepared for both organisms tested, meaning six descending concentrations were created. Primers were sourced from Integrated DNA Technologies, Inc. A master mix of 10 µL 2x EvaGreen, 1 µL of forward primer, 1 µL of reverse primer, and 6 µL of dH_2O per well tested was prepared. It was then dispensed on a 96-well PCR plate. Each sample was allocated 3 wells to create a triplicate redundancy. 18 µL of the master mix was added to every well that would be tested with genomic DNA or cDNA. 18 wells were prepped with 2 µL of each genomic DNA dilution, while all other wells were prepped with 2µL of the samples of cDNA. The well plate was sealed with adhesive and checked for bubbles in the wells. It was then centrifuged in a well centrifuge.

Table 2. Primers Used for qPCR

Primers	**Sequence (5'→ 3')**	**Organism Tested**
DNA Oligo Forward Primer	TGC TCC CAACAG AAT GCT TAC	*D. arcticus*
DNA Oligo Reverse Primer	CGA TCT GTG CCC TTC CAA AT	*D. arcticus*
DNA Oligo Forward Primer	ATC GGT AGC AGG AGT ATG ACA	*D. psychrophila*
DNA Oligo Reverse Primer	AAG CCG TGG CAA CAA GT	*D. psychrophila*

Amplification occurred using a BioRad Cfx96 Real-Time PCR (Hercules, CA). The protocol used was standard and did not differ between the two organisms apart from one step. The annealing temperature for *D. arcticus* was adjusted to 56°C while the annealing temperature for *D. psychrophila* was adjusted to 59°C.

The qPCR program started at 90°C for 30 seconds. It then repeated the next steps 11 times. The temperature stayed at 90°C for 15 seconds, then 59°C or 56°C for 15 seconds depending on the organism, then 72°C for 60 seconds.

The temperature rose to 90°C for 15 seconds, then dropped to 59°C or 56°C for 60 seconds depending on the organism, then rose to 72°C for 30 seconds before reading the plate and repeating these steps 35 more times. The temperature then went to 72°C for 3 minutes, increased to 90°C for 15 seconds, dropped to 65°C for 3 minutes, then performed a melting curve from 75°C to 90°C at increments of 0.1°C every 5 seconds while also reading the plate at each interval.

Results

Table 3 (See Appendix) describes the results of the qPCR procedure on the *D. arcticus* samples. The goal to be marked as a positive result was to have at least three out of six duplicates of each sample from a single cDNA prep yield a C_q value. If the sample triplicate yielded three C_q values after the first trial, that sample was not repeated.

Table 4 (See Appendix) describes the averaging of the C_q value triplicates for each sample of *D. arcticus*. The calculation done in this table is simply taking the average of the three C_q values obtained for a triplicate of a sample. If less than three C_q values were obtained for a particular sample, the sample is marked as "n/a" instead of with an average C_q value.

Table 5. Experimental Standard Curve DNA C_q Values for *D. arcticus* (Part 1)

Sample	plate 1 standard (triplicate)	plate 2 standard (triplicate)	plate 3 standard (triplicate)
1	17.41054351	18.60763746	14.28896852
2	21.10462745	23.28037873	18.01700632
3	23.05025511	25.55755324	21.54471724
4		28.59310913	25.49821428
5			21.92813244
6			27.8984511
1	17.57647464	18.43711258	14.62340029
2	21.08648394	23.25926026	18.63793816
3	23.22201478	25.88291049	21.74916133
4	28.99022032	27.11261545	25.05060208
5			23.27730504
6			22.78205335
1	17.73439304	18.71621311	14.90453783
2	20.83661309	24.15882342	19.19322594
3	24.63536007	28.18543454	21.60324069
4			24.72201688

5		26.67914932	23.4853159
6			28.40449644

Table 6. Experimental Standard Curve DNA C_q Values for *D. arcticus (part 2)*

Dilution	plate 4 standard (triplicate)	plate 5 standard (triplicate)	plate 6 standard (triplicate)
1	18.34507308	15.12140663	16.0818499
2	19.97925364	26.39270425	26.24016733
3	27.49205994	23.50519259	26.15323115
4	28.91408491	30.15845753	
5	23.31122596	29.4976296	
6			
1	18.68954562	15.22220616	16.44395724
2	20.98520015	25.68384896	26.67083674
3	22.54885025	23.64046788	26.15480464
4			
5	22.69843741	26.88293853	
6			28.0490363
1	19.41395767	15.40526594	15.87691853
2	21.03864178	26.46120961	25.6515485
3	26.44331768	24.19367274	28.02220882
4			29.54971157
5	22.95623607	28.93697433	
6		28.97027908	

Table 5 and Table 6 describe the experimental DNA C_q values for the DNA isolated from *D. arcticus* and the subsequent dilutions. C_q values are increasing, indicating that concentration is decreasing. Blanks in several positions indicate that there was simply not enough DNA amplification to be measured.

Table 7. Average Experimental Standard Curve DNA Concentrations (ng/µL) vs Measured Average DNA C_q Values for *D. arcticus* (Part 1)

	plate 1 standard curve			plate 2 standard curve	
	Concentration	Avg C_q value		Concentration	Avg C_q value
1	7.14	17.5738037		7.14	18.58698772
2	1.428	21.0092415		1.428	23.56615414
3	0.2856	23.6358767		0.2856	26.54196609
4	0.05712	28.9902203		0.05712	27.85286229
5					
6					

Table 8. Average Experimental Standard Curve DNA Concentrations (ng/µL) vs Measured C_q Values for *D. arcticus* (Part 2)

	plate 3 standard curve			plate 4 standard curve	
	concentration	avg C_q value		concentration	avg C_q value
1	3.66	14.6056355		3.66	18.8161921
2	0.732	18.6160568		0.732	20.6676985
3	0.1464	21.6323731		0.1464	25.4947426
4	0.02928	25.0902777		0.02928	28.9140849
5	0.005856	22.8969178		0.005856	22.9886331
6	0.0011712	26.361667		0.0011712	

Table 9. Average Experimental Standard Curve DNA Concentrations (ng/µL) vs Measured DNA C_q Values for *D. arcticus* (Part 3)

	plate 5 standard curve			plate 6 standard curve	
	concentration	avg C_q value		concentration	avg C_q value
1	3.5	15.2496262		3.5	20.1916145
2	0.7	26.1792543		0.7	22.9293075

3	0.14	23.7797777			0.14	25.9865281
4	0.028	30.1584575			0.028	28.0222088
5	0.0056	28.4391808			0.0056	29.5497116
6	0.00112	28.9702791			0.00112	28.0490363

Tables 7, 8, and 9 describe the C_q value triplicates averaged and set against the measured concentrations of each dilution level in ng/µL. In the concentration column are the measured concentrations of each dilution level. In the avg C_q value column are averages of each dilution level triplicate shown in full in tables 5 and 6.

Figure 4. Plate 1 DNA concentration vs Average C_q Value

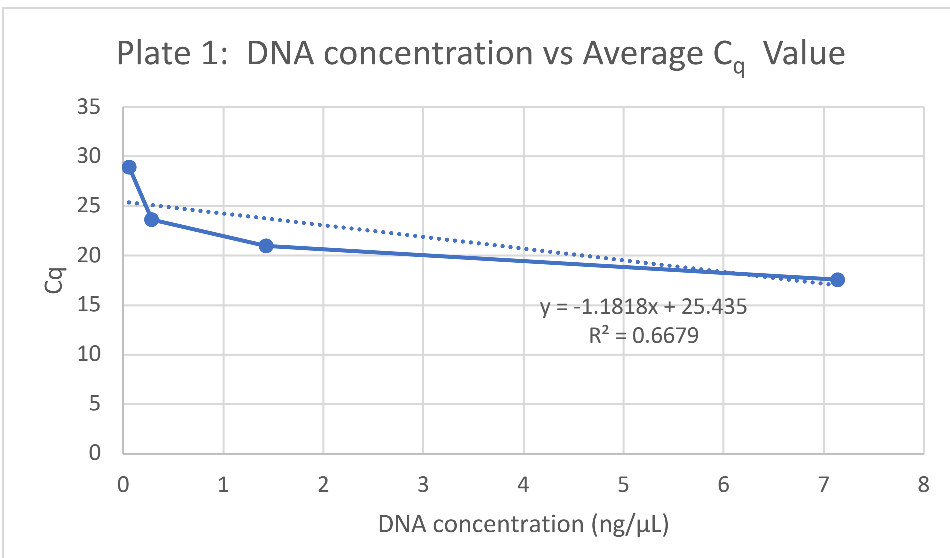


Figure 5. Plate 2 DNA concentration vs Average C_q Value

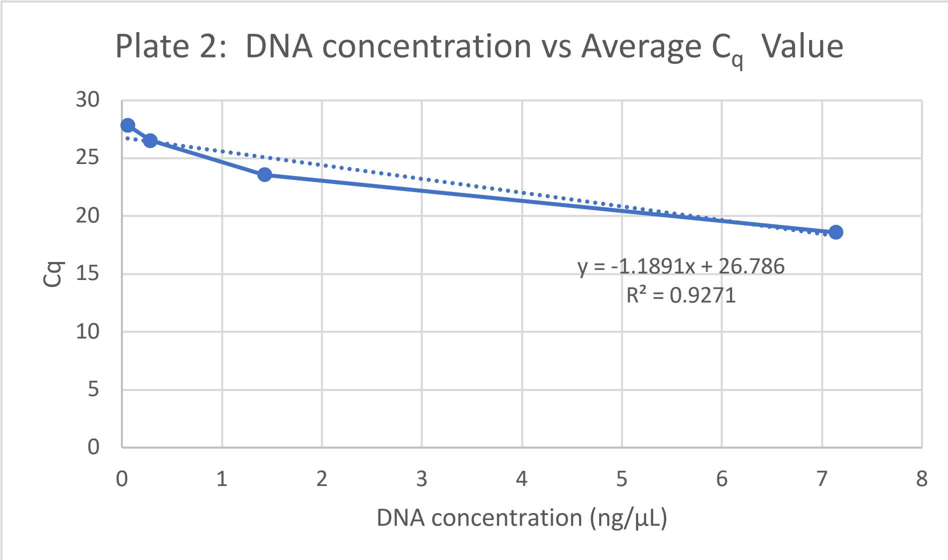


Figure 6. Plate 3 DNA concentration vs Average C_q Value

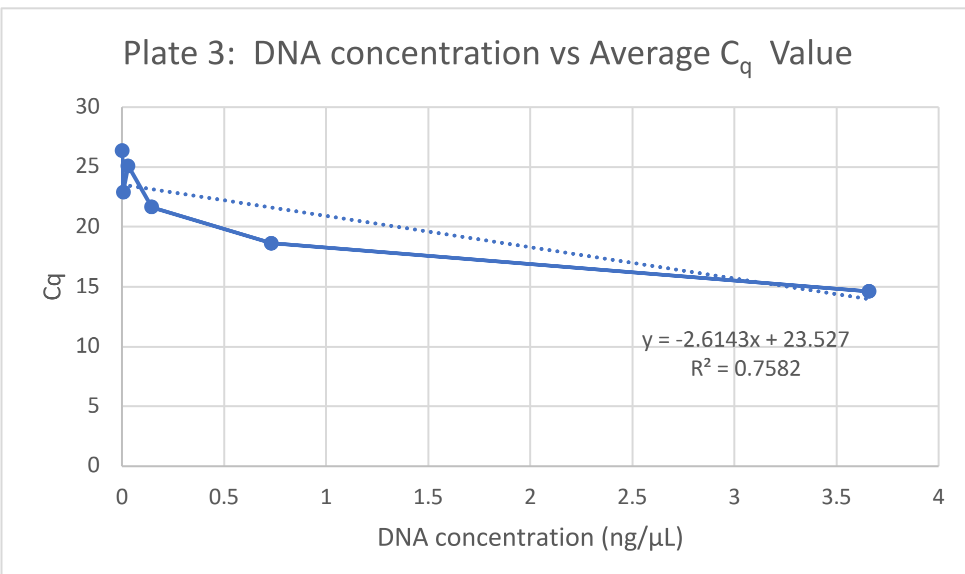


Figure 7. Plate 4 DNA concentration vs Average C_q Value

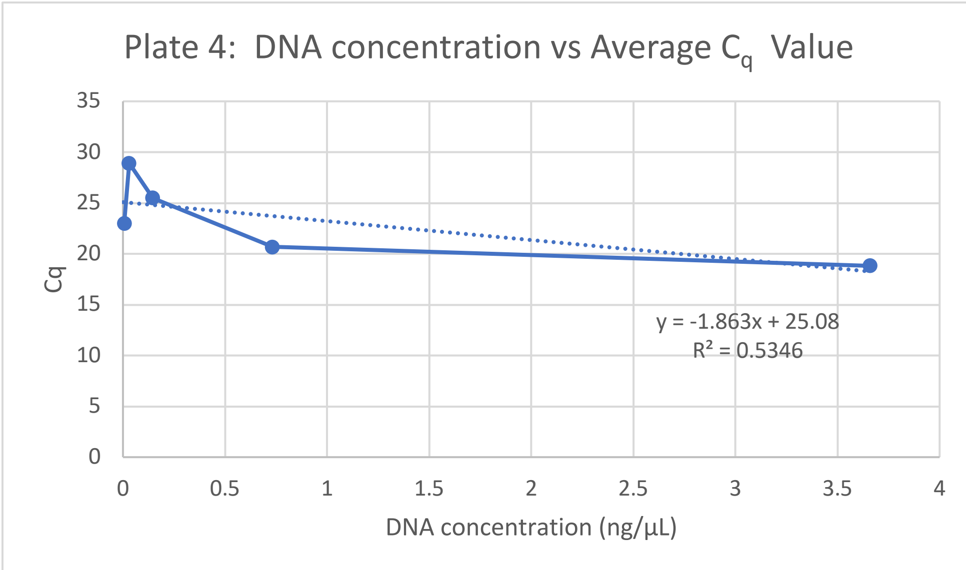


Figure 8. Plate 5 DNA concentration vs Average C_q Value

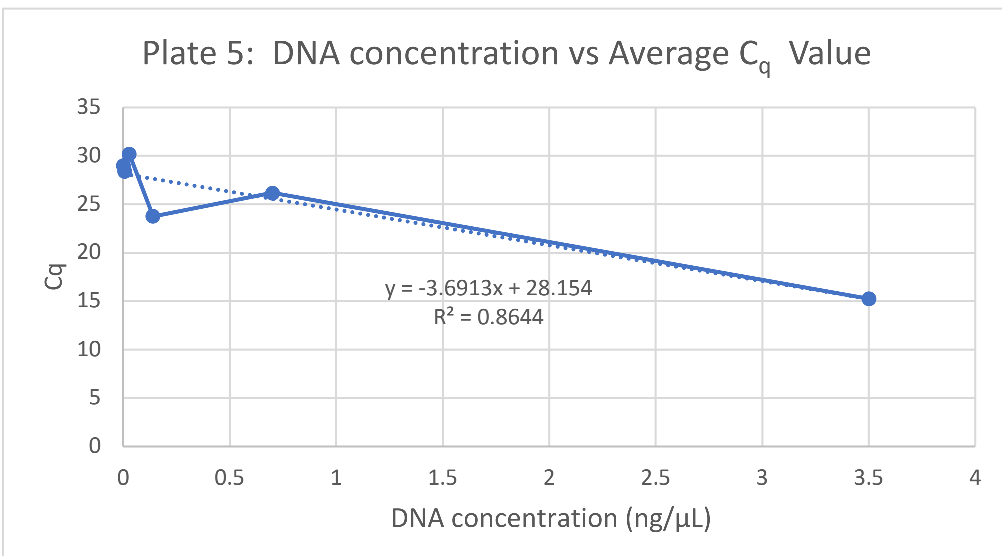
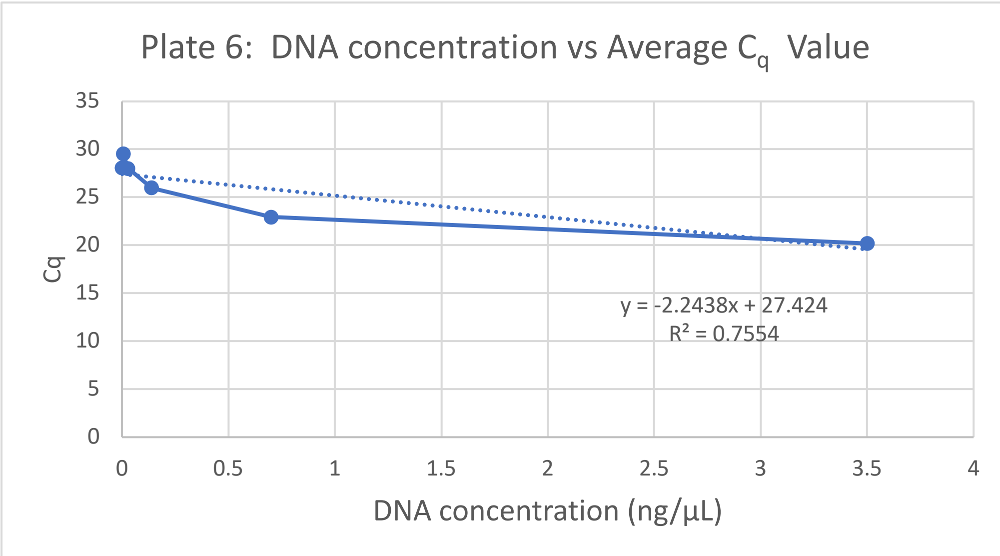


Figure 9. Plate 6 DNA concentration vs Average C_q Value



Figures 4-9 are charts of the data presented in tables 7, 8, and 9. Through these charts the inverse relationship of concentration and C_q value become obvious. In every chart, as DNA concentration increases, the average C_q value decreases. The goal of generating these charts is to find the formula for the line of best fit, which is used to calculate the RNA concentration based on the standard curve. The data are presenting in table 10.

Table 10 (See Appendix) presents the RNA concentrations of each sample quantified using the standard curve line of best fit generated by the charts. They are arranged by well-plate in order to match to their appropriate standard curve. These RNA concentrations were found by plugging in the average C_q values found in table 4 (See Appendix) to their corresponding standard curve lines of best fit formulas. Many of the values are marked "n/a" because an average C_q value was not recorded for those samples in table 4 at all (they failed to present at least three C_q values in the triplicates). Several of the values are also negative. This does not mean that there is a negative RNA concentration as that is not possible. This instead indicates that the C_q value used to calculate the concentration fell below the line of best fit.

There are several possible reasons for this occurrence that are discussed in the discussion section. Out of all *D. arcticus* samples tested, only one sample, sample 104, came out as a positive result. An abbreviated table is shown as table 11.

Table 11. Abbreviated Table 10 Showing No Concentration, Positive, and Negative RNA Concentrations

Sample	Plate 1 Conc.	Plate 2 Conc.	Plate 3 Conc.	Plate 4 Conc.
103	x	n/a	x	x
104	x	0.116243862	x	x
105	x	x	-1.842326097	x

Table 12 (See Appendix) displays a breakdown of each sample and whether the sample yielded positive or negative results. Of all samples tested, only sample 104, which is the Sodium Acetate Positive Control, exhibited expression. It is very likely that this was an anomaly in the experiment considering that all other 2% sodium acetate samples yielded no results.

Table 13. RNA Expression Sorted by Carbon Source and Percentage without Repetitions for *D. arcticus*

Carbon Source	**%**	**Result (pos/neg)**
Dextrose	0.5	n
Dextrose	1	n
Dextrose	2	n
Dextrose	3	n
Dextrose	4	n
Glycerol	0.5	n
Glycerol	1	n
Glycerol	2	n
Glycerol	3	n

Glycerol	4	n
Sodium Acetate	0.5	n
Sodium Acetate	1	n
Sodium Acetate	2	n
Sodium Acetate	3	n
Sodium Acetate	4	n
Isobutyric Acid	0.5	n
Isobutyric Acid	1	n
Isobutyric Acid	2	n
Isobutyric Acid	3	n
Isobutyric Acid	4	n

Table 13 displays the final results of carbon sources and their percentages that led to expression. In order to pass as a positive result, two out of three triplicates must yield a positive RNA concentration in table 10. As evident in this table, no percentage of any of the tested carbon sources yielded positive results. The only positive result was an individual 2% sodium acetate positive control, but that is highly likely to have been an anomaly. All other negative controls and positive controls yielded a negative result.

Table 14 (See Appendix) describes the results of the qPCR procedure on the *D. psychrophila* samples. The goal to be marked as a positive result was to have at least three out of six duplicates of each sample from a single cDNA prep yield a C_q value. If the sample triplicate yielded three C_q values after the first trial, that sample was not repeated. This was the case with most of the samples, with the exception of sample 19, a positive control for glycerol that was repeated and still yielded negative results. The only case where negative results were not repeated were in negative control samples where negative results were expected.

Table 15 (See Appendix) describes the averaging of the C_q value triplicates for each sample of *D. psychrophila*. The calculation done in this table is simply taking the average of the three C_q values obtained for a triplicate of a sample, which can be found in table 14. If less than three C_q values were obtained for a particular sample, the sample is marked as “n/a” instead of with an average C_q value. The “/” indicate expected negative results for negative controls, with the exception of 19, which was an unexpected negative result.

Table 16. Experimental Standard Curve DNA C_q Values for *D. psychrophila*

Sample	plate 1 standard (triplicate)	plate 2 standard (triplicate)	plate 3 standard (triplicate)
1	13.44170726	13.61181782	13.54501866
2	14.60266193	14.61582704	16.61656211
3	18.11091638	17.52574655	18.2953073
4	19.05759004	20.63840293	21.66001627
5	24.55471048	26.0993874	27.13480717
6	26.03683175	26.38728166	26.73367282
1	13.62676746	13.46476023	13.7888848
2	14.77454893	14.57617233	18.49004293
3	17.81506062	18.70333	19.81328732
4	19.62552783	20.7274041	23.37424786
5	23.58504922	24.21413722	25.75304721
6	24.06364638	26.55290029	25.98518291
1	13.74633814	13.65417707	14.00641252
2	15.47053894	14.67344448	19.35955746
3	17.59288883	18.95001379	18.77370144
4	19.3117499	20.5861461	23.74423993
5	22.78638817	26.27407786	27.18675169
6	26.41565223	26.67482703	25.79621107

Table 16 describes the experimental DNA C_q values for the DNA isolated from *D. psychrophila* and the subsequent dilutions. C_q values are increasing, indicating that concentration is decreasing.

Table 17. Average Experimental Standard Curve DNA Concentrations (ng/µL) vs Measured DNA C_q Values for *D. psychrophila (Well Plate 1)*

	Plate 1 Standard Curve	
	Concentration	Avg C_q Value
1	83	13.6049376
2	16.6	14.9492499
3	3.32	17.8396219
4	0.664	19.3316226
5	0.1328	23.6420493
6	0.02656	25.5053768

Table 18. Average Experimental Standard Curve DNA Concentrations (ng/µL) vs Measured DNA C_q Values for *D. psychrophila (Well Plate 2)*

	Plate 2 Standard Curve	
	Concentration	Avg C_q Value
1	83	13.57691837
2	16.6	14.62181462
3	3.32	18.39303011
4	0.664	20.65065104
5	0.1328	25.52920083
6	0.02656	26.53833633

Table 19. Average Experimental Standard Curve DNA Concentrations (ng/µL) vs Measured DNA C_q Values for *D. psychrophila (Well Plate 3)*

	Plate 3 Standard Curve	
	Concentration	Avg C_q Value
1	83	13.78010532
2	16.6	18.1553875
3	3.32	18.96076535

4	0.664	22.92616802
5	0.1328	26.69153536
6	0.02656	26.17168893

Tables 17, 18, and 19 describe the C_q value triplicates averaged and set against the measured concentrations of each dilution level in ng/µL. In the concentration column are the measured concentrations of each dilution level. In the avg C_q value column are averages of each dilution level triplicate as shown in table 16.

Figure 10. Plate 1 DNA concentration vs Average C_q Value

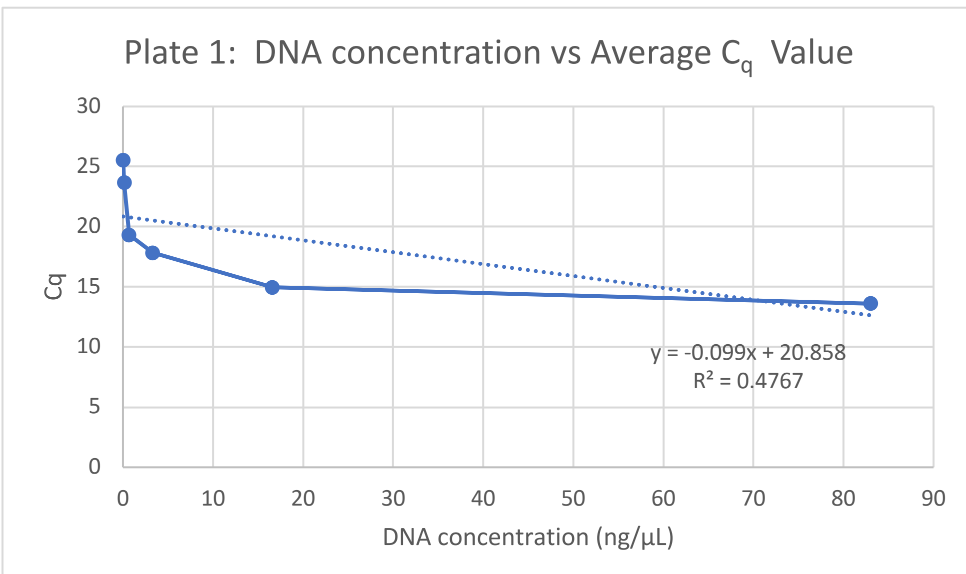


Figure 11. Plate 2 DNA concentration vs Average C_q Value

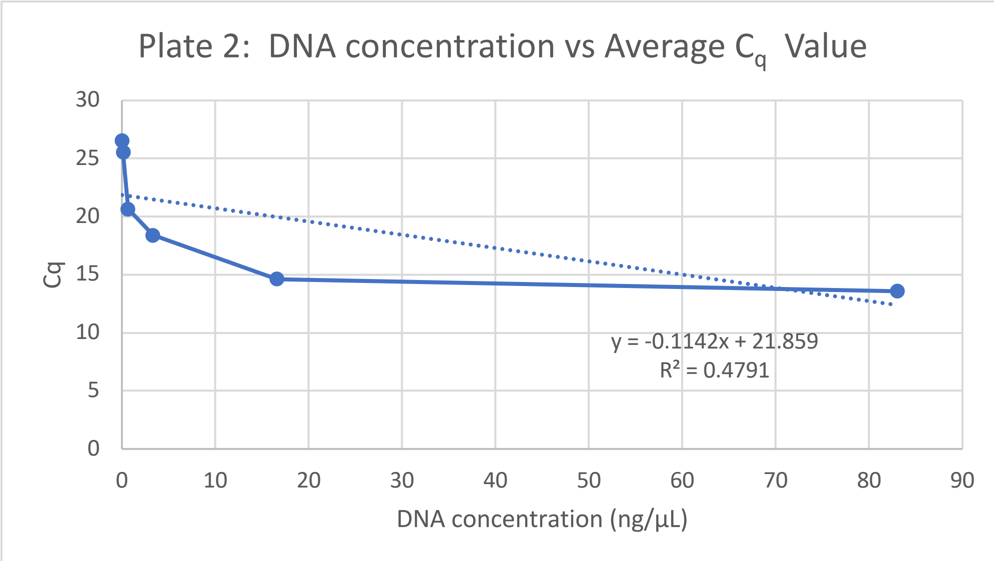
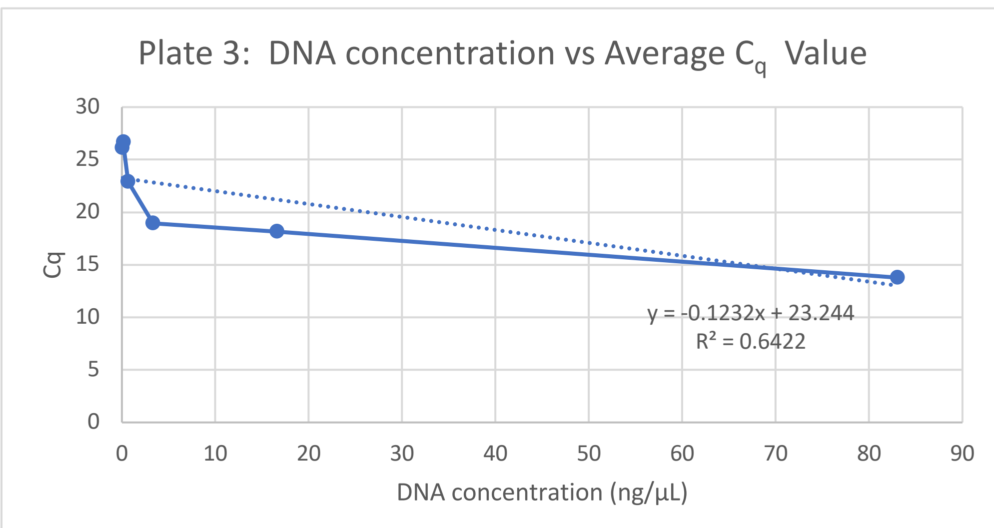


Figure 12. Plate 3 DNA concentration vs Average C_q Value



Figures 10, 11, and 12 are charts of the data presented in tables 16, 17, and 18. Through these charts the inverse relationship of concentration and C_q value become obvious. In every chart, as DNA concentration increases, the average C_q value decreases. The goal of generating these charts is to find the formula for the line of best fit, which is used to calculate the RNA concentration based on the standard curve. The data are presenting in table 20.

Table 20 (See Appendix) presents the RNA concentrations of each sample quantified using the standard curve line of best fit generated by the charts. They are arranged by well plate to match each value to the appropriate standard curve. These RNA concentrations were found by plugging in the average C_q values, found in table 15, to their corresponding standard curve lines of best fit formulas depending on the well-plate that they were processed with. Several of the values are negative. This does not mean that there is a negative RNA concentration as that is not possible. This instead indicates that the C_q value used to calculate the concentration fell below the line of best fit. There are several possible reasons for this occurrence that are discussed in the discussion section. With regard to the *D. psychrophila* samples tested, several samples came out with a positive result. An abbreviated table is shown in table 21.

Table 21. Abbreviated Table 20 Showing Positive Concentration, No Concentration, and Negative Concentration

Sample	Plate 1 RNA Concentrations	Plate 2 RNA Concentrations	Plate 3 RNA Concentrations
17	99.19344496		
18	/		
19	/		
20	-21.23652552		

Table 22 (See Appendix) displays a breakdown of each sample and whether the sample yielded positive or negative results. Of all samples tested, several came out as positive results. The data are compiled accounting for the triplicates in the next table.

Table 23. RNA Expression Sorted by Carbon Source and Percentage without Repetitions for *D. psychrophila*

Carbon Source	%	Result (pos/neg)
Dextrose	0.5	n
Dextrose	1	n
Dextrose	2	p
Dextrose	3	p
Dextrose	4	p
Glycerol	0.5	n
Glycerol	1	p
Glycerol	2	p
Glycerol	3	n
Glycerol	4	n
Sodium Acetate	0.5	p
Sodium Acetate	1	p
Sodium Acetate	2	n
Sodium Acetate	3	n
Sodium Acetate	4	n
Isobutyric Acid	0.5	n
Isobutyric Acid	1	n
Isobutyric Acid	2	n
Isobutyric Acid	3	n
Isobutyric Acid	4	n

Table 23 displays the final results of carbon sources and their percentages that led to expression. In order to pass as a positive result, two out of three triplicates must yield a positive RNA concentration in table 20. As evident in this table, above 2% dextrose, 1% and 2% glycerol, and below 2% sodium acetate yielded positive results. There is sufficient evidence that these carbon sources are able to be metabolized by *D. psychrophila* at certain concentrations. The exception is isobutyric acid, which yielded all negative results regardless of the percentage used. This carbon source is unlikely to be easily metabolized by *D. psychrophila* at all. All negative controls acted in a way concurrent with expectations. All positive controls did as well with exception to sample 19, the glycerol positive control, which yielded a negative result.

Discussion

Based on the data presented, there is strong evidence that none of the four tested carbon sources are compatible for the growth of *D. arcticus*. As exemplified by table 10, there was only one example of any carbon source producing significant results in terms of replication. Furthermore, only one well out of a triplicate produced any result, meaning that two other wells included in that triplicate produced no result. From this, it can reasonably be concluded that the positive result was an outlier.

Also evident in table 10 are several negative RNA values, which should be impossible. However, these RNA values were calculated based on C_q values against the standard curve of the plate. The fact that C_q values were obtained for those samples is indicative of expression of the dsrAB gene. Negative calculated RNA concentrations demonstrate that there was not enough expression of the dsrAB gene to be measured according to the standard curve.

One significant point of contention with the data collected is that there is visible growth and clear evidence of sulfate reduction within most of the vials. However, based on table 12, there are definitive data suggesting that the dsrAB gene was not greatly expressed with the presence of any of the tested carbon sources. This can potentially be explained with the concept of incomplete reduction. It is possible that the preceding steps in the electron transport chain of *D. arcticus* occurred, but the conditions of growth proved too suboptimal for complete reduction. In essence, this means that the last enzyme was either never reached or reached very seldom, resulting in the incomplete reduction of sulfate.

Another possible explanation for the lack of result is the complexity of the carbon sources. It is known that *D. arcticus* can grow in hydrogen and carbon dioxide. The simplest carbon used in this project is a two-length carbon, and the expression of the dsrAB gene in the two-length carbon and all other complex carbons tested for that matter were negligible according to the standard curves. This potentially means that *D. arcticus* has adapted to only grow using short length carbons, likely single-length carbons. The reason that expression was not significant would be because the carbons utilized in this experiment were too complex for the organism to metabolize.

Also based on the data presented, there is strong evidence that three of the four carbon sources are compatible for the growth of *D. psychrophila.* Based on table 14, there was a recorded C_q value for every sample triplicate, indicating that there was some degree of expression with every sample. However, after analyzing the samples against the standard curves generated to estimate the RNA concentrations in table 19, it is evident that not all of the samples yielded statistically significant dsrAB expression. Some of the RNA concentrations are negative, indicating expression to some degree. However, as before, these RNA concentrations demonstrate that expression was not great enough to be measured according to the appropriate standard curve. After accounting for the repetitions, it is evident that only 2%, 3%, and 4% dextrose, 1% and 2% glycerol, and 0.5% and 1% sodium acetate yielded positive results of reproducible upticks in expression. All others did not yield results that indicate consistent RNA expression.

One potential reason for the failure of isobutyric acid to be metabolized by *D. psychrophila* may be that the specific structure of isobutyric acid and its subsequent properties are not compatible for the growth of *D. psychrophila.* Since this bacteria was able to metabolize carbon sources both longer and shorter than isobutyric acid, it can be reasonably concluded that the length of this carbon source did not play a factor in of itself.

Appendix

Table 3. Sample C_q Values for *D. arcticus* with Triplicates

Sample	Raw C_q Value	Repeat? (yes/no)	Raw C_q Value	Result (pos/neg)
70		y	27.4275278	n
70		y		n
70		y		n
71		y		n
71		y		n
71		y		n
72	34.77935914	n		p
72	27.69791178	n		p
72	28.19155233	n		p
73		y	28.61264005	n
73		y		n
73	29.55155961	y		n
74	29.16481588	n		p
74	29.0783896	n		p
74	30.16799732	n		p
75		y		n
75		y		n
75		y		n
76		y	29.60155722	n
76		y		n
76		y		n
77	29.46505451	n		p
77	28.11576033	n		p
77	29.10006775	n		p
78		y		n
78	30.12057105	y		n
78		y		n
79		y		n
79		y		n
79		y		n
80	27.16066124	n		p
80	27.5209918	n		p
80	28.56029908	n		p
81	29.22255258	n		p

81	29.95749302	n		p
81	27.43466772	n		p
82	28.35359723	y	29.27444858	p
82		y		p
82		y	29.10503994	p
83	28.26616452	y	29.60776883	p
83	30.47001291	y	29.09873358	p
83		y	29.06528961	p
84	29.0119431	y		n
84		y		n
84	29.38716396	y		n
85		y		n
85	29.2549748	y		n
85		y		n
86		y		n
86	30.14865928	y		n
86		y		n
87		y		n
87		y		n
87		y		n
88	27.44141322	y		n
88		y		n
88		y		n
89		y		n
89	29.38943531	y		n
89	28.65887525	y		n
90		y		n
90		y		n
90	30.17410895	y		n
91		y	30.37551192	n
91		y		n
91		y		n
92		y		n
92	28.60123295	y		n
92		y		n
93		y		n
93		y		n
93		y		n
94		y		n

94	35.66936698	y		n
94		y		n
95		y	30.94391636	p
95	27.70760912	y		p
95	28.31305461	y		p
96	27.35675513	n		p
96	30.43348966	n		p
96	28.38012453	n		p
97		y		n
97	34.51923829	y		n
97	29.21829854	y		n
98	29.35741124	y	31.47050861	p
98		y	29.38307113	p
98	27.88583285	y	29.42400679	p
99	30.17057875	n		p
99	29.42495368	n		p
99	29.64818368	n		p
100		y	30.56044863	p
100	32.69171622	y		p
100		y	2.050223247	p
101		y		n
101	28.02932286	y		n
101	29.54339831	y		n
102		y	28.68005921	n
102		y		n
102		y	31.49272705	n
103	33.35354806	y		n
103	29.13865084	y		n
103		y		n
104	26.4660481	n		p
104	26.73966004	n		p
104	26.73761513	n		p
105	28.17890282	n		p
105	28.45896073	n		p
105	28.3923158	n		p
106	29.97336791	y	30.69443884	n
106		y		n
106		y		n
107	27.81686688	n		p

107	28.93128278	n		p
107	27.62858113	n		p
108		y	31.4758048	p
108	29.48722804	y	33.03567489	p
108		y		p
109	27.85626388	n		p
109	26.87127346	n		p
109	26.60519799	n		p
110	28.24413064	n		p
110	29.58647396	n		p
110	29.58473573	n		p
111	28.54738724	y		n
111		y		n
111	29.87144571	y		n
112	28.60056456	y	28.568245	p
112	28.84155493	y		p
112		y	30.32344814	p
113	30.12301579	n		p
113	29.66096522	n		p
113	28.43269698	n		p
114	30.256165	n		p
114	29.21499927	n		p
114	34.3885984	n		p
115	33.08868954	y		n
115		y		n
115		y		n
116		y		n
116		y		n
116		y		n
117	29.75926602	y	29.92806447	p
117		y	28.91932062	p
117		y		p
118	28.05399448	n		p
118	29.20650346	n		p
118	34.26831622	n		p
119		y		n
119		y		n
119	28.1829258	y		n
120		y		n

120	27.85012521	y		n
120		y		n
121		y		n
121		y		n
121		y		n
122		y		n
122		y		n
122		y		n
123		y	28.91010969	n
123		y		n
123		y		n
124		y		n
124	28.96627151	y		n
124		y		n
125		y		n
125		y		n
125		y		n
126		y		n
126		y		n
126		y		n
127		y	30.20218671	p
127	29.57778009	y		p
127	29.66521939	y		p
128		y		n
128		y		n
128		y		n
129		y		n
129		y		n
129		y		n
130		y		n
130		y		n
130	28.59114081	y		n
131		y		n
131		y		n
131		y		n
132		y		n
132		y		n
132		y		n
133		y		n

133		y		n
133		y		n
134		y		n
134	28.2086745	y		n
134		y		n
135		y		n
135		y		n
135		y		n
136		y		n
136		y		n
136		y		n
137		y		n
137		y		n
137		y		n

Table 4. Average C_q Value per Sample for *D. arcticus*

Sample	Average C_q
70	n/a
71	n/a
72	30.22294108
73	n/a
74	29.47040093
75	n/a
76	n/a
77	28.89362753
78	n/a
79	n/a
80	27.74731738
81	28.87157111
82	28.91102859
83	29.30159389
84	n/a
85	n/a
86	n/a
87	n/a
88	n/a
89	n/a

90	n/a
91	n/a
92	n/a
93	n/a
94	n/a
95	28.98819336
96	28.72345644
97	n/a
98	29.50416612
99	29.74790537
100	n/a - outlier
101	n/a
102	n/a
103	n/a
104	26.64777442
105	28.34339312
106	n/a
107	28.12557693
108	31.33290258
109	27.11091178
110	29.13844678
111	n/a
112	29.08345316
113	29.40555933
114	31.28658755
115	n/a
116	n/a
117	29.53555037
118	30.50960472
119	n/a
120	n/a
121	n/a
122	n/a
123	n/a
124	n/a
125	n/a
126	n/a
127	29.81506206
128	n/a

129	n/a
130	n/a
131	n/a
132	n/a
133	n/a
134	n/a
135	n/a
136	n/a
137	n/a

Table 10. Average RNA concentrations (ng/µL) for *D. arcticus*

Sample	Plate 1 RNA Concentrations	Plate 2 RNA Concentrations	Plate 3 RNA Concentrations	Plate 4 RNA Concentrations
70	n/a	x	x	x
71	n/a	x	x	x
72	-4.051397093	x	x	x
73	n/a	x	x	x
74	-3.414622554	x	x	x
75	n/a	x	x	x
76	n/a	x	x	x
77	-2.926576011	x	x	x
78	n/a	x	x	x
79	n/a	x	x	x
80	x	-0.808441154	x	x
81	x	-1.753907246	x	x
82	x	-1.787089888	x	x
83	x	-2.115544437	x	x
84	x	n/a	x	x
85	x	n/a	x	x
86	x	n/a	x	x
87	x	n/a	x	x
88	x	n/a	x	x
89	x	n/a	x	x
90	x	n/a	x	x
91	x	n/a	x	x
92	x	n/a	x	x

93	x	n/a	x	x
94	x	n/a	x	x
95	x	-1.851983317	x	x
96	x	-1.629346936	x	x
97	x	n/a	x	x
98	x	-2.285902047	x	x
99	x	-2.490879967	x	x
100	x	n/a - outlier present	x	x
101	x	n/a	x	x
102	x	n/a	x	x
103	x	n/a	x	x
104	x	0.116243862	x	x
105	x	x	-1.842326097	x
106	x	x	n/a	x
107	x	x	-1.759008886	x
108	x	x	-2.985848057	x
109	x	x	-1.370887723	x
110	x	x	-2.146443323	x
111	x	x	n/a	x
112	x	x	-2.125407626	x
113	x	x	-2.248616965	x
114	x	x	-2.968132025	x
115	x	x	n/a	x
116	x	x	n/a	x
117	x	x	-2.298340042	x
118	x	x	-2.670927101	x
119	x	x	n/a	x
120	x	x	n/a	x
121	x	x	n/a	x
122	x	x	n/a	x
123	x	x	n/a	x
124	x	x	n/a	x
125	x	x	n/a	x
126	x	x	n/a	x
127	x	x	-2.405256498	x
128	x	x	n/a	x
129	x	x	n/a	x
130	x	x	x	n/a

131	x	x	x	n/a
132	x	x	x	n/a
133	x	x	x	n/a
134	x	x	x	n/a
135	x	x	x	n/a
136	x	x	x	n/a
137	x	x	x	n/a

Table 12. Results of RNA Expression Sorted by Sample Source without Triplicates for *D. arcticus*

Sample ID	Carbon Source	rep/%	Result (pos/neg)
70	Dextrose	control (+)	n
71	Dextrose	Rep1-0.5%	n
72	Dextrose	Rep1-1%	n
73	Dextrose	Rep1-2%	n
74	Dextrose	Rep1-3%	n
75	Dextrose	Rep1-4%	n
76	Dextrose	Rep2-0.5%	n
77	Dextrose	Rep2-1%	n
78	Dextrose	Rep2-2%	n
79	Dextrose	Rep2-3%	n
80	Dextrose	Rep2-4%	n
81	Dextrose	Rep3-0.5%	n
82	Dextrose	Rep3-1%	n
83	Dextrose	Rep3-2%	n
84	Dextrose	Rep3-3%	n
85	Dextrose	Rep3-4%	n
86	Dextrose	Control (-)	n
87	Glycerol	Control (+)	n
88	Glycerol	Rep1-0.5%	n
89	Glycerol	Rep1-1%	n
90	Glycerol	Rep1-2%	n
91	Glycerol	Rep1-3%	n
92	Glycerol	Rep1-4%	n
93	Glycerol	Rep2-0.5%	n
94	Glycerol	Rep2-1%	n

95	Glycerol	Rep2-2%	n
96	Glycerol	Rep2-3%	n
97	Glycerol	Rep2-4%	n
98	Glycerol	Rep3-0.5%	n
99	Glycerol	Rep3-1%	n
100	Glycerol	Rep3-2%	n
101	Glycerol	Rep3-3%	n
102	Glycerol	Rep3-4%	n
103	Glycerol	Control (-)	n
104	Sodium Acetate	Control (+)	p
105	Sodium Acetate	Rep1-0.5%	n
106	Sodium Acetate	Rep1-1%	n
107	Sodium Acetate	Rep1-2%	n
108	Sodium Acetate	Rep1-3%	n
109	Sodium Acetate	Rep1-4%	n
110	Sodium Acetate	Rep2-0.5%	n
111	Sodium Acetate	Rep2-1%	n
112	Sodium Acetate	Rep2-2%	n
113	Sodium Acetate	Rep2-3%	n
114	Sodium Acetate	Rep2-4%	n
115	Sodium Acetate	Rep3-0.5%	n
116	Sodium Acetate	Rep3-1%	n
117	Sodium Acetate	Rep3-2%	n
118	Sodium Acetate	Rep3-3%	n
119	Sodium Acetate	Rep3-4%	n
120	Sodium Acetate	Control (-)	n
121	Isobutyric Acid	Control (+)	n
122	Isobutyric Acid	Rep1-0.5%	n
123	Isobutyric Acid	Rep1-1%	n
124	Isobutyric Acid	Rep1-2%	n
125	Isobutyric Acid	Rep1-3%	n
126	Isobutyric Acid	Rep1-4%	n
127	Isobutyric Acid	Rep2-0.5%	n
128	Isobutyric Acid	Rep2-1%	n
129	Isobutyric Acid	Rep2-2%	n
130	Isobutyric Acid	Rep2-3%	n
131	Isobutyric Acid	Rep2-4%	n
132	Isobutyric Acid	Rep3-0.5%	n
133	Isobutyric Acid	Rep3-1%	n

134	Isobutyric Acid	Rep3-2%	n
135	Isobutyric Acid	Rep3-3%	n
136	Isobutyric Acid	Rep3-4%	n
137	Isobutyric Acid	Control (-)	n

Table 14. Sample C_q Values for *D. psychrophila* with Triplicates

Sample	Raw C_q Value	Repeat?	Result (pos/neg)
2	17.41	n	p
2	17.24	n	p
2	17.08	n	p
3	26.21	n	p
3	26.21	n	p
3	27.30	n	p
4	25.76	n	p
4	25.52	n	p
4	26.16	n	p
5	10.31	n	p
5	10.39	n	p
5	10.53	n	p
6	22.79	n	p
6	22.03	n	p
6	21.76	n	p
7	10.16	n	p
7	10.19	n	p
7	10.22	n	p
8	21.89	n	p
8	21.53	n	p
8	21.60	n	p
9	20.33	n	p
9	20.50	n	p
9	20.56	n	p
10	9.16	n	p
10	8.74	n	p
10	9.14	n	p
11	10.17	n	p
11	10.12	n	p
11	10.03	n	p

12	12.24	n	p
12	12.13	n	p
12	12.23	n	p
13	21.20	n	p
13	21.47	n	p
13	21.23	n	p
14	21.63	n	p
14	21.48	n	p
14	21.49	n	p
15	10.71	n	p
15	10.54	n	p
15	10.99	n	p
16	12.24	n	p
16	12.19	n	p
16	12.39	n	p
17	10.88	n	p
17	11.14	n	p
17	11.09	n	p
18	0.00	n	p
18	0.00	n	p
18	0.00	n	p
*19	0.00	y	n
*19	0.00	y	n
*19	0.00	y	n
20	22.94	n	p
20	22.82	n	p
20	23.12	n	p
21	22.31	n	p
21	22.43	n	p
21	22.57	n	p
22	21.69	n	p
22	18.93	n	p
22	21.44	n	p
23	28.12	n	p
23	29.88	n	p
23	29.31	n	p
24	23.75	n	p
24	24.27	n	p
24	24.34	n	p

25	15.26	n	p
25	15.21	n	p
25	15.36	n	p
26	9.95	n	p
26	10.07	n	p
26	9.77	n	p
27	20.41	n	p
27	20.09	n	p
27	20.04	n	p
28	22.59	n	p
28	22.54	n	p
28	22.73	n	p
29	25.81	n	p
29	25.98	n	p
29	26.32	n	p
30	23.69	n	p
30	23.44	n	p
30	23.82	n	p
31	14.06	n	p
31	13.93	n	p
31	13.93	n	p
32	14.63	n	p
32	14.38	n	p
32	14.58	n	p
33	25.95	n	p
33	25.55	n	p
33	26.19	n	p
34	30.17	n	p
34	29.09	n	p
34	29.82	n	p
35	0.00	n	p
35	0.00	n	p
35	0.00	n	p
36	25.69	n	p
36	25.53	n	p
36	25.31	n	p
37	14.99	n	p
37	15.04	n	p
37	14.89	n	p

38	21.23	n	p
38	21.20	n	p
38	21.44	n	p
39	28.53	n	p
39	27.42	n	p
39	27.70	n	p
40	23.65	n	p
40	24.14	n	p
40	23.86	n	p
41	23.21	n	p
41	23.26	n	p
41	23.15	n	p
42	19.48	n	p
42	19.49	n	p
42	19.67	n	p
43	19.48	n	p
43	19.26	n	p
43	19.11	n	p
44	21.48	n	p
44	21.25	n	p
44	21.19	n	p
45	26.92	n	p
45	26.04	n	p
45	25.63	n	p
46	21.36	n	p
46	21.39	n	p
46	21.77	n	p
47	14.50	n	p
47	14.66	n	p
47	14.42	n	p
48	15.55	n	p
48	15.83	n	p
48	15.68	n	p
49	22.45	n	p
49	22.35	n	p
49	22.73	n	p
50	23.58	n	p
50	23.62	n	p
50	23.53	n	p

51	23.03	n	p
51	23.14	n	p
51	23.24	n	p
52	0.00	n	p
52	0.00	n	p
52	0.00	n	p
53	23.33	n	p
53	23.38	n	p
53	23.82	n	p
54	25.65	n	p
54	25.94	n	p
54	26.13	n	p
55	23.26	n	p
55	23.28	n	p
55	23.49	n	p
56	23.27	n	p
56	23.54	n	p
56	23.37	n	p
57	28.70	n	p
57	30.07	n	p
57	29.10	n	p
58	24.44	n	p
58	24.43	n	p
58	24.50	n	p
59	28.02	n	p
59	28.01	n	p
59	29.35	n	p
60	24.30	n	p
60	25.10	n	p
60	24.41	n	p
61	24.32	n	p
61	24.32	n	p
61	24.23	n	p
62	20.75	n	p
62	20.57	n	p
62	20.63	n	p
63	21.21	n	p
63	21.04	n	p
63	21.60	n	p

64	20.65	n	p
64	20.77	n	p
64	20.34	n	p
65	22.30	n	p
65	22.47	n	p
65	22.22	n	p
66	21.41	n	p
66	21.19	n	p
66	21.45	n	p
67	20.38	n	p
67	20.49	n	p
67	20.79	n	p
68	22.30	n	p
68	22.43	n	p
68	22.08	n	p
69	0.00	n	p
69	0.00	n	p
69	0.00	n	p

Table 15. Average C_q Value per Sample for *D. psychrophila*

Sample	Average C_q
2	17.24
3	26.57
4	25.81
5	10.41
6	22.20
7	10.19
8	21.68
9	20.46
10	9.02
11	10.11
12	12.20
13	21.30
14	21.53
15	10.75
16	12.27
17	11.04

18	/
*19	/
20	22.96
21	22.44
22	20.69
23	29.11
24	24.12
25	15.27
26	9.93
27	20.18
28	22.62
29	26.04
30	23.65
31	13.97
32	17.38
33	25.90
34	29.69
35	/
36	25.51
37	14.97
38	21.29
39	27.88
40	23.88
41	23.21
42	19.55
43	19.29
44	21.31
45	26.20
46	21.51
47	14.53
48	15.69
49	22.51
50	23.58
51	23.14
52	/
53	23.51
54	25.91
55	23.34
56	23.39

57	29.29
58	24.46
59	28.46
60	24.60
61	24.29
62	20.65
63	21.28
64	20.59
65	22.33
66	21.35
67	20.56
68	22.27
69	/

Table 20. Average RNA concentrations (ng/µL) for *D. psychrophila*

Sample	Plate 1 RNA Concentrations	Plate 2 RNA Concentrations	Plate 3 RNA Concentrations
2	36.50865715		
3	-57.71076286		
4	-50.05976971		
5	105.5292549		
6	-13.53183482		
7	107.7690825		
8	-8.264417755		
9	4.009030501		
10	119.6232621		
11	108.5965452		
12	87.46396348		
13	-4.432142852		
14	-6.780904718		
15	102.1360162		
16	86.72434054		
17	99.19344496		
18	/		
19	/		
20	-21.23652552		
21	-15.93974975		
22	1.713374323		

23	-83.30689349		
24	-32.91506072		
25	56.41515379		
26	110.3778776		
27		14.70523815	
28		-6.665349734	
29		-36.57563053	
30		-15.70678519	
31		69.07218582	
32		39.18677252	
33		-35.34170707	
34		-68.60012545	
35		/	
36		-31.97913029	
37		60.29829975	
38		4.986269532	
39		-52.75222943	
40		-17.72439398	
41		-11.8347598	
42		20.25793948	
43		22.53883636	
44		4.845401462	
45		-37.99566276	
46		3.082659794	
47		64.18725996	
48		54.04873574	
49		-5.675932633	
50		-15.03875686	
51		-11.20229716	
52			/
53			-2.165243832
54			-21.61489619
55			-0.818668658
56			-1.215419201
57			-49.07235313
58			-9.866687277
59			-42.34558974
60			-11.03742076
61			-8.495918565

62			21.04878479
63			15.92552602
64			21.56323054
65			7.406725421
66			15.37101936
67			21.8163442
68			7.911266131
69			/

Table 22. Results of RNA Expression Sorted by Sample Source without Triplicates for *D. psychrophila*

Sample ID	Carbon Source	rep/%	Result (pos/neg)
2	Dextrose	control (+)	p
3	Dextrose	Rep1-0.5%	n
4	Dextrose	Rep1-1%	n
5	Dextrose	Rep1-2%	p
6	Dextrose	Rep1-3%	n
7	Dextrose	Rep1-4%	p
8	Dextrose	Rep2-0.5%	n
9	Dextrose	Rep2-1%	p
10	Dextrose	Rep2-2%	p
11	Dextrose	Rep2-3%	p
12	Dextrose	Rep2-4%	p
13	Dextrose	Rep3-0.5%	n
14	Dextrose	Rep3-1%	n
15	Dextrose	Rep3-2%	p
16	Dextrose	Rep3-3%	p
17	Dextrose	Rep3-4%	p
18	Dextrose	Control (-)	n
19	Glycerol	Control (+)	n
20	Glycerol	Rep1-0.5%	n
21	Glycerol	Rep1-1%	n
22	Glycerol	Rep1-2%	p
23	Glycerol	Rep1-3%	n
24	Glycerol	Rep1-4%	n
25	Glycerol	Rep2-0.5%	p

26	Glycerol	Rep2-1%	p
27	Glycerol	Rep2-2%	p
28	Glycerol	Rep2-3%	n
29	Glycerol	Rep2-4%	n
30	Glycerol	Rep3-0.5%	n
31	Glycerol	Rep3-1%	p
32	Glycerol	Rep3-2%	p
33	Glycerol	Rep3-3%	n
34	Glycerol	Rep3-4%	n
35	Glycerol	Control (-)	n
36	Sodium Acetate	Control (+)	p
37	Sodium Acetate	Rep1-0.5%	p
38	Sodium Acetate	Rep1-1%	p
39	Sodium Acetate	Rep1-2%	n
40	Sodium Acetate	Rep1-3%	n
41	Sodium Acetate	Rep1-4%	n
42	Sodium Acetate	Rep2-0.5%	p
43	Sodium Acetate	Rep2-1%	p
44	Sodium Acetate	Rep2-2%	p
45	Sodium Acetate	Rep2-3%	n
46	Sodium Acetate	Rep2-4%	p
47	Sodium Acetate	Rep3-0.5%	p
48	Sodium Acetate	Rep3-1%	p
49	Sodium Acetate	Rep3-2%	n
50	Sodium Acetate	Rep3-3%	n
51	Sodium Acetate	Rep3-4%	n
52	Sodium Acetate	Control (-)	n
53	Isobutyric Acid	Control (+)	p
54	Isobutyric Acid	Rep1-0.5%	n
55	Isobutyric Acid	Rep1-1%	n
56	Isobutyric Acid	Rep1-2%	n
57	Isobutyric Acid	Rep1-3%	n
58	Isobutyric Acid	Rep1-4%	n
59	Isobutyric Acid	Rep2-0.5%	n
60	Isobutyric Acid	Rep2-1%	n
61	Isobutyric Acid	Rep2-2%	n
62	Isobutyric Acid	Rep2-3%	p
63	Isobutyric Acid	Rep2-4%	p
64	Isobutyric Acid	Rep3-0.5%	p

65	Isobutyric Acid	Rep3-1%	p
66	Isobutyric Acid	Rep3-2%	p
67	Isobutyric Acid	Rep3-3%	p
68	Isobutyric Acid	Rep3-4%	p
69	Isobutyric Acid	Control (-)	n

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