

Disseminating Probiotic Yogurt Recipe and Educational Materials to Underserved People with Type II Diabetes in Albany County

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The project that I am about to describe is a culmination of the efforts of many individuals seeking to solve the problems of food insecurity and the prevalence of Type II diabetes in an underserved population of people in Laramie, Wyoming. The following will first give a detailed project layout as to the proposed lab research for a probiotic yogurt to address an imbalanced gut microbiome in these individuals, followed by the results of that experimentation. It will then discuss further research that was done at home to modify a probiotic yogurt recipe and make the beneficial probiotic food more accessible to these patients. The paper will discuss a brochure that was created, which incorporated different research components. Lastly, the future of this continuing project will be explored.

Introducing *Bifidobacterium lactis*, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus*
Probiotics to Traditional Yogurt: Suggestions for the Microbiome of Type 2 Diabetics

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Project Summary:

Impoverished populations across the globe are faced with food insecurity, or the inability to access a sufficient quantity of affordable and nutritious food. Through the gain and expansion of medical knowledge, scientists have discovered that nutritious food is not only necessary to the individual to merely survive, but nutritious food is also essential for the health of that person, beginning with the gut microbiota. Medical research has also linked the relationship of the composition of the microbial communities to the development of chronic diseases. Researchers have discovered that Type 2 diabetics lack certain microbes that are essential for the human body to operate, such as a reduction in endotoxemia and improved glucose tolerance and insulin secretion (Cani et al., 2007). The bacteria that are shown to specifically have an effect on these functions are *Bifidobacterium lactis*, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus* (Panwar et al., 2013). These would be beneficial to diabetics across the globe, but especially to low income patients in Laramie, Wyoming. Individuals who have a low income and no insurance are approved to be seen at the Albany County Downtown Clinic (DTC), where they are provided with comprehensive primary health care. One in three patients at the DTC have Type 2 diabetes, and while DTC has a probiotic that they administer to patients (TruBiotic® by Bayer), it does not include all of the bacteria that are in reduced quantity in the diabetic's gut microflora and is generally not prescribed for chronic illnesses such as Type 2 diabetes. Our research will create a probiotic yogurt specialized for a Type 2 diabetic microbiome by isolating *Bifidobacterium lactis*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* and inoculating these five bacteria into a singular probiotic yogurt. We will thus be able to simultaneously provide DTC patients with instructions to make this healthy food.

To successfully isolate these similar lactic acid bacteria, we will create media that select for each individual bacterium, as described by Dave and Shah (1996) and Tharmaraj and Shah (2003). Once the bacteria have been successfully isolated and identified, they will all be inoculated into yogurt, where their longevity and viability will be observed to ensure that a consumer could obtain appropriate amounts of each bacterium. It is expected that the bacteria will be able to survive in yogurt together and that each bacterium will still be viable after four days post inoculation. Through the creation of this probiotic yogurt, we will be providing diabetic patients at DTC with the recipe for a healthy food source and bacteria for their microbiome that are associated with improvements in Type 2 Diabetes. If successful, this study will furthermore allow other free health clinics with a probiotic recipe to improve the health of their diabetic patients.

Statement of Significance:

The rising rates of Type 2 diabetes are posing a threat to human health and contributing to the increasing cost of healthcare. The Centers for Disease Control and Prevention show that 26 million Americans have diabetes and that approximately 33% of adults in the United States are classified as prediabetic (Hirahatake, 2014). The Downtown Clinic located in Laramie, Wyoming, serves the uninsured community of the Laramie Valley. The clinic is open once a week in the evening for appointments, with visiting specialists once a month. With the expansion of the clinic into their own building they are also able to offer pharmaceutical services. With this came the Tuesday night refill clinic for those patients who need their prescriptions filled. The clinic is funded completely by the United Way and private donors. Physicians, Nurse practitioners, and other health care specialists volunteer their time to see patients. It is important to note that residents of Wyoming are not required to have health insurance, even with the Medicare expansion. For this reason, the clinic is increasingly busy seeing patients with common chronic conditions such as asthma, diabetes, high blood pressure and chronic pain.

On average, a third of the patients seen by the nurses at the Downtown clinic have Type 2 diabetes. With this in mind, an alternative is needed to allow those without insurance to control their diabetes. With the location of the clinic and the patients being in such a rural area, there are limited resources for proper nutrition. The local soup kitchen can feed these low-income clients two meals a day and provide some breads donated by local grocers. However, these are high in simple (high glycemic index) carbohydrate. This makes it difficult for our patients to manage their blood glucose levels. If diabetics cannot balance their own blood glucose levels, there is a good likelihood that there will be recurrence in cellulitis, issues with the kidneys, neuropathy, or numbness, and even blindness (Department of Health, 2011). Our proposed technique of establishing an affordable and effective way for diabetic patients to culture their own probiotic yogurt, using strains that have demonstrated benefit in the gut microbiome of Type 2 diabetics, will provide them with a way to gain control over both their daily nutrition and their diabetes.

Introduction:

Relevant Literature

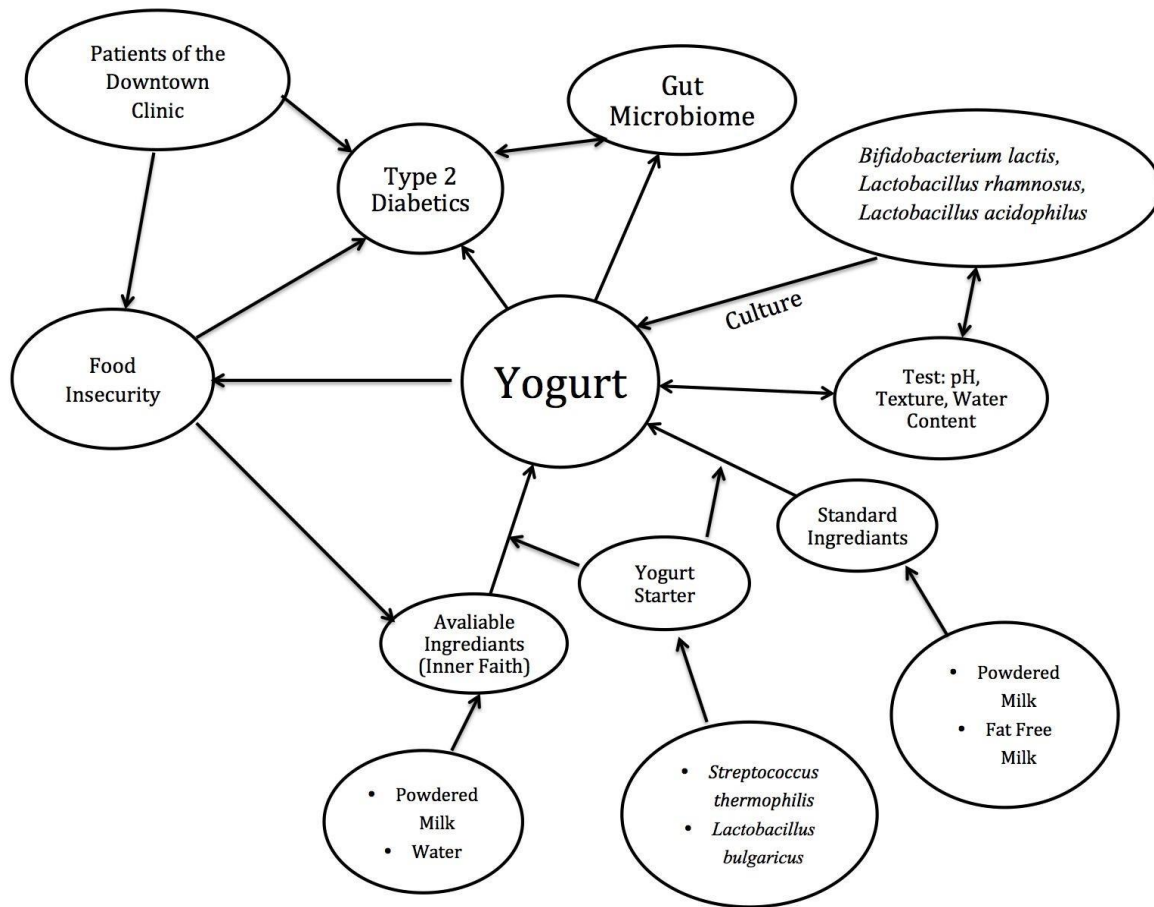
The human gut microbiome is colonized by several microbial communities (including prokaryotes, viruses, and eukaryotes) that are associated with host energy regulation and homeostasis (Kasai et al., 2015). These tiny living organisms must form a mutualistic relationship and cohabitate with the host to ensure a healthy existence. Several studies of the microbiome have determined remarkable microbial differences in gut microflora between healthy individuals and people with chronic diseases, including obesity, metabolic syndrome, autoimmune disorders, and inflammatory bowel syndrome (Kuperman and Koren, 2015). One chronic disease that is threatening many individuals throughout the world is diabetes mellitus, specifically Type 2. The introduction of a probiotic, or a dietary supplement that contains live bacteria, can offer a health benefit to these diabetics, especially if that probiotic contains the healthy bacteria that is missing from their regular microflora (Al-Salami et al., 2008).

Normal and healthy commensal gut microflora is stably conserved in people. Any disturbance in the structure, composition, or proportion indicates an effect on the individual's health. This can be due to changes in lifestyle, stress levels, genetic predisposition, and immunity weaknesses and can result in the development of medical conditions (Panwar et al., 2013). Modifying the intestinal microbiota by probiotics has the potential to expedite the management of several clinical conditions and may also potentially be the key to help insulin resistance therapies (Gomes et al. 2014). Several studies have observed the contents of a diabetic's gut microflora and found that the ratios of *Bacteroidetes* to *Proteobacteria* was somewhat higher in diabetic patients compared to healthy individuals (Larsen et al., 2010). Concurrently, studies discovered a reduction in *Bifidobacterium* and *Lactobacillus*. Diabetic individuals who consumed yogurt that contained *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis* showed a noteworthy decrease in fasting blood glucose as well as an increase in erythrocyte superoxide dismutase and overall antioxidant status (Panwar et al., 2013). These results prove that consumption of these bacteria on a regular basis will improve the health of Type 2 diabetics. The use of homemade probiotics for Type 2 diabetics has the potential to be a strong step in increasing the health of underprivileged Type 2 diabetics, while also simultaneously providing them with a nutritious food source.

Preliminary Data

Cultivation of yogurt starter culture *Lactobacillus bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*) has been successfully completed. Cultivation of Swanson Probiotics *Lactobacillus rhamnosus* (*L. rhamnosus*) has been successfully completed. The *Lactobacillus* strains have been successfully differentiated using MRS agar with a pH indicator. Likewise, cultivation of *Bifidobacterium lactis* (*B. lactis*) has been successfully completed using *Bifidobacterium* agar media, and selection and differentiation of *Lactobacillus acidophilus* (*L. acidophilus*) has also been successful. Currently at the Downtown clinic the only form of a probiotic available is TruBiotic® by Bayer, which is only prescribed for patients who are on antibiotics. In previous years, students in the UW Microbiology Capstone Lab were able to isolate and grow the bacterial stains, *Lactobacillus acidophilus* and *Bifidobacterium animalis* from TruBiotic onto Tryptic Soy Agar (TSA) and *Lactobacillus* on (MRS). However, they could not be subcultured. Because we are helping the low income community of the Laramie Valley, we are striving to use probiotics in a yogurt recipe that will have a long lasting effect on the diabetic patient, as yogurt is both easy to make and inexpensive.

Conceptual Model



Justification of Methods

Previous studies have shown that *B. lactis*, *L. acidophilus*, and *L. rhamnosus* have a much lower quantity in the gut microflora of Type 2 diabetics as compared to healthy gut microflora. Studies have also revealed the importance of these bacteria, including increase natural killer cells (NKT), improved insulin resistance, and reduction in inflammation (Panwar et al., 2013). Additionally, these bacteria were discovered to be associated with a reduction of intestinal endotoxin levels as well as an improvement of the mucosal barrier function (Diamant et al., 2010). Thus, incorporating these bacteria into a probiotic specific to Type 2 diabetics would be justifiable.

The yogurt starter culture contains *S. thermophilus* and *L. bulgaricus*, which is required by law to be present for it to be called yogurt. Likewise, studies have revealed that several *Bifidobacterium* species, in addition to *L. rhamnosus*, have been added to several probiotic yogurts, proving that these lactic acid bacteria can be successfully cultured in yogurt (Shah and Lankaputhra, 1997). Although, there is a possibility that all five strains will not be able to be cultured together in yogurt. Therefore, we will test the longevity and viability of these bacteria after inoculation. The use of probiotic yogurt rather than a pill form of probiotics offers a food source to DTC diabetic clientele.

All techniques regarding selection on media are well-documented procedures. All of the media can be created in the lab and (theoretically) specifically selects each bacteria (Dave and Shah, 1996; Tharmaraj and Shah, 2003).

Objectives:

- 1) To gain a better understanding of the significance of food security and the impacts that it has on the health of individuals in Laramie, Wyoming.
- 2) To successfully culture the bacterial strains *B. lactis*, *L. rhamnosus*, and *L. acidophilus* into yogurt.
- 3) To provide a yogurt recipe and instructions on making a beneficial food and probiotic source to diabetic patients at the Laramie Downtown Clinic.
- 4) To suggest approaches by which partners of the DTC (Interfaith Good Samaritan and the Laramie Soup Kitchen) might provide the suggested augmented, homemade yogurt ingredients to the DTC patients. This may eventually create a more balanced microbiome in individuals within the underprivileged diabetic population in Laramie.

Hypotheses:

- 1) Traditional yogurt made in our lab with the starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) will be equivalent in texture, pH, and water content to Dannon original yogurt with the same bacteria.
- 2) If the probiotic bacteria *Bifidobacterium lactis*, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus* are added to basic yogurt containing its usual live cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), then all five bacteria will remain viable and at concentration greater than or equal to 10^6 colony forming units per gram at a 24 hour timeframe post incubation.
- 3) If the probiotic bacteria *Bifidobacterium lactis*, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus* are added to basic yogurt containing its usual live cultures (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*), then the texture, pH, and water content of the yogurt will be unchanged as compared to that with only the original live cultures.

Specific Aims:

- After the addition of *B. lactis*, *L. acidophilus*, and *L. rhamnosus* to basic yogurt culture (*S. thermophilus* and *L. bulgaricus*), titers of the bacteria will be enumerated by dilution and plate count.
- Isolation, identification, and cultivation of all probiotics (from yogurt and supplement to plate) will be completed.
- Using a pH meter, we will measure the pH content of each yogurt culture created.
- We will determine differences in the texture of the yogurt culture created.
- Using a fume hood to dry the yogurt and comparing initial weight and final weight after drying, we will test the water content of each yogurt culture created.

Experimental Design:

Our overarching goal will be to provide a way for Downtown Clinic patients to obtain a healthy food source (yogurt) that individuals can culture and eat. Through traditional microbiology methods, we will culture said bacteria on various selective and differential medias, allowing us to determine if specific probiotic bacterial strains can be successfully cultured into an individual probiotic yogurt.

This will be done through the production of our own yogurt with the required strains (*S. thermophilus* and *L. bulgaricus*), production of the same yogurt with the addition of variable strains of bacteria (*L. acidophilus*, *L. rhamnosus*, *B. lactis*), enumerating all bacteria strains in yogurt containing variable bacteria (if successful) to determine concentration of each bacterium present, and testing the yogurt containing variable strains for characteristics including texture, pH and water content.

Materials and Methods:

The first part of our experiment will be to test bacterial culture sources and isolate bacteria from these sources. It is important that the bacteria can grow from the pills (*L. acidophilus*), capsules (*L. rhamnosus*), and blends (*S. thermophilus* and *L. bulgaricus*) and (isolation of *B. lactis* from a mix). Frozen cultures will be created once successful isolation is completed. Bacteria will be in liquid solution with phosphate-buffered saline (PBS) in order to remain viable in the cold freezer. Yogurt will then be produced using the necessary ingredients (milk and starter culture with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*), in the incubator in the lab at 44 degrees Celsius in 90 milliliter (mL) sterile containers, and stored in the refrigerator at 2 degrees Celsius post production. Milk used will be pasteurized.

These yogurts will be evaluated through pH measurements with a digital meter and wet mass and dry mass through drying out the yogurt at room temperature in the fume hood and measuring mass before and after. It is important that the pH is close to a neutral 7. Although, it is understood that lactic acid fermentation will result in a slightly acidic yogurt. In our experiment, taste, texture, spreadability, and smell will also be qualitatively analyzed in comparison to plain store-bought yogurt. It is important that our yogurt has a taste that has no oddities from any bacterial byproducts. Measurements of titers of each yogurt will be performed at 6, 12, and 24 hours during production. We will take 1 gram of yogurt out of the culture and mix it in a dilution scheme with phosphate-buffered saline (PBS) and plate onto media. It is important that we determine if we have a good balance of microbes after 24 hours post production of final product to ensure that the bacteria will not die out before consumption by our patients and be introduced to the gut. Desired titer will be the recommended level bacteria of $\geq 10^6$ colony forming units per gram (Ashraf, 2011). The first evaluation and comparison will be between our original yogurt and Dannon yogurt original flavor with the same two bacteria.

We will then create a yogurt with *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium lactis*, as well as a separate yogurt culture using *S. thermophilus*, *L. bulgaricus*, and *Lactobacillus rhamnosus*. Lastly, a yogurt will be created with all 4 bacteria and the same measurement will be taken, as previously described.

With all of these yogurts, the bacteria in them must be able to subculture onto another plate from same type of plate they were taken from, coming from the yogurt culture. This is to make sure that these bacteria can grow for generations to come. We will create frozen stocks of all five bacteria from our pure, isolated colonies on the subcultured plates. Gram staining and colony morphology and characteristics will also be important tools to use in making sure that the bacteria on the plate are our desired bacteria.

We will grow and isolate colonies from all five bacterial types, according to the work of Tharmaraj and their study. *Streptococcus thermophilus* agar (ST agar) will be used to isolate *S. thermophilus*, which will have tiny (0.1–0.5 mm) colonies at 37 degrees Celsius by aerobic incubation after 24 hours. Likewise, *Bifidobacteria* will be isolated and enumerated on *Bifidobacterium* agar. *Lactobacilli* MRS (de Man, Rogosa and Sharpe) agar when the pH of MRS agar was reduced to 4.58 and the incubation temperature increased to 45 degrees Celsius, *L. rhamnosus*, which will form shiny smooth white colonies. On the same media, *L. acidophilus* will theoretically form colonies that are 0.1 to 0.5 mm in size, brown, and rough and irregular. MRS agar and anaerobic incubation (using anaero packs) at 43 degrees Celsius could be used to enumerate *L. acidophilus* by itself. If we are unable to isolate and select for the bacteria based on these methods, we will consult relevant literature (e.g. Dave and Shah, 1996).

Throughout this study, it will be crucial to use proper aseptic techniques. For example, for yogurt production, a 1000mL beaker will be autoclaved, we will measure powdered milk into this sterile beaker. Bacterial cultures will be measured into sterile falcon tubes, using a scoopula that was dipped in alcohol and flamed off. Gloves will always be worn and tools will all be either autoclaved or sterilized with fire.

References:

1. Al-Salami, H., Butt, G., Tucker, I., Skrbic, R., Golocorbin-Kon, S., and M. Mikov, "Probiotic Pre-treatment Reduces Gliclazide Permeation (ex vivo) in Healthy Rats but Increases it in Diabetic Rats to the Level Seen in Untreated Healthy Rats," *Arch Drug Info*, 1(2008)35-41.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2817444/>>

This publication looked at the pre-treatment with probiotics on the permeation of the antidiabetic drug gliclazide in both healthy and diabetic rats. This publication was found using National Center for Biotechnology Information Database.

2. "BD Bifidobacterium Agar, Modified." (n.d.): n. pag. BD, June 2013. Web. 2 Oct. 2015. Retrieved From:

<https://www.bd.com/europe/regulatory/Assets/IFU/HB/CE/PA/PA-254546.pdf>>

This publication provided us with a description of the medium that we will use to isolate *B. lactis* from the other strains of bacteria we will be using us. This source provided us with the recipe to make the medium. This source was found using BD's product list.

3. Cani, P.D., Neyrinck, A.M., Fava, F., Knauf, C., Burcelin, R.G., Tuohy, K.M., Gibson, G.R., and N.M. Delzenne, "Selective Increases in Bifidobacteria in Gut Microflora Improve High- Fat-Induced Diabetes in Mice through a Mechanism Associated with Endotoxaemia," *Diabetologia*, 50(2007)2374-2383.

<http://link.springer.com/article/10.1007%2Fs00125-007-0791-0>>

This publication looked at the effects that a high-fat diet can lead to increased endotoxaemia and reduce *Bifidobacterium* species in mice. The source discussed how the gut microbiota can set the tone of inflammation for diabetes or obesity. The source was found through the UW web of knowledge and was cited and has been cited 392 times.

4. Dave, R.I., and N.P. Shah, "Evaluation of Media for Selective Enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, and Bifidobacteria," *Journal of Dairy Science*, 79.9(1996)1529-1536.

<http://www.sciencedirect.com/science/article/pii/S002203029676513X>>

This publication provides techniques used for the isolation and enumeration of *S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, and *Bifidobacterium* species. The source was found through the UW web of knowledge and has been cited 277 times.

5. Diamant, M., Blaak, E.E., and W. M. de Vos, "Do Nutrient-Gut-Microbiota Interactions Play a Role in Human Obesity, Insulin Resistance, and Type 2 Diabetes?" *The Obesity Reviews*, 12:4(2010)272-281.

<http://www.ncbi.nlm.nih.gov/pubmed/20804522>>

This publication reviewed the evidence that the gut microbiota is different in obese and lean people and in diabetic and non-diabetic individuals. They looked at the potential benefit of

prebiotic and probiotics to help modify the gut microbiota composition. This publication was found through the UW web of knowledge and has been cited 35 times.

6. Ejtahed, H.S., et. al. (2011). "Effect of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* on lipid profile in individuals with Type 2 diabetes mellitus." *Journal of Dairy Science*. Retrieved from:

<<http://www.sciencedirect.com/science/article/pii/S0022030211003213>>

This publication looked at study where Type 2 diabetics ate either 300 grams of probiotic yogurt, containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* or conventional yogurt daily for six weeks. They evaluated their lipid profile before and after consuming the yogurt. This publication was found through the UW web of knowledge and had been cited 68 times.

7. Hartstra, Annick, et. al. (2015). "Insights Into the Role of the Microbiome in Obesity and Type 2 Diabetes." *American Diabetes Association*. Retrieved from:

<<http://care.diabetesjournals.org/content/38/1/159.long>>

This publication discusses the relationship between obesity and the composition of the intestinal microbiome. From this article we were able to get insight into how the microbiome can influence insulin sensitivity in Type 2 diabetics with increased intestinal microbial diversity. This publication was found through the UW web of knowledge and has been cited 100 times.

8. Kuperman, A.A., and O. Koren, "The Era of Fecal Microbiota Transplantation," *The Israel Medical Association Journal*, 17(2015)515-516.

<<https://www.ima.org.il/FilesUpload/IMAJ/0/164/82282.pdf>>

9. Larsen, Nadja, et. al. (2010). "Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults." *PLOS ONE*.

< <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0009085>>

This publication compared the intestinal microbiota in humans with Type 2 diabetes and humans without diabetes. This particular study used fecal samples to assess the bacterial composition of each participant. This publication was found using National Center for Biotechnology Information Database and has been cited 172 time.

10. Larsen, N., Vogensen, F.K., van der Berg, F.W.J., Nielsen, D.S., Andreasen, A.S., Pedersen, B.K., Al-Soud, W.A., Sorensen, S.J., Hansen, L.H, and M. Jakobsen, "Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults," *PLoS One*, 5:2(2010)1-10.

<<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0009085>>

This publication studied the composition of the intestinal microbiota in humans with Type 2 diabetes and compared it with non-diabetic persons. They used PCR to compare fecal samples of patients with a broad range of age and BMIs. This publication was found through the UW web of knowledge and has been cited 384 times.

11. Panwar, H., Rashmi, H. M., Batish, V. K. and Grover, S. (2013), "Probiotics as potential biotherapeutics in the management of Type 2 diabetes – prospects and perspectives." *Diabetes Metab. Res. Rev.*, 29: 103–112.

<<http://onlinelibrary.wiley.com/doi/10.1002/dmrr.2376/citedby>>

This publication shows how the different probiotics affect the different aspects of diabetic health. Table one was the most beneficial showing the anti-diabetic efficacy of probiotic interventions in animal models. From this publication we were able to select the probiotics to add to the yogurt. This source was provided by our professor and has been cited 17 times.

12. Shah, N.P., and W.E.V. Lankaputhra, "Improving Viability of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in Yogurt," *International Dairy Journal*, (1997)349-356.

<<http://www.sciencedirect.com/science/article/pii/S095869469700023X>>

This publication explored the viability of probiotic bacteria cells in yogurt that were either whole or had been ruptured. They looked for viable cells after two log cycles and again after 6 weeks. It was concluded that the viability was not affected whether the cells were ruptured or whole. This publication was found through the UW web of knowledge and has been cited 60 times.

13. "Streptococcus thermophilus Isolation Agar." Fluka Analytical Chemistry Anal. Chem. 59.6 (1987): n. pag. Sigma-aldrich. Web. 1 Oct. 2015.

<<http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Fluka/Datasheet/17257dat.pdf>>

This publication provided us with a description of the medium that we will use to isolate *S. thermophilus* from the other strains of bacteria we will be using. This source provided us with the recipe to make the medium. This source was found using Sigma-Aldrich's product list

14. Tharmaraj, N., Shah, N.P. (2003) "Selective Enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacteria*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Propionibacteria*" *Journal of Dairy Science*, Volume 86, Issue 7, July, Pages 2288–2296

<<http://www.sciencedirect.com/science/article/pii/S0022030203738211>>

This publication is the source of several media making techniques. There is specific growth techniques listed for *Lactobacillus* and *Bifidobacteria*. The source was found through the UW web of knowledge and had been cited 122 times.

15. "What Complications (Problems) Are Caused by Diabetes?" New York State Department of Health, Jan. 2015. Web. 1 Oct. 2015.

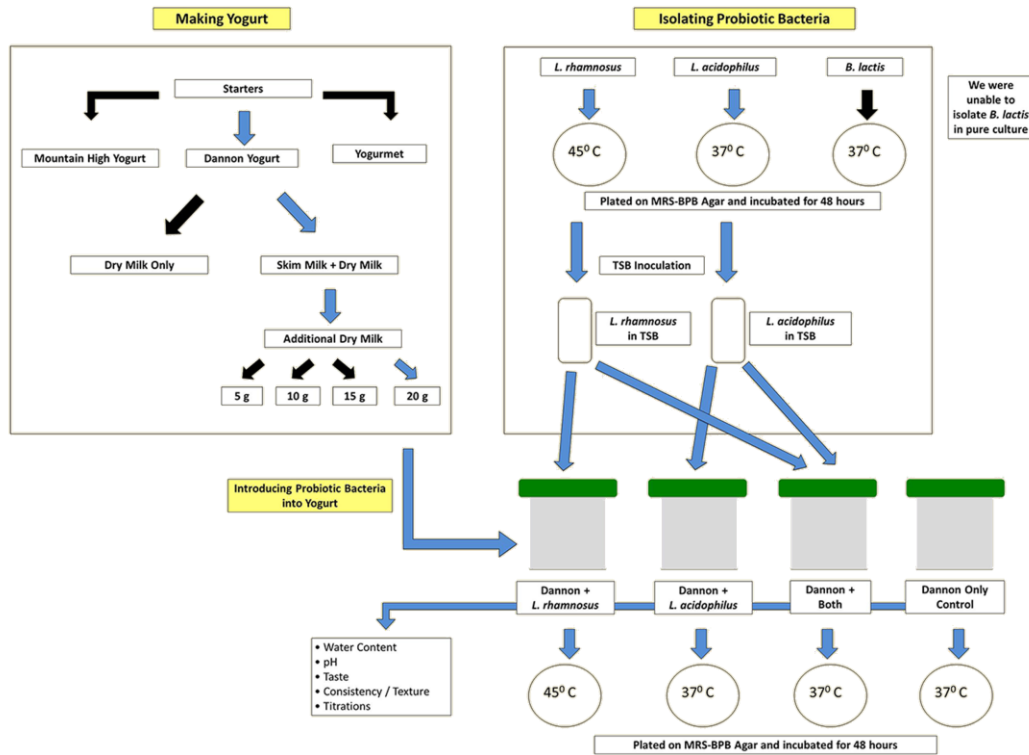
<http://www.health.ny.gov/diseases/conditions/diabetes/problems_caused_by_diabetes.htm>

This publication describes complications of mismanaged Type 2 diabetes. The source specifically discusses complications that arise from high blood sugars which is more common than low blood sugars in Type 2 diabetics.

16. Ashraf, Rabia and Shah, P. Nagendra. 2011. “Selective and differential enumerations of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium* spp. in yoghurt — A review”
<<http://www.sciencedirect.com/science/article/pii/S0168160511004004>>

17. <http://aboutyogurt.com/index.asp?bid=5>
Requirement for the Yoplait active yogurt label

Results of the lab research:



Figures Page:

Figure 1: Introducing Probiotic Bacteria to Yogurt. This figure shows the process we went through to make our



yogurt and to introduce different probiotic bacteria into our yogurt.

Figure 2 (left): Yogurt Mass With Addition of Probiotic Bacteria This graph shows the changes of mass that occur when drying our yogurt in a 55°C oven for 72 hours for yogurt containing the probiotic bacteria *L. rhamnosus* and *L. acidophilus* to determine the water content of each treatment group

Figure 3 (right): Probiotic Bacteria Concentrations in Yogurt This graph looks at the enumeration of *S. thermophilus*, *L. acidophilus*, and *L. rhamnosus* on MRS-BPB plates from yogurt with variable bacteria. Dannon + *L. rhamnosus* was incubated at 45° Celsius to select for growth of *L. rhamnosus* and inhibit *S. thermophilus*. All other plates were incubated at 37°C.

* *S. thermophilus* was not grown on MRS-BPB from Dannon + *L. rhamnosus*. The expected growth was predicted from other values.

Results:

- We were able to isolate *S. thermophilus*, *L. acidophilus*, and *L. rhamnosus* in pure cultures. We were not able to isolate *L. bulgaricus* and *B. lactis* in pure culture.
- Yogurt produced with only dry milk proved to be more runny than the desired store-bought yogurt consistency
- Even on the same MRS+ BPB plate, all 3 bacteria (excluding the *Bifidobacteria* that we could not isolate, as well as *L. bulgaricus*) have distinct colony morphologies (Table 1).
- The addition of both *L. rhamnosus* and *L. acidophilus* did not change the pH (ranging from 4.16 to 4.19), water content (ranging from 59% to 65%) (Fig. 2), nor the concentration of *S. thermophilus* (ranging from 1.68×10^6 to 2.08×10^7 cells/mL) (Fig. 3)
- The concentrations of *L. rhamnosus* and *L. acidophilus* were higher when they were added together compared to separately (Fig. 3)

Discussion:

Our data indicates *L. acidophilus* and *L. rhamnosus* can be added to yogurt without dramatically changing the microbial titers, pH, or moisture content. The concentration of *S. thermophilus* growth being similar in all treatment groups shows that the bacteria are able to grow together without inhibition. Increased concentration of *L. acidophilus* and *L. rhamnosus* when added together in yogurt compared to inoculation with just one bacterium suggests that there is a synergistic effect between the bacteria. The Dannon yogurt we used had a pH of 4.1, causing the yogurt we produced to have an average pH of 4.1 from lactic acid production. Because our control yogurt had a pH similar to the yogurt inoculated with *L. acidophilus* and *L. rhamnosus*, we believe that these probiotic bacteria had limited effects on the pH.

Future studies would include trying to introduce the probiotic bacterium *B. lactis*, which also has beneficial characteristics for individuals diagnosed with Type 2 diabetes. Due to time constraints, we were unable to isolate the bacterium from a probiotic pill which also possessed other *Lactobacilli* species. *Bifidobacterium* is an anaerobic bacterium, which we attempted to grow aerobically, explaining our adversity of isolating the bacterium. We were likewise unable to determine concentrations of *L. bulgaricus*, but the concentration must be equal to or higher than 10^6 cells/mL in accordance with yogurt laws passed by the FDA (16). *L. bulgaricus* likely formed tiny, white colonies that could not be distinguished easily from *S. thermophilus* on MRS + BPB (4). This strain was difficult to isolate and enumerate and could only be accomplished with Lactobacillus Streptococcus Agar.

Conclusion:

Our experiment demonstrated the possibility of adding probiotic strains to yogurt, based on quantitative and qualitative analysis of the yogurt. We have titrations after 24 hour yogurt production that met 10^6 cells per gram of yogurt.

Our probiotic yogurt recipe for patients with Type 2 Diabetes at the Downtown Clinic:

2 cups of yogurt: 1 $\frac{3}{4}$ cup Skim Milk, 4 Tsp Dry Milk, 4 Spoonful's of Dannon Yogurt (Can be replaced by other yogurt including homemade yogurt that has been made)

Addition of other beneficial probiotics for Type 2 diabetes can involve:

- 1 pill of NatureMade *L. acidophilus* and 1 pill of Swanson's probiotics *L. rhamnosus*

*Although our research shows that *L. acidophilus* and *L. rhamnosus* did not affect the yogurt we produced. The pills we suggest are not pure cultures and the concentrations of bacteria and other bacteria present may alter results.

Continued Research:

Through the above experimentation we were able to create a probiotic yogurt that was catered to the gut microbiome of patients diagnosed with Type II Diabetes. I started volunteering at the Downtown Clinic to better understand how the largely volunteer-run organization functions and to better understand the clientele. In order to next implement our lab research findings within the Albany County Community, more research was required. The next step of the project was to evaluate the possibility of patients being able to create the yogurt at home. This consisted of taking the recipe and trying different ways of creating yogurt through various cost-effective methods. This involved looking at different way to incubate yogurt including using a at-home yogurt incubator (\$20), towel wraps or tin foil wraps, incubation in the microwave, a crockpot, in the oven, and in a cooler/ice chest. These all created a quality yogurt with a good curd. I worked with different yogurt starters (Dannon and Great Value) and different low fat milks. Many controls were used with testing of all variable, which were tested one at a time.

The most important experimentation revolved around the probiotic yogurt culture. I had difficulty making good yogurt with added *L. acidophilus*. This could have been due to the fact that the only plain yogurt available in the store already containing live cultures of *L. acidophilus*. For this reason, it was decided that the starter culture purchased at a store for the recipe should contain: *L. acidophilus*, *S. thermophilus*, and *L. bulgaricus*, instead of just containing the later two bacteria. The next variable tested was adding *L. rhamnosus*. Experimentation demonstrated that adding an entire capsule of Swanson *L. rhamnosus* was far too much. This is largely due to the initial concentration in the capsule being 5 billion cfu. These variables all came together to provide a modified yogurt recipe which is as follows:

The recipe (after modification):

-3 $\frac{1}{2}$ Cups Skim Milk -3 Tbs dry milk

-1/4 Cup of starter Yogurt (containing Live Cultures including *Lactobacillus acidophilus*)

-1/4 capsule of *Lactobacillus rhamnosus*

This recipe, along with a comprehensive step-by-step guide to making yogurt was the next step to disseminating the yogurt recipe. This was done in the format of a brochure, incorporating information about the gut and probiotics, resources available in Laramie (such as the Laramie Soup Kitchen and Interfaith Good Samaritan), as well as an important survey done by Claire Dinneen. This survey involved 53 patients of the Downtown Clinic who were asked important questions regarding probiotics. They were asked if they had ever taken probiotics, what they knew about probiotics, if they thought probiotics would reduce gastrointestinal disease and other illnesses, whether they preferred to take probiotics in the form of a food or as a pill, and the possibility of changing one's diet to accommodate what a doctor desires for their patient. This actually largely affected the start of this entire probiotic yogurt project. Not that a brochure has been created, it is my goal to make it clear and concise.

The next components to this project are talk to Ann Marie Hart at the Downtown Clinic to evaluate if the brochure is patient-centered. Also, John Willford will help better incorporate Claire's research into a more legible format. The brochure will then be translated in Spanish due to a large amount of Downtown Clinic patients being Spanish-speaking. Yogurt demonstrations this summer may also help to teach clients how to make yogurt. Lastly, it may be possible to team up with the Laramie Soup Kitchen to have a volunteer make yogurt for their clients.

My continued research beyond the lab has helped to take the next steps, where lab research meets practicality. Through the holistic approach with research and education, we hope to make a positive impact in fighting food insecurity and Type II Diabetes in Albany County. Thank you for taking the time to read about this project. The brochure can be found on the next page.

For more information, feel free to contact Caleb Brackett: cdwbrackett@gmail.com

The Brochure:

DOWNTOWN CLINIC PATIENTS

Research was done with 53 patients at the Downtown Clinic by Claire Duggan to see what the average patient knew about probiotics and live cultures. Here is what you all said:

1. Only 13% had taken probiotics
2. 53% said they were somewhat knowledgeable about probiotics and live cultures
3. 80% believed that taking probiotics could reduce gastrointestinal illness
4. 58% said that primary healthcare providers have never talked to them about probiotics
5. 66% said they would rather take a probiotic food than a capsule or pill
6. 53% were impartial in the belief that it was important to take probiotics to prevent illness
7. 53% said a great majority of their meals are prepared by themselves or a member of the household



With this in mind, yogurt will be a beneficial way to incorporate beneficial probiotics into your diet. Making yogurt could fit into your everyday schedule, and it will provide a delicious breakfast or snack.

Additional Resources:

Yogurt and health:

<http://www.webmd.com/diet/benefits-of-yogurt> <https://en.wikipedia.org/wiki/Yogurt>
http://www.medscape.com/viewarticle/820273#vp_2

Making yogurt:

<http://www.epicurious.com/archive/howtocook/primers/homemade-yogurt>

Need Food?

Interfaith Good Samaritan
(307) 742-4240

Laramie Soup Kitchen: Open 11:30am-1pm Mon.-Sat.



611 S. 2nd St, Laramie, WY 82070

Phone (307) 745-8445

[http://](http://www.downtownclinic.or)

www.downtownclinic.or

Type II Diabetes & Probiotic Yogurt: Your Resource Guide



In Collaboration with the University of Wyoming College of Agriculture, Microbiology Capstone Class

Caleb Brackett, Rachel Watson, Daulton Grube, Callie Wilson, Jennifer Rife,

BACTERIA & YOU

You are not alone with your Type II Diabetes diagnosis. The Centers for Disease Control and Prevention found that approximately 29.1 million people in the United States have diabetes. At the Albany County Downtown Clinic (DTC), about one-third of the patients seen by the nurses have Type 2 diabetes. Improper maintenance of diabetes can damage blood vessels, nerves, and organs. This could lead to heart disease, stroke, damaged vision, and kidney disease, among other serious conditions.



Bacteria are everywhere, including in our gut, which contains billions of bacteria. These small microorganisms help with digesting foods and

many other functions,



including prevention of certain diseases. Taking a probiotic, or a dietary supplement that contains live bacteria (yogurt) can replace good bacteria that are missing from your gut. Two such bacteria are

Lactobacillus rhamnosus and *Lactobacillus acidophilus*. University researchers demonstrated that patients who take these bacteria demonstrate a decrease in fasting blood glucose and insulin resistance, and improved antioxidant status. *Lactobacillus acidophilus* also improves bad cholesterol.

The following yogurt recipe and instructions will help you to make quality yogurt that will provide a delicious meal or snack, while also helping control your diabetes!

1 QUART YOGURT RECIPE:

- 3 1/2 Cups Skim Milk
- 3Tbs dry milk
- 1/4 Cup of starter Yogurt (containing Live cultures including *Lactobacillus acidophilus*)
- 1/4 capsule of *Lactobacillus rhamnosus*



THE PROCESS

1. On a stove, heat milk to 180°F, just below boiling. This effectively kills off unwanted bacteria in the milk. Stir while heating to prevent scalding. Add dry milk while the milk is hot.
2. Let the milk cool to 108-112°F (or until a spoonful no longer feels too hot on the tongue). While the milk is cooling: add yogurt and probiotic pill to a separate bowl and mix with a half cup of warm milk to the bacteria to get the bacteria ready.
3. Add the yogurt to the milk and gently mix. Then add this mixture to a container (Mason jar, circular Pyrex dish, etc.) that was cleaned very well with hot water and soap.

4. Incubate 8-12 hours to desired thickness in a yogurt incubator (or other incubator options below). Longer incubation means thicker, larger yogurt. The incubator needs to be around 100°F for the entire incubation time. Refrigerate to stop the process.

Tips: An ice bath will cool milk down faster (optional). Also, remove skim or burnt milk before pouring into your glass to be incubated. You can use a yogurt product you created to start a new batch! Lastly, do not disturb incubation!

INCUBATOR OPTIONS

Preferred method: purchase a \$20 yogurt incubator from Amazon / Walmart. Turn it on and then turn it off when time is up (set an alarm).

Warm Towel Wrap or Microwave: (Milk can be heated up in the microwave instead of on the stove). For incubation, fill a water bottle with hot water from your faucet (this will be wrapped alongside your container with yogurt). Wrap both of these together with a towel and let incubate on your counter or in the microwave (while off). You may need to cover your yogurt container with a lid (but don't seal it). Also, a tin foil wrap may work better than a towel.

Oven: Heat milk to 180 and then set oven to 110°F. Make sure <http://www.kitchenaid.com> is oven-safe.

Crockpot: Heat to 180 and cool to ~110. Turn it off or keep it on low. Wrap the crockpot with a towel.

Camping cooler/ice chest: Create either a hot water bath or fill water bottles/jugs with hot water and place them in the cooler alongside your soon-to-be yogurt. Close the lid.

*Processes and incubators may need to be adjusted to create the best possible yogurt.