Received: 24 July 2020 | Revised: 27 September 2020 | Accepted: 2 October 2020

DOI: 10.1002/mgg3.1535

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

Microbiota profile and efficacy of probiotic supplementation on laxation in adults affected by Prader-Willi Syndrome: A randomized, double-blind, crossover trial

Zainab Alyousif[1] | **Jennifer L. Miller**[2] | **Jeremie Auger**[3] | **Mariana Sandoval**[3] | **Amanda Piano**[3] | **Thomas A. Tompkins**[3] | **Wendy J. Dahl**[1]

[1]Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, USA

[2]Division of Endocrinology, Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL, USA

[3]Rosell Institute for Microbiome and Probiotics, Montreal, QC, Canada

Correspondence
Wendy Dahl, Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, USA.
Email: wdahl@ufl.edu

Funding information
This study was funded by Lallemand Health Solutions Inc. (University of Florida grant AWD03033) and in-kind support from the Department of Food Science and Human Nutrition, University of Florida/Institute of Food and Agricultural Sciences. ZA received a doctoral scholarship through the King Abdullah Scholarship Program, Saudi Arabian Cultural Mission (SACM).

Abstract

Background: Probiotics may provide a benefit for adults with Prader-Willi syndrome (PWS) experiencing constipation. The primary aim was to determine if *Bifidobacterium animalis* ssp. *lactis* B94 (*B. lactis* B94) improves stool frequency, with secondary aims of stool form and gastrointestinal symptoms. Exploratory aims included diet quality and fecal microbiota composition.

Methods: Following a 4-week baseline, 25 adults with PWS were randomized to consume *B. lactis* B94 by capsule (15 billion) or placebo for 4 weeks, followed by 4-week washout in a double-blind, crossover design. Stool frequency and Bristol Stool Form (BSF) were assessed daily, and Gastrointestinal Symptom Rating Scale (GSRS) and dietary intake (7-days food records), per period. Fecal microbiota per period was analyzed using 16S rRNA gene amplicon sequencing and taxa of interest by qPCR ($n = 24$).

Results: No adverse events were reported. Stool frequency at baseline ($n = 25$; 2.0 ± 0.1 stools/day), GSRS syndromes, and microbiota composition did not differ with the probiotic intervention overall; however, a delayed, carry-over effect on BSF types 6 and 7 was seen. Diet quality by HEI-2015 was 65.4 ± 8.5.

Conclusion: In adults with PWS, *B. lactis* B94 exhibited little effect on laxation over 4 weeks; however, further research is needed.

KEYWORDS

Bristol Stool Form, constipation, HEI-2015, Prader-Willi syndrome, probiotic, stool frequency

1 | INTRODUCTION

Prader–Willi syndrome (PWS) is a rare genetic syndrome presenting with altered body composition, reduced energy expenditure, and hyperphagia, typically leading to obesity (Alsaif et al., 2017). The energy requirements of individuals with PWS are thought to be 3040% less than people without the syndrome (Alsaif et al., 2017). The recommendation for energy intake in PWS for ensuring weight maintenance is typically around 1200 kcal/day, while to achieve weight

reduction, the recommended range is 800-1000 kcal/day (Butler et al., 2019). Given their lower energy requirement in combination with dysfunctional satiation and food seeking behavior (Martinez Michel et al., 2016), individuals affected by PWS require restricted access to food to prevent obesity and ensure their safety and wellbeing. Individuals with PWS prefer quantity over quality once they develop hyperphagia because having an increased appetite in the face of limited calorie intake is extremely difficult to live with. However, limiting energy-dense foods may not necessarily result in healthful, nutrient-dense food choices. Of particular concern is the possibility of low fiber intake, which may contribute to constipation (Mitsuhashi et al., 2018). Constipation symptoms, including less than three defecations per week, sensation of anorectal obstruction, straining during defecation, and having hard stools, have been reported in as many as 40% of adults with PWS (Kuhlmann et al., 2014). Fiber intake of children affected by PWS has been reported to fall below recommendations (Mackenzie et al., 2018), as has the fiber intake of the adult PWS population (Woods et al., 2018).

Although implementing a high fiber diet may seem a prudent approach to improving laxation in adults with PWS, whether or not low fiber intake is associated with constipation in this syndrome is not known. Probiotics have been suggested as a potential therapeutic agent for alleviating symptoms of constipation through regulating motility, although specific mechanisms remain unclear (Dimidi et al., 2017). Certain probiotics have been shown to increase stool frequency and improve stool consistency in adults with functional constipation, and some evidence suggests that *Bifidobacterium animalis* ssp. *lactis* strains may be effective (Dimidi et al., 2014). Thus, probiotics may offer a safe and potentially effective means for alleviating symptoms of constipation in the PWS population. Hence, the aims of the study were to determine the effects of *B. lactis* B94 on stool frequency, stool form, and gastrointestinal symptoms in adults with PWS. As *Bifidobacterium animalis* ssp. *lactis* B94 (*B. lactis* B94) has been shown to improve constipation in children with irritable bowel syndrome (Basturk et al., 2016), it was hypothesized that *B. lactis* B94 would increase stool frequency, decrease the percentage of hard stools (suggestive of slow transit), and thereby, improve gastrointestinal symptoms in adults affected by PWS. Exploratory aims were to assess diet quality and fiber intake, as well as assess the effect of the intervention on microbiota profile. As *Akkermansia muciniphila* (Derrien et al., 2017) and *Fecalibacterium prausnitzii* (Ferreira-Halder et al., 2017) are considered by some to be next-generation probiotics (Martin & Langella, 2019), and protective *Bifidobacterium* spp. are suppressed in constipation (Khalif et al., 2005), these three taxa were quantitatively explored.

2 | METHODS

2.1 | Ethical compliance

The study was approved by the University of Florida Institutional Review Board 1 (IRB201701976, version 3). Study procedures were carried out in accordance to the Declaration of Helsinki. All subjects provided written informed consent. The study is registered at clinicaltrials.gov (NCT03277157).

2.2 | Study design

A 20-week-, randomized, double-blind, placebo-controlled crossover study was carried out in Florida, United States. Participants were recruited in May and June 2018 with the trial per se concluding in October 2018. Details of the study protocol and sample size determination are reported elsewhere (Alyousif et al., 2018). In brief, we recruited 25 adults with genetically confirmed PWS, who were living in PWS-specialized residential care, where food access was strictly controlled, and dietary intake was closely monitored and recorded. Randomization was by sealed envelope method prepared by an individual not otherwise involved with the study. Participants, researchers, and the statistician were blinded until the completion of the analysis. Participants completed a 4-week baseline period and were randomized on Day 29 to consume one capsule per day containing 15 billion *B. lactis* B94 or placebo for 4 weeks, followed by a 4-week washout, 4 weeks on the alternative treatment, and a second 4-week washout.

2.3 | Gastrointestinal outcomes

As previously described (Alyousif et al., 2018), subjects completed a daily record of stool frequency, Bristol Stool Form Scale (BSF) (Lewis & Heaton, 1997), and compliance to capsule intake (during intervention periods). The Gastrointestinal Symptom Rating Scale (GSRS) (Kulich et al., 2008) was administered at the end of each study period.

2.4 | Dietary intake and diet quality

Dietary intake was assessed during weeks 4, 8, 12, 16, and 20 from 7-day food records and analyzed using Food Processor Nutrition Analysis Software (ESHA version 11.3.2). Nutrient intakes of the group were compared to their respective Estimated Average Requirement (EAR) to assess risk of inadequacy (Institute of Medicine, 2005). Food group intakes were compared to the 2015–2020 Dietary Guidelines

of Americans MyPlate recommendations for an energy level of 1400 kcal/day (USDHHS & USDA, 2015). Diet quality at baseline was assessed using the Healthy Eating Index (HEI) 2015 (Krebs-Smith et al., 2018).

2.5 | Microbiota composition

Single stools were collected at the end of each 4-week period and stored at −80°C. Total DNA was extracted from stool samples using the QIAmp Fast DNA Stool Mini Kit (Qiagen) with previously described modifications (Ford et al., 2020). Extracted DNA was used for the absolute quantitation of *B. animalis* to ensure participant compliance and relative abundance of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Bifidobacterium* genus as microbes of interest. Detailed Real-Time PCR methods were previously reported (Ford et al., 2020). In brief, Real-Time PCR reactions were prepared with a final volume of 10 µl, including 300 nM of both forward and reverse primers (Table S1), 1X SYBR Select Master Mix (Thermo Fisher Scientific) and 1 µl of five-fold diluted DNA. Mastermix and DNA was added to a 384-well plate using the epMotion 5075tc liquid handling robot and plates were analyzed on the CFX384 Touch Real-Time PCR Detection System (Bio-Rad).

The bacterial DNA extracted from the fecal samples was also used for community-wide taxonomic profiling via 16S amplicon sequencing. The libraries were assessed for quantity and quality of DNA and indexed in a second PCR for multiplexing, following Illumina's protocols. The parallel sequencing was performed on a MiSeq platform using the V3 chemistry kit with 2 × 250 bp reads. The fastq files containing 9,883,101 sequences from 122 samples (median 80,368 sequences per sample) were exported for bioinformatics analyses. The forward reads were inspected for quality and trimmed at 240 pb (since the quality remained high throughout). The reads were quality filtered using QIIME™'s dedicated module and were used to generate (cluster the sequences) and count the amplicon sequence variants (ASVs) present in each sample (Bolyen et al., 2019). Taxonomic profiles were generated for each sample by taxonomic attribution of the ASVs using the QIIME™ 2 feature-classifier machine learning based tool and the database, GreenGenes (DeSantis et al., 2006). The taxonomic profiles for Baseline, Probiotic, Placebo, and Washout periods were generated and compared globally on group averages and on individual taxa and strains.

Using QIIME™'s visualization tools alpha diversity profiles, including Pielou's evenness, Faith's phylogenetic diversity, Shannon diversity index, observed Operational Taxonomic Units (OTUs), and individual taxonomic profiles were generated and examined (Amir et al., 2017; Bokulich et al., 2013; Bolyen et al., 2019; Caporaso et al., 2010; Chang et al., 2011; Chen et al., 2012; Halko et al., 2011; Katoh & Standley, 2013; Legendre & Legendre, 2012; Lozupone et al., 2007; Lozupone & Knight, 2005; McDonald et al., 2012, 2018; McKinney, 2010; Price et al., 2010; Stackebrandt & Goodfellow, 1991; Vázquez-Baeza et al., 2013, 2017; Weiss et al., 2017).

2.6 | Statistical analysis

For the outcomes of stool frequency, stool form, gastrointestinal symptoms, and nutrient and food groups intakes, data were analyzed using a general linear mixed model with treatment and sequence as the main fixed effects, and where treatments were baseline, probiotic, probiotic washout, placebo, and placebo washout. Differences in an individual's measurements were accounted for by treating individual as a random effect, and to account for repeated measurements of the same individual, an autoregressive correlation structure was included. Alpha was set at 0.05. Data are presented as mean ± SEM unless otherwise indicated. Kruskal-Wallis test was used to compare alpha-diversities.

3 | RESULTS

The study flow diagram is presented as Figure 1. Of the 28 participants consented, 25 completed the 20-week study. One participant withdrew prior to completing any study procedures due to lack of interest, and during the baseline, two participants were withdrawn due to non-compliance. No adverse events were reported. Participant characteristics are shown in Table 1. Mean body weight did not change over the duration of the study.

3.1 | Gastrointestinal outcomes

The primary outcome, stool frequency and secondary outcome, gastrointestinal symptom syndrome scores by period, are presented in Table 2. Stool frequency during the baseline averaged 2.0 ± 0.1 stools/day and ranged from 0.3 to 5.9 stools/day; there were no significant differences reported between periods. No significant treatment effects were found for gastrointestinal symptom syndromes of the GSRS, with the mean scores for abdominal pain, reflux, indigestion and diarrhea syndromes reported at a mean of <2 out of 7, considered to be below clinical significance, with the exception of constipation. Twelve participants reported mild to moderate discomfort related to constipation at baseline. An additional secondary outcome, stool form, in percentages of types 1 and 2, types 3, 4 and 5, and types 6 and 7, is presented in Table 2. At baseline, stool form types 1 and 2 were reported at 31.3% and showed no

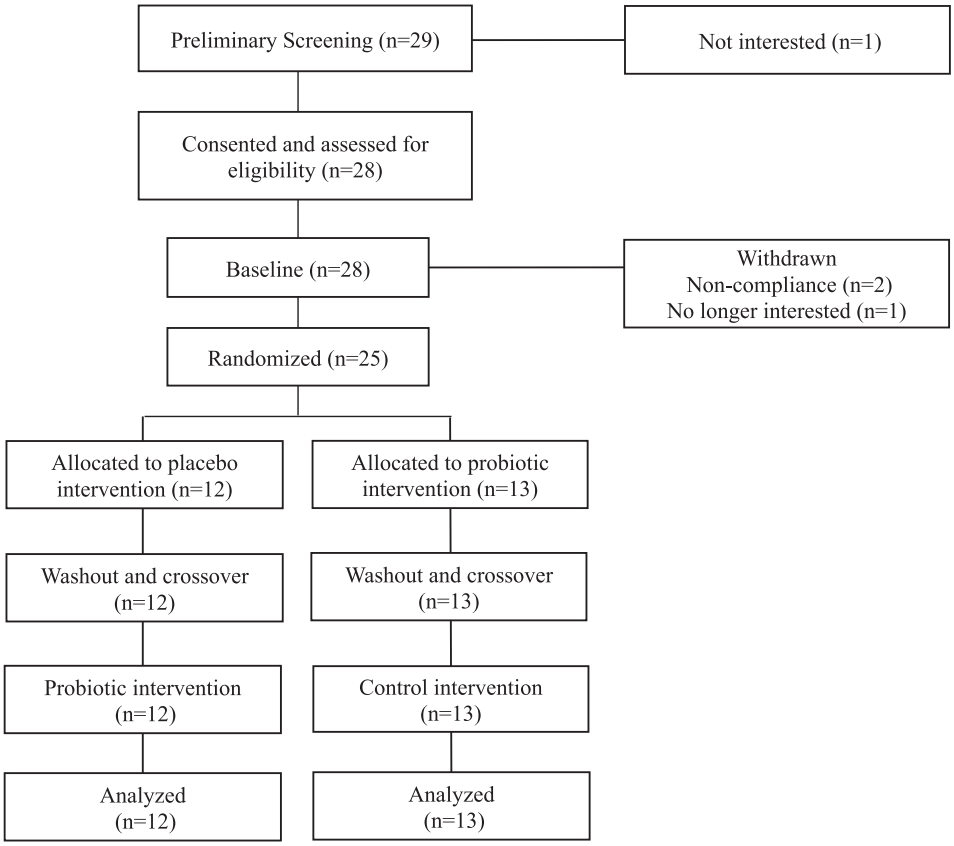

FIGURE 1 Participant recruitment, randomization, and study flow

TABLE 1 Characteristics of adult study participants with Prader-Willi syndrome

	***n* = 27**
Gender (M/F), *n*	12/15
Age, mean ±SD (range)	34.9 ± 10.2 (19–56)
Race, n (%)	
White	23 (85.2)
Black or African American	1 (3.7)
American Native	1 (3.7)
More than one race	2 (7.4)
Ethnicity, n (%)	
Hispanic	2 (7.4)
Not Hispanic	25 (92.6)
	n = 25
BMI, kg/m^2 mean ± SD	30.2 ± 6.3
BMI categories, n (%)	
Normal (18.5–24.9)	3 (12)
Overweight (25–29.9)	13 (52)
Obese (more than 30)	9 (36)

Abbreviation: BMI, body mass index

significant differences between periods. However, types 6 and 7 were higher in the probiotic washout compared to the placebo, and specifically for participants randomized to the placebo first, there was a significant treatment × sequence effect for percentage of stool form types 6 and 7. Placebo + placebo washout differed from probiotic + probiotic washout ($p < 0.0004$). For the period comparisons of the group receiving the placebo first, the probiotic washout differed from the placebo ($p < 0.01$) and placebo washout ($p < 0.0001$) periods for stool form types 6 and 7. Differences were not seen for those participants receiving the probiotic first.

3.2 | Dietary intake

Participants consumed a diet providing 43%, 34%, and 23% of energy from carbohydrate, fat and protein, respectively. Nutrient and food group intakes for each period are presented in Table S2. No significant differences between periods were observed for food groups, energy, macronutrients, or select micronutrients (calcium, iron, vitamin D, potassium and sodium). Protein intake exceeded the Recommended Dietary Allowance (RDA) in all but one

TABLE 2 Stool frequency, stool form, and gastrointestinal symptom syndrome scores by period of adults affected by Prader-Willi Syndrome

	Baseline	Probiotic	Probiotic washout	Placebo	Placebo washout	*p*-value P × S	*p*-value T × S
Stool frequency (stools/week; mean ± SE)							
	13.7 ± 1.0	17.9 ± 1.3	17.6 ± 1.3	15.7 ± 1.1	17.3 ± 1.3	0.46	
BSF (%)							
Types 1 and 2	31.3	26.1	21.1	26.4	28.0	0.36	0.07
Types 3, 4 and 5	54.7	56.6	51.9	63.6	59.6	0.36	0.47
Types 6 and 7	14.0^{ab}	17.2^{ab}	27.0^{a}	10.0^{b}	12.4^{ab}	0.007	0.01
GSRS syndromes (mean ± SE)							
Abdominal pain	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	0.2	
Reflux	1.4 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	0.5	
Indigestion	1.9 ± 0.3	1.5 ± 0.2	1.4 ± 0.2	1.5 ± 0.2	1.3 ± 0.1	0.7	
Constipation	2.3 ± 0.3	2.0 ± 0.3	1.4 ± 0.1	1.7 ± 0.3	1.7 ± 0.2	0.6	
Diarrhea	1.6 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	0.6	

Note:: Values with letters that differ indicate a significant difference ($p < 0.05$).

Abbreviations: BSF, Bristol Stool Form; GSRS, Gastrointestinal Symptom Response Scale; P, period; S, sequence; T, treatment.

individual. From food sources alone, 100% of participant intakes fell below the EAR for vitamin D, 80% for calcium, and 28% for iron. Fiber intake was 19.4 ± 7.7 g/day at baseline, which given their low energy intake, exceeded the Adequate Intake recommendation of 14 g/1000 kcal/day (Institute of Medicine, 2005). No differences were seen between baseline intakes of total grains, vegetables, fruit, protein, and dairy, and intervention or washout periods. Based on food group recommendations for a 1400 kcal/day diet (USDHHS & USDA, 2015), participants consumed the recommended number of servings of fruit, vegetables, and protein, but less than the recommendations for dairy and grains. Diet quality as assessed by the HEI-2015 was 65.4 ± 8.5 out of a possible 100 total points.

3.3 | Microbiota composition

Stool collections were complete for 24 participants. One participant was unable to provide stools during washout and the placebo collection periods due to severe constipation. *B. animalis* was detected by qPCR in all but one participant's stool collected during the probiotic intervention period (Figure 2). In addition, *B. animalis* was detected in three participants at baseline, seven participants in the probiotic washout, seven during placebo, and six during the placebo washout. No differences were seen in the relative fold changes in abundance of *Akkermansia muciniphila*, *Fecalibacterium prausnitzii*, and total *Bifidobacterium* genus comparing placebo to probiotic periods (Figure 3). Diversity indices are presented in Figure 4 and did not differ with interventions. Shannon diversity was 6.5 ± 0.1 at baseline. The relative abundance of taxa observed at the phyla, order and genus levels is shown in Figure 5. No significant effects of treatment on the taxonomic profiles were observed.

4 | DISCUSSION

Research into the efficacy of probiotics on indicators of laxation has focused on otherwise healthy individuals with functional constipation (Zhang et al., 2020) and constipation-predominant irritable bowel syndrome (Wen et al., 2020), yet infrequently in other patient populations experiencing constipation (Barichella et al., 2016). This trial of individuals with PWS, a population reported to exhibit a high prevalence of constipation (Kuhlmann et al., 2014), is the first study to evaluate the potential of *B. lactis* B94 to modulate stool frequency and stool form in adults. Of participants in the study, 84% reported normal bowel frequency (3-21 per week) compared to the general population reporting 96% (Mitsuhashi et al., 2018), and surprisingly, only one participant reported <3 stools/week at baseline. However, the percentage of what is considered normal stool form (BSF types 3–5) was lower in the PWS subjects (59%) compared to the general population (86%), and percentage of BSF types 1–2, suggestive of slow transit, was higher, 31% versus 6% (Mitsuhashi et al., 2018). The findings suggest that stool form may be a more informative indicator of constipation than stool frequency in the PWS population. The percentage of BSF types 1 and 2 stool forms for the group as a whole did not significantly improve with the probiotic intervention, although types 6 and 7 were highest during the probiotic washout, and throughout the probiotic and washout in the group randomized to the placebo-probiotic sequence. This finding may suggest a delayed,

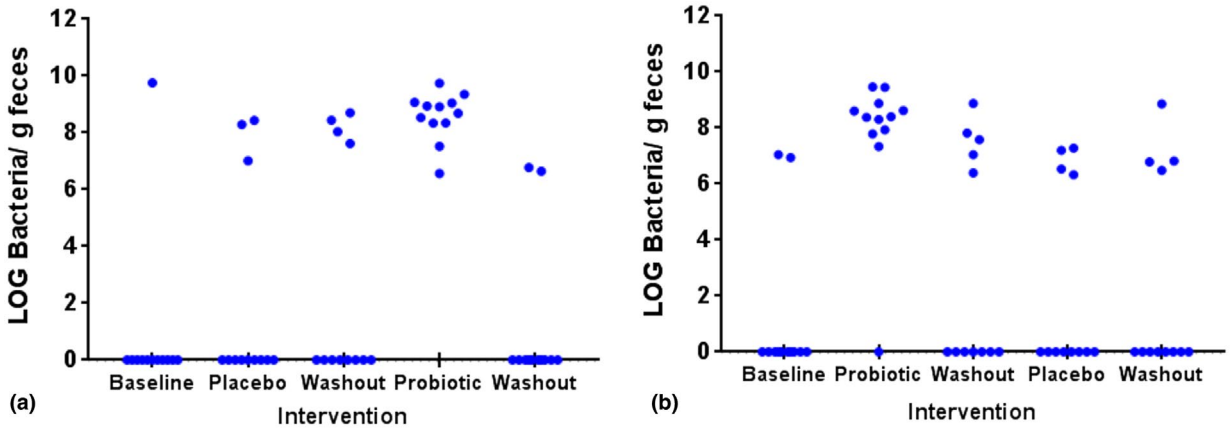

FIGURE 2 Abundance of *Bifidobacterium animalis* by study period. (a) Group that received Placebo → Probiotic ($n = 12$). (b) Group that received Probiotic → Placebo ($n = 12$)

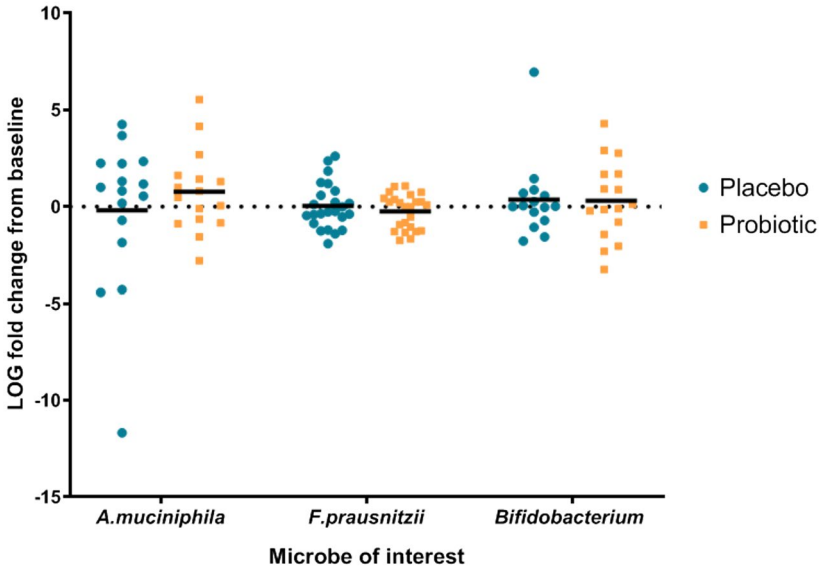

FIGURE 3 Relative fold change of *Akkermansia muciniphila*, *Fecalibacterium prausnitzii*, and *Bifidobacterium* genus over each intervention period in participants with complete stool collections ($n = 24$)

carry-over effect on motility with the probiotic administration. As the mechanism by which probiotics impact laxation may be strain specific, there are conflicting findings regarding *B. lactis* strains and indicators of laxation (Dimidi et al., 2014; Zhang et al., 2020). *B. lactis* NCC2818 (1.5×10^{10}) was assessed in adults with self-reported functional constipation and showed no significant effects on transit time, stool frequency or BSF (Dimidi et al., 2019). *B. lactis* HN01 was tested at two doses (10^9 and 10^{10}) using a parallel design in adults diagnosed with functional constipation and although no differences were seen for stool frequency for the group as a whole, 65 subjects with <3 bowel movements per week showed an increase (Ibarra et al., 2018). Similar to what is seen with dietary fiber (Christodoulides et al., 2016), probiotics may modulate stool frequency in those individuals with low frequency and less so in those exhibiting stool frequency within the normal range. Thus, participants in the present study, exhibiting a normal stool frequency of 2 stools/day on average, may not be expected to respond. Of note, the participant exhibiting stool frequency of <3 stools/week at baseline, reported increased stool frequency during the probiotic (2.2 stools/day) and probiotic washout (and 2.5 stools/day), but returned to infrequency during placebo and placebo washout. Stool frequency has previously been shown to be a poor proxy for colonic transit time (Saad et al., 2010). There is evidence that probiotic supplementation reduces intestinal transit time in adults and is most effective in individuals presenting with constipation (Miller et al., 2016). Future research may need to utilize precise measures of transit time versus reliance on stool frequency or stool form as proxies to evaluate probiotic efficacy in patient populations such as PWS.

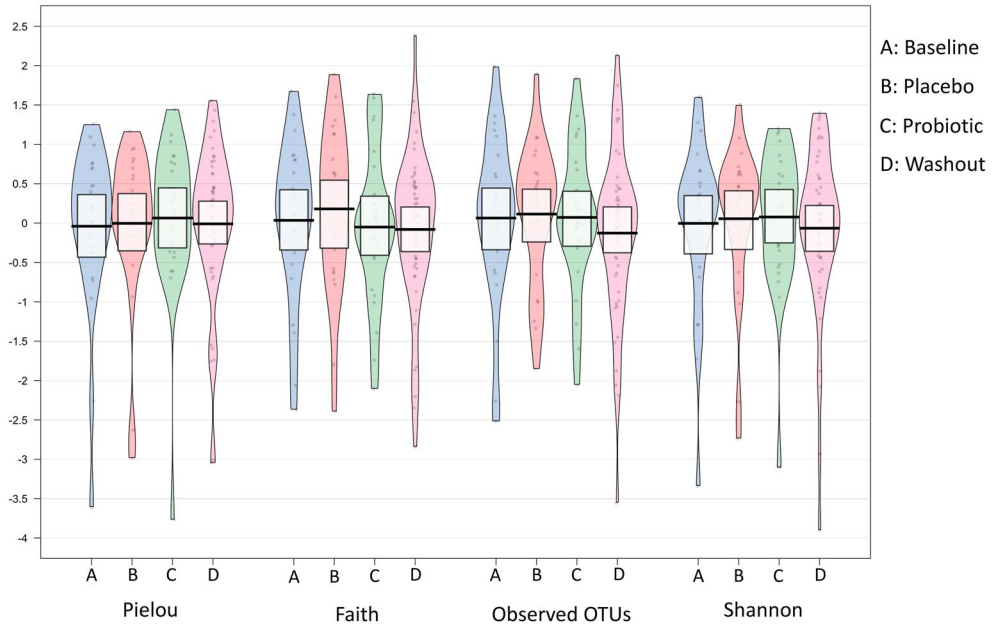

FIGURE 4 A Pirateplot relative comparison of Pielou, Faith, and Shannon diversity indices during baseline, probiotic, placebo, and washout periods

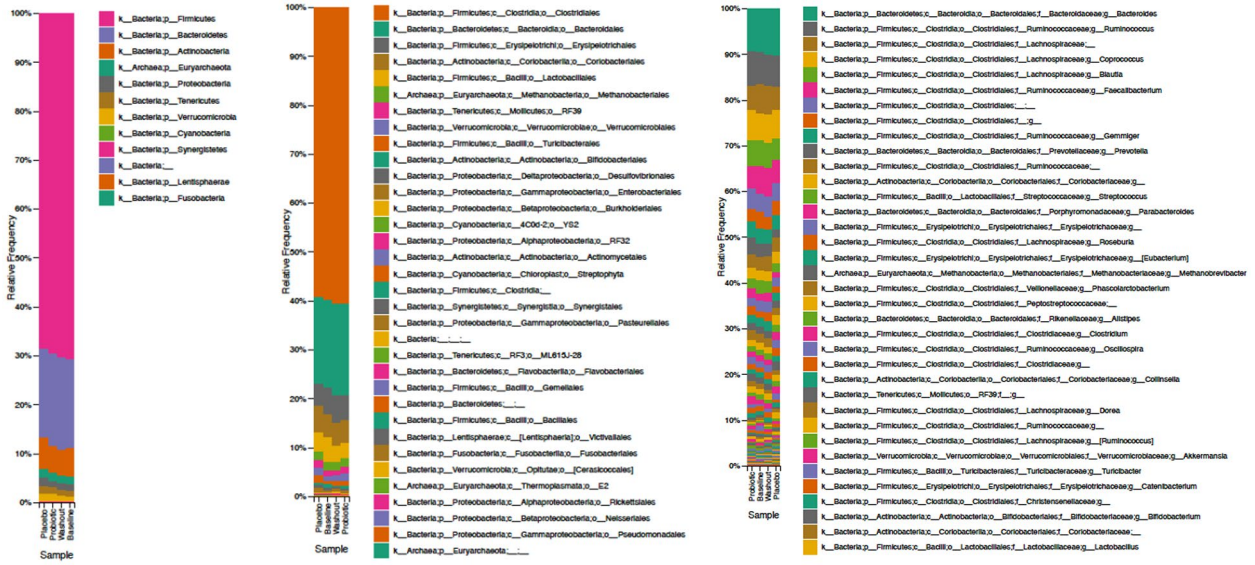

FIGURE 5 Taxonomic classification by phyla, order, and genus of fecal microbiome of adults affected by Prader-Willi syndrome during placebo, baseline, washout, and probiotic periods

Data representative of the US population has shown that fiber intake of >20 g per day was associated with normal bowel habits (Mitsuhashi et al., 2018). In the present study, adults affected by PWS on energy-controlled diets consumed 19.4 ± 7.7 g/day of fiber, an intake similar to the general US population (Reicks et al., 2014). Grains typically contribute a higher percentage of fiber intake than do fruits and vegetables (Reicks et al., 2014). In contrast, the adults with PWS in the present study consumed a diet exceeding the recommended number of servings of fruits and vegetables for their average energy requirement, but not grains. Limited intake of grains, specifically the lack of whole grains, contributed to an average fiber intake of <20 g/day. Although participants' fiber intake exceeded the AI for fiber, this recommendation, intended for healthy individuals, may not be appropriate for patient populations, specifically those requiring lower energy intakes. Microbial fermentation of fruit and vegetable fiber is extensive (Cummings & Englyst, 1987), and thus contributes little to the fecal bulking needed for laxation. The relative lack of cereal fiber, known for its fecal bulking potential (Nyman

et al., 1986), may have contributed to symptoms of constipation in some participants. Consumption of whole grain fiber, particularly whole wheat, has been shown to support microbial diversity (Jefferson & Adolphus, 2019). Although the PWS participants consumed few grains and virtually no whole grains, microbiota diversity was higher than was seen in generally healthy, non-affected adults from a similar geographical area (Tremblay et al., 2020). However, given that the association of microbial diversity with health *vs*. disease risk has recently been questioned (Ma et al., 2019), the health implications of their high diversity is unclear.

This study had limitations. The sample size was determined based on a study of constipated adults, as no known published research has explored probiotic administration and gastrointestinal outcomes in PWS subjects. Individuals with PWS were recruited independent of constipation status due to the rarity of the condition. Surprisingly, most participants exhibited normal stool frequency, which undermined this outcome as individuals with normal frequency may not respond to *B. lactis* strains (Ibarra et al., 2018). *B. animalis* were found in samples that were collected in a least one of the periods other than the probiotic intervention in 52% of the participants suggesting that participants were consuming *B. animalis* strains from food sources. The proliferation of probiotics as food ingredients poses a significant challenge for community-based clinical trials and few studies confirm compliance using qPCR (Tremblay et al., 2020). As the data suggest a potential delayed effect on motility extending into the probiotic washout period, a parallel design may be more appropriate for future studies. An additional limitation was the length of the intervention periods. The 4-week intervention period was based on previous trials (Dimidi et al., 2014), as well as feasibility related to participant burden. However, an intervention period of greater than 4 weeks may be needed to elicit a probiotic effect on gastrointestinal symptom reporting (Guglielmetti et al., 2011), and thus possibly for functional outcomes also. Although low water intake is associated with constipation (Markland et al., 2013), non-caloric beverage consumption was not tracked in this study, and thus we cannot report on the impact of water intake on outcomes. Aberrant drinking behaviors have been reported in individuals with PWS. One study found that 77% of the subjects with PWS drank "extremely small" amounts of water, and 13% drank "small" volumes of water (Akefeldt, 2009).

Health professionals practicing in primary care recommend probiotics, including for constipation (Johnson et al., 2019). As PWS is a unique patient population, their response to probiotics, including *B. lactis* strains, may differ from those without such a diagnosis due to potential confounding effects of syndrome-related motility issues, an energy-controlled diet, and possibly, their baseline microbiota profile. Recent research has suggested that the microbiota profile of individuals affected by PWS differ non-affected adults and children (Olsson et al., 2019; Peng et al., 2020). As multi-strain probiotics have recently shown efficacy in the management of constipation (Zhang et al., 2020), such formulations require testing in patient populations such as PWS. This study provides preliminary evidence of a motility effect of *B. lactis* B94 impacting stool form but not stool frequency. Future studies should specifically assess intestinal transit time. In addition, further research is needed to determine if increased dietary fiber through prudent food choice, fortification or supplementation helps to alleviate hard stool form, suggestive of slow transit, in adults affected by PWS.

ACKNOWLEDGMENTS

The authors thank James Colee, Institute of Food and Agricultural Sciences, University of Florida for his assistance with the statistical analysis, and the study participants for their contribution to science. ZA, JLM, and WJD declare no conflict of interest. JA, MS, AP, and TAT are employees of the Rosell Institute for Microbiome and Probiotics, the research group of Lallemand Health Solutions Inc.

AUTHOR CONTRIBUTIONS

WJD, ZA, MS, JM, and TA designed the study. ZA and WJD conducted the clinical trial. AP and MS carried out the microbiota analysis, and JA, the bioinformatics. ZA, WJD, JA, and AP wrote the manuscript. All authors read and approved the final manuscript.

ORCID

Thomas A. Tompkins https://orcid.org/0000-0002-2990-2265
Wendy J. Dahl https://orcid.org/0000-0003-2061-4731

REFERENCES

Akefeldt, A. (2009). Water intake and risk of hyponatraemia in Prader-Willi syndrome. *Journal of Intellectual Disability Research*, *53*(6), 521-528. https://doi.org/10.1111/j.1365-2788.2009.01169.x

Alsaif, M., Elliot, S. A., MacKenzie, M. L., Prado, C. M., Field, C. J., & Haqq, A. M. (2017). Energy metabolism profile in individuals with Prader-Willi syndrome and implications for clinical management: A systematic review. *Advances in Nutrition*, *8*(6), 905-915.

Alyousif, Z., Miller, J. L., Sandoval, M. Y., MacPherson, C. W., Nagulesapillai, V., & Dahl, W. J. (2018). The effects of Bifidobacterium animalis ssp. lactis B94 on gastrointestinal wellness in adults with Prader-Willi syndrome: Study protocol for a randomized controlled trial. *Trials*, *19*(1), 256.

Amir, A., McDonald, D., Navas-Molina, J. A., Kopylova, E., Morton, J. T., Zech Xu, Z., Kightley, E. P., Thompson, L. R., Hyde, E. R., Gonzalez, A., & Knight, R. (2017). Deblur rapidly resolves single-nucleotide community sequence patterns. *mSystems*, *2*(2), e00191-16. https://doi.org/10.1128/mSystems.00191-16

Barichella, M., Pacchetti, C., Bolliri, C., Cassani, E., Iorio, L., Pusani, C., Pinelli, G., Privitera, G., Cesari, I., Faierman, S. A., Caccialanza, R., Pezzoli, G., & Cereda, E. (2016). Probiotics and prebiotic fiber for constipation associated with Parkinson disease:

An RCT. *Neurology*, *87*(12), 1274-1280. https://doi.org/10.1212/wnl.0000000000003127

Basturk, A., Artan, R., & Yilmaz, A. (2016). Efficacy of synbiotic, probiotic, and prebiotic treatments for irritable bowel syndrome in children: A randomized controlled trial. *Turkish Journal of Gastroenterology*, *27*(5), 439-443. https://doi.org/10.5152/tjg.2016.16301

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., Mills, D. A., & Caporaso, J. G. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods*, *10*(1), 57-59. https://doi.org/10.1038/nmeth.2276

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9

Butler, M. G., Miller, J. L., & Forster, J. L. (2019). Prader-Willi syndrome - clinical genetics, diagnosis and treatment approaches: An update. *Current Pediatric Reviews*, *15*, 207–244. https://doi.org/10.2174/1573396315666190716120925

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335-336. https://doi.org/10.1038/nmeth.f.303

Chang, Q., Luan, Y., & Sun, F. (2011). Variance adjusted weighted UniFrac: A powerful beta diversity measure for comparing communities based on phylogeny. *BMC Bioinformatics*, *12*, 118. https://doi.org/10.1186/1471-2105-12-118

Chen, J., Bittinger, K., Charlson, E. S., Hoffmann, C., Lewis, J., Wu, G. D., Collman, R. G., Bushman, F. D., & Li, H. (2012). Associating microbiome composition with environmental covariates using generalized UniFrac distances. *Bioinformatics*, *28*(16), 2106-2113. https://doi.org/10.1093/bioinformatics/bts342

Christodoulides, S., Dimidi, E., Fragkos, K. C., Farmer, A. D., Whelan, K., & Scott, S. M. (2016). Systematic review with meta-analysis: Effect of fibre supplementation on chronic idiopathic constipation in adults. *Alimentary Pharmacology and Therapeutics*, *44*(2), 103-116. https://doi.org/10.1111/apt.1366

Cummings, J. H., & Englyst, H. N. (1987). Fermentation in the human large intestine and the available substrates. *American Journal of Clinical Nutrition*, *45*(5 Suppl), 1243-1255.

Derrien, M., Belzer, C., & de Vos, W. M. (2017). Akkermansia muciniphila and its role in regulating host functions. *Journal of Microbiology and Pathology*, *106*, 171-181. https://doi.org/10.1016/j.micpath.2016.02.005

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied Environmental Microbiology*, *72*(7), 5069-5072.

Dimidi, E., Christodoulides, S., Fragkos, K. C., Scott, S. M., & Whelan, K. (2014). The effect of probiotics on functional constipation in adults: A systematic review and meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition*, *100*(4), 1075-1084. https://doi.org/10.3945/ajcn.114.089151

Dimidi, E., Christodoulides, S., Scott, S. M., & Whelan, K. (2017). Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation. *Advances in Nutrition*, *8*(3), 484-494. https://doi.org/10.3945/an.116.014407

Dimidi, E., Zdanaviciene, A., Christodoulides, S., Taheri, S., Louis, P., Duncan, P. I., Emami, N., Crabbé, R., De Castro, C. A., McLean, P., Bergonzelli, G. E., Whelan, K., & Mark Scott, S. (2019). Randomised clinical trial: Bifidobacterium lactis NCC2818 probiotic vs placebo, and impact on gut transit time, symptoms, and gut microbiology in chronic constipation. *Alimentary Pharmacology and Therapeutics*, *49*(3), 251-264. https://doi.org/10.1111/apt.15073

Ferreira-Halder, C. V., Faria, A. V. S., & Andrade, S. S. (2017). Action and function of Faecalibacterium prausnitzii in health and disease. *Best Practice and Research: Clinical Gastroenterology*, *31*(6), 643-648. https://doi.org/10.1016/j.bpg.2017.09.011

Ford, A. L., Nagulesapillai, V., Piano, A., Auger, J., Girard, S.-A., Christman, M., Tompkins, T. A., & Dahl, W. J. (2020). Microbiota stability and gastrointestinal tolerance in response to a high protein diet with and without a prebiotic, probiotic and synbiotic: A randomized, double-blind, placebo-controlled trial in older women. *Journal of the Academy of Nutrition and Dietetics*, *120*(4), 500-516.

Guglielmetti, S., Mora, D., Gschwender, M., & Popp, K. (2011). Randomised clinical trial: Bifidobacterium bifidum MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life–A double-blind, placebo-controlled study. *Alimentary Pharmacology and Therapeutics*, *33*(10), 1123-1132. https://doi.org/10.1111/j.1365-2036.2011.04633.x

Halko, N., Martinsson, P.-G., Shkolnisky, Y., & Tygert, M. (2011). An algorithm for the principal component analysis of large data sets. *SIAM Journal on Scientific Computing*, *33*(5), 2580-2594.

Ibarra, A., Latreille-Barbier, M., Donazzolo, Y., Pelletier, X., & Ouwehand, A. C. (2018). Effects of 28-day Bifidobacterium animalis subsp. lactis HN019 supplementation on colonic transit time and gastrointestinal symptoms in adults with functional constipation: A double-blind, randomized, placebo-controlled, and dose-ranging trial. *Gut Microbes*, *9*(3), 236-251. https://doi.org/10.1080/19490976.2017.1412908

Institute of Medicine. (2005). *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. The National Academies Press.

Jefferson, A., & Adolphus, K. (2019). The effects of intact cereal grain fibers, including wheat bran on the gut microbiota composition of healthy adults: A systematic review. *Frontiers in Nutrition*, *6*, 33. https://doi.org/10.3389/fnut.2019.00033

Johnson, A. J., Vangay, P., Al-Ghalith, G. A., Hillmann, B. M., Ward, T. L., Shields-Cutler, R. R., Kim, A. D., Shmagel, A. K., Syed, A. N., Walter, J., Menon, R., Koecher, K., & Knights, D. (2019). Daily sampling reveals personalized diet-microbiome associations in humans. *Cell Host & Microbe*, *25*(6), 789-802.e785. https://doi.org/10.1016/j.chom.2019.05.005

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biololgy and Evolution*, *30*(4), 772-780. https://doi.org/10.1093/molbev/mst010

Khalif, I. L., Quigley, E. M., Konovitch, E. A., & Maximova, I. D. (2005). Alterations in the colonic flora and intestinal permeability and

evidence of immune activation in chronic constipation. *Digestive and Liver Disease*, *37*(11), 838–849. https://doi.org/10.1016/j.dld.2005.06.008

Krebs-Smith, S. M., Pannucci, T. R. E., Subar, A. F., Kirkpatrick, S. I., Lerman, J. L., Tooze, J. A., Wilson, M. M., & Reedy, J. (2018). Update of the healthy eating index: HEI-2015. *Journal of the Acad Nutrition and Dietetics*, *118*(9), 1591-1602. https://doi.org/10.1016/j.jand.2018.05.021

Kuhlmann, L., Joensson, I. M., Froekjaer, J. B., Krogh, K., & Farholt, S. (2014). A descriptive study of colorectal function in adults with Prader-Willi syndrome: High prevalence of constipation. *BMC Gastroenterology*, *14*, 63. https://doi.org/10.1186/1471-230x-14-63

Kulich, K. R., Madisch, A., Pacini, F., Piqué, J. M., Regula, J., Van Rensburg, C. J., Újszászy, L., Carlsson, J., Halling, K., & Wiklund, I. K. (2008). Reliability and validity of the Gastrointestinal Symptom Rating Scale (GSRS) and Quality of Life in Reflux and Dyspepsia (QOLRAD) questionnaire in dyspepsia: A six-country study. *Health and Quality of Life Outcomes*, *6*, 12. https://doi.org/10.1186/1477-7525-6-12

Legendre, P., & Legendre, L. F. (2012). *Numerical ecology*. Elsevier.

Lewis, S. J., & Heaton, K. W. (1997). Stool form scale as a useful guide to intestinal transit time. *Scandinavian Journal of Gastroenterolgy*, *32*(9), 920-924. https://doi.org/10.3109/00365529709011203

Lozupone, C. A., Hamady, M., Kelley, S. T., & Knight, R. (2007). Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Applied Environmental Microbiology*, *73*(5), 1576-1585. https://doi.org/10.1128/aem.01996-06

Lozupone, C., & Knight, R. (2005). UniFrac: A new phylogenetic method for comparing microbial communities. *Applied Environmental Microbiology*, *71*(12), 8228-8235. https://doi.org/10.1128/aem.71.12.8228-8235.2005

Ma, Z. S., Li, L., & Gotelli, N. J. (2019). Diversity-disease relationships and shared species analyses for human microbiome-associated diseases. *ISME Journal*, *13*(8), 1911-1919. https://doi.org/10.1038/s41396-019-0395-y

Mackenzie, M. L., Triador, L., Gill, J. K., Pakseresht, M., Mager, D., Field, C. J., & Haqq, A. M. (2018). Dietary intake in youth with Prader-Willi syndrome. *American Journal of Medical Genetics A*, *176*(11), 2309-2317. https://doi.org/10.1002/ajmg.a.40491

Markland, A. D., Palsson, O., Goode, P. S., Burgio, K. L., Busby-Whitehead, J., & Whitehead, W. E. (2013). Association of low dietary intake of fiber and liquids with constipation: Evidence from the National Health and Nutrition Examination Survey. *American Journal of Gastroenterology*, *108*(5), 796-803. https://doi.org/10.1038/ajg.2013.73

Martin, R., & Langella, P. (2019). Emerging health concepts in the probiotics field: Streamlining the definitions. *Frontiers in Microbiology*, *10*, 1047. https://doi.org/10.3389/fmicb.2019.01047

Martinez Michel, L., Haqq, A. M., & Wismer, W. V. (2016). A review of chemosensory perceptions, food preferences and food-related behaviours in subjects with Prader-Willi syndrome. *Appetite*, *99*, 17-24. https://doi.org/10.1016/j.appet.2015.12.021

McDonald, D., Clemente, J. C., Kuczynski, J., Rideout, J. R., Stombaugh, J., Wendel, D., Wilke, A., Huse, S., Hufnagle, J., Meyer, F., Knight, R., & Caporaso, J. G. (2012). The Biological Observation Matrix (BIOM) format or: How I learned to stop worrying and love the ome-ome. *Gigascience*, *1*(1), 7. https://doi.org/10.1186/2047-217x-1-7

McDonald, D., Vázquez-Baeza, Y., Koslicki, D., McClelland, J., Reeve, N., Xu, Z., Gonzalez, A., & Knight, R. (2018). Striped UniFrac: Enabling microbiome analysis at unprecedented scale. *Nature Methods*, *15*(11), 847-848. https://doi.org/10.1038/s41592-018-0187-8

McKinney, W. (2010). *Data structures for statistical computing in python*. Paper presented at the Proceedings of the 9th Python in Science Conference.

Miller, L. E., Zimmermann, A. K., & Ouwehand, A. C. (2016). Contemporary meta-analysis of short-term probiotic consumption on gastrointestinal transit. *World Journal of Gastroenterology*, *22*(21), 5122-5131. https://doi.org/10.3748/wjg.v22.i21.5122

Mitsuhashi, S., Ballou, S., Jiang, Z. G., Hirsch, W., Nee, J., Iturrino, J., Cheng, V., & Lembo, A. (2018). Characterizing normal bowel frequency and consistency in a representative sample of adults in the United States (NHANES). *The American Journal of Gastroenterology*, *113*(1), 115.

Nyman, M., Asp, N. G., Cummings, J., & Wiggins, H. (1986). Fermentation of dietary fibre in the intestinal tract: Comparison between man and rat. *British Journal of Nutrition*, *55*(3), 487-496. https://doi.org/10.1079/bjn19860056

Olsson, L. M., Poitou, C., Tremaroli, V., Coupaye, M., Aron-Wisnewsky, J., Bäckhed, F., Clément, K., & Caesar, R. (2019). Gut microbiota of obese subjects with Prader-Willi syndrome is linked to metabolic health. *Gut*, 1229–1238. https://doi.org/10.1136/gutjnl-2019-319322

Peng, Y. E., Tan, Q., Afhami, S., Deehan, E. C., Liang, S., Gantz, M., Triador, L., Madsen, K. L., Walter, J., Tun, H. M., & Haqq, A. M. (2020). The gut microbiota profile in children with Prader-Willi syndrome. *Genes (Basel)*, *11*(8), 904. https://doi.org/10.3390/genes11080904

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS One*, *5*(3), e9490.

Reicks, M., Jonnalagadda, S., Albertson, A. M., & Joshi, N. (2014). Total dietary fiber intakes in the US population are related to whole grain consumption: Results from the National Health and Nutrition Examination Survey 2009 to 2010. *Nutrition Research*, *34*(3), 226-234. https://doi.org/10.1016/j.nutres.2014.01.002

Saad, R. J., Rao, S. S. C., Koch, K. L., Kuo, B., Parkman, H. P., McCallum, R. W., Sitrin, M. D., Wilding, G. E., Semler, J. R., & Chey, W. D. (2010). Do stool form and frequency correlate with whole-gut and colonic transit? Results from a multicenter study in constipated individuals and healthy controls. *American Journal of Gastroenterology*, *105*(2), 403-411. https://doi.org/10.1038/ajg.2009.612

Stackebrandt, E., & Goodfellow, M. (1991). *Nucleic acid techniques in bacterial systematics*. Wiley.

Tremblay, A., Fatani, A., Ford, A. L., Piano, A., Nagulesapillai, V., Auger, J., MacPherson, C. W., Christman, M. C., Tompkins, T. A., & Dahl, W. J. (2020). Safety and effect of a low- and high-dose multi-strain probiotic supplement on microbiota in a general adult population: A randomized, double-blind. Placebo-controlled study. *Journal of Dietary Supplements*, *6*, 1–21. https://doi.org/10.1080/19390211.2020.1749751

U.S. Department of Health and Human Services, U.S. Department of Agriculture. (2015). 2015–2020 Dietary Guidelines for Americans. 8th Edition. http://health.gov/dietaryguidelines/2015/guidelines/

Vázquez-Baeza, Y., Gonzalez, A., Smarr, L., McDonald, D., Morton, J. T., Navas-Molina, J. A., & Knight, R. (2017). Bringing the dynamic microbiome to life with animations. *Cell Host & Microbe*, *21*(1), 7-10. https://doi.org/10.1016/j.chom.2016.12.009

Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A., & Knight, R. (2013). EMPeror: A tool for visualizing high-throughput microbial community data. *Gigascience*, *2*(1), 16. https://doi.org/10.1186/2047-217x-2-16

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J. R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E. R., & Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, *5*(1), 27. https://doi.org/10.1186/s40168-017-0237-y

Wen, Y., Li, J., Long, Q., Yue, C. C., He, B., & Tang, X. G. (2020). The efficacy and safety of probiotics for patients with constipation-predominant irritable bowel syndrome: A systematic review and meta-analysis based on seventeen randomized controlled trials. *International Journal of Surgery*, *79*, 111–119. https://doi.org/10.1016/j.ijsu.2020.04.063

Woods, S. G., Knehans, A., Arnold, S., Dionne, C., Hoffman, L., Turner, P., & Baldwin, J. (2018). The associations between diet and physical activity with body composition and walking a timed distance in adults with Prader-Willi syndrome. *Food & Nutrition Research*, *62*. https://doi.org/10.29219/fnr.v62.1343

Zhang, C., Jiang, J., Tian, F., Zhao, J., Zhang, H., Zhai, Q., & Chen, W. (2020). Meta-analysis of randomized controlled trials of the effects of probiotics on functional constipation in adults. *Clinical Nutrition*, *39*(10), 2960–2969. https://doi.org/10.1016/j.clnu.2020.01.005

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Alyousif Z, Miller JL, Auger J, et al. Microbiota profile and efficacy of probiotic supplementation on laxation in adults affected by Prader-Willi Syndrome: A randomized, double-blind, crossover trial. *Mol Genet Genomic Med*. 2020;8:e1535. https://doi.org/10.1002/mgg3.1535