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Original Article

Prebiotic supplements correct oral probiotic deficiency for lasting allergy relief

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Abstract: The prevalence of allergic rhinitis (common allergies) has increased in the last fifty years, from less than one percent to more than twenty-six percent of the population. Today, more than one hundred million people in the US suffer seasonal or yearlong allergies. The hygiene hypothesis was proposed 30 years ago as a potential explanation for this phenomenon, and we built on it with the specific oral hygiene hypothesis. Our longitudinal pilot study suggested that oral probiotic deficiency is the cause of allergic rhinitis. This clinical trial served to verify our theory and evaluate the effectiveness of AllerPops for allergy relief. We carried out a phase II, randomized, double-blind, controlled, single-center 21-day study to investigate the efficacy of AllerPops to reduce nasal symptoms in 72 adult volunteers with seasonal/year-long nasal allergies and its impact on oral microbiome using amplicon sequencing of 16S ribosome RNA genes. The volunteers were randomly separated into two equally sized groups: a control group and an investigational group. Both groups were given at least three doses of AllerPops, taken every other day, and asked to answer questions about observed allergy symptoms. Volunteers in the investigational group cleaned their mouths before taking a dose and slowly dissolve the lozenge, while those in the control did not. Through this trial, we show that AllerPops prebiotic supplements are effective in providing sustained allergy relief ($P = 0.002$) and can modulate oral beneficial bacteria that produce short-chain fatty acids (SCFA), such as Fusobacteria, Butyrivibrio, and Peptostreptococcus. The clinical improvements correlated with changes in the relative abundance of probiotics significantly: Fusobacteria ($R = 0.32$, $P = 0.009$), Butyrivibrio ($R = 0.25$, $P = 0.044$), and Peptostreptococcus ($R = 0.34$, $P = 0.005$). These results point to the root cause of allergic rhinitis: the lack of oral probiotics that produce SCFA to pacify the immune systems. Future study of AllerPops' theory will help society redefine the best oral hygiene practice to protect oral probiotics so that we may prevent allergic and autoimmune diseases and dental/gum infections. The trial was retrospectively registered at clinicaltrials.com, with registration number NCT05956691, on 21/07/2023.

Keywords: Prebiotics, probiotics, allergic rhinitis, oral microbiome, oral probiotic deficiency

Introduction

Allergic rhinitis (AR) was described in 1929 as a process that includes three cardinal symptoms: sneezing, nasal obstruction, and mucus discharge [1]. AR is clinically defined as a symptomatic disorder of the nose induced by an immunoglobulin (IgE)-mediated inflammation after allergen exposure of the membranes lining the nose. AR is a widely prevalent condition in Canada (i.e., affecting 20 to 25% of the population) [2] and the United States [3]. Significant physical sequelae and recurrent or persistent morbidities often accompany it.

More than 30 years ago, Dr. Strachan proposed the hygiene hypothesis to explain the increase in allergy diseases [4]. Subsequently, many later studies suggest that personal and social hygiene practices are associated with the allergy disease epidemic [5]. Increasingly common literature on animals and humans has indicated that changes in the gut microbiome are associated with the development of allergic disease [6-9], which were shown to be associated with insufficient early microbial exposure [10]. Changes in the nasal [11-15] and oral microbiota [16-19] were also implicated.

Some investigations have shown that the oral administration of probiotics and prebiotics may benefit allergic rhinitis patients [20-22]. Moreover, the local nasal administration of *Lactococcus lactis* NZ9000 can affect the local

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and systemic immune responses against *Streptococcus pneumonia* [23].

Probiotics are a promising therapeutic approach for allergic airway diseases. However, attempts at using probiotics to cure or prevent allergy diseases have had limited success [24]. In those trials, researchers used probiotics that live in the gut. Instead of focusing on this remote interaction between the gut and the airway, we examined the local interaction between microbiota living in the airway, especially in the mouth, and the immune system residing around the respiratory tracts during our pilot study [25]. That study illustrated that restructuring oral microbiota can lead to lasting remissions of common allergies (allergic rhinitis).

The primary purpose of this clinical study was to assess the effectiveness of AllerPops [26] in relieving nasal symptoms in volunteers with seasonal or year-long allergies, as indicated by results on the total nasal symptoms score (TNSS) questionnaire and peak nasal inspiratory flow (PNIF) measurement device. The secondary purpose was to identify the changes in the oral microbiome during the intervention and to verify the oral probiotic deficiency hypothesis, that the lack of short-chain fatty acid (SCFA)-producing bacteria causes allergic rhinitis.

Materials and methods

Study design

The study was conducted as a phase II, a randomized, double-blind, controlled, single-center, 21-day study designed to investigate the efficacy of AllerPops in reducing nasal symptoms in adult participants with seasonal/year-long nasal allergies. Participants were dosed every other day for a minimum of 3 doses. Or until the participant was satisfied with the relief of the nasal allergy symptoms.

Ethics statements

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by Health Canada under protocol number 21-SAHSE-01, and Advarra Institutional Review Board (Aurora, Ontario, Canada). Informed consent was obtained from all subjects before participation.

Participants

All participants were between 18 and 70 years old and clinically diagnosed with persistent or intermittent AR according to the Allergic Rhinitis and Its Impact on Asthma (ARIA) classification [27]. Participants meeting any exclusion criteria were not eligible for admission to the study. A baseline TNSS was obtained from all participants. TNSS is a questionnaire developed to assess AR symptoms. In this subjective assessment, AR symptoms were scored by the participant from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe) [28]. Peak nasal inspiratory flow (PNIF) scores were also obtained. This device assesses nasal airway potency by measuring nasal volume and flow [29], and provides an objective score.

Using block randomization, participants were separated into two groups (i.e., control and investigational). Both groups received AllerPops as the intervention but with different product consumption instructions. The investigational group was asked to clean their mouth first and let the product slowly dissolve in their mouth for over one hour. The control group was not required to clean their mouth and was instructed to quickly swallow one lozenge every other day for a minimum of 3 doses or until they were satisfied with the symptom relief, up to and including Day 13.

Outcome measures

At baseline and after 7, 14, and 21 days, the TNSS questionnaire was completed by all participants, and PNIF measurements were obtained. Similarly, blood samples were also taken at baseline, day 7, and day 21, to measure total IgE (Immunoglobulin E) and eosinophils (known immune mediators), and to monitor standard safety parameters. Any out-of-range parameter was flagged and assessed by the Principal Investigator. Heart rate, blood pressure, and body temperature were collected at baseline, day 7, and day 21 to assess safety outcomes. Finally, the emergence, severity, and causality of adverse events were measured on day 7, day 14, and day 21. Saliva samples were obtained from participants at baseline, day 7, and day 21 to evaluate the effect of the AllerPops administration on the oral microbial presentation/microbiota using Amplicon Metagenomics Sequencing. The analysis was performed separately by Novogene.

Statistics

Continuous data were tested for normal distribution using the Shapiro-Wilk test and were confirmed to be nonparametric. Demographic data were evaluated using mean and standard deviation. Baseline and follow-up values were compared between the investigational and control groups using the Mann-Whitney U test or, where appropriate, Fisher's exact test. Baseline and follow-up values were also compared within each group using repeated measures (RM) ANOVA over several periods, followed by the Wilcoxon signed-rank test for each specific period. All analyses were done using RStudio (v. 2022.07.1+554), Microsoft Excel (v. 16.67), and the Real Statistics Resource Pack (Rel 8.3.1).

16S profiling of saliva sample

Saliva was collected after fasting for at least 8 hours without stimulation at baseline and after 7, 14, and 21 days and stored at -70°C immediately. The samples were sent to Novogene (Sacramento, California, USA) for DNA extraction, 16S amplification, and sequencing. The amplicon sequences were analyzed through Novogene's pipeline, including quality control, diversity analysis, and abundance comparison. A detailed description can be found in our pilot study [25]. We also did a correlation test between improvements in clinical symptoms score and variation of the species abundance, using the CORREL function in Microsoft Excel.

Results

Demographics and groups

Seventy-two (72) individuals clinically diagnosed with persistent or intermittent allergic rhinitis were enrolled randomly into the two arms of the double-blinded phase II clinical trial. Two participants in the experimental treatment arm did not complete the study. Both participants withdrew from the study after baseline values were taken (CONSORT (Consolidated Standards of Reporting Trails) Diagram, Figure S1).

Participants included 23 males and 49 females, with an average age of 37.8 years ± SD 14.1 (range 18-70), a height of 168.9 cm ± SD 9.6 (range 147.5-197.0), a weight of 68.7 kg ± SD 12.2 (range 47.3-106.9), and a BMI of 24.0 kg/m^2 ± SD 3.1 (range 18.6-29.9).

Participants were randomized to either the control group or the investigational group. No statistically significant differences between the control group (n = 36) and the investigational group (n = 36) were seen in any of the baseline measurements aside from body temperature (See **Table 1**); the slight mean difference of 0.15°C can be explained by more females being enrolled in the control group.

Allerpops relieves allergies according to TNSS and PNIF score

Baseline TNSS scores were obtained for all participants, with overall scores averaging 4.6 ± SD 1.8, based on the following breakdown: (1) nasal obstruction score of 1.7 ± SD 0.7, (2) itchiness score of 1.4 ± SD 0.7, and (3) runny nose score of 1.5 ± SD 0.9.

PNIF measurements averaged 76.1 L/min ± SD 34.7. A study by Dor-Wojnarowska in 2011 established reference PNIF values. Participants in this study had a mean PNIF value 57.7 L/min lower than they were expected to have given their sex and height (i.e., 133.8 L/min), a difference anticipated with all participants having been clinically diagnosed with allergic rhinitis.

To assess treatment impact, the change in TNSS scores was compared between the control and investigational groups (See **Table 2**). Each group improved on both the overall score and the three sub-scores during all three assessments (i.e., day 7, day 14, and day 21), with this change only being statistically significant between the two groups on day 14 for the overall score (P = 0.016) and the nasal subscore (P = 0.032).

PNIF scores also improved in both groups, although the difference between the two groups was only statistically significant on the day 14 mark (P = 0.045) (See **Table 2**).

Table 1. Demographics and baseline measurements for the control and investigational groups

Measurement	Control Group *(n = 36)*	Investigational Group *(n = 36)*	*p*-value
Age *(mean, SD, in years)*	39.4 (13.9)	35.3 (14.3)	0.11
Height *(mean, SD, in cm)*	169.7 (10)	167.8 (9.4)	0.40
Weight *(mean, SD, in kg)*	70 (13.2)	67.6 (11.7)	0.38
BMI *(mean, SD, kg/m^2)*	24.2 (3.1)	23.9 (3.2)	0.72
Female	69.4%	66.7%	0.84
TNSS *(mean, SD)*			
Nasal Obstruction	1.8 (0.62)	1.5 (0.65)	0.10
Itching/Sneezing	1.4 (0.77)	1.4 (0.73)	0.98
Secretion/Runny Nose	1.5 (0.91)	1.4 (0.81)	0.66
Overall	4.8 (1.92)	4.4 (1.78)	0.46
PNIF *(mean, SD, in L/min)*	72.8 (32.2)	79.5 (37.2)	0.41
IgE *(mean, SD, IU/mL)*	121.7 (154.3)	140.7 (191.6)	0.99
Eosinophils *(mean, SD, ×10^9/L)*	0.18 (0.17)	0.21 (0.15)	0.43
Heart Rate *(mean, SD, beats/min)*	72.4 (10.8)	74.4 (10)	0.30
Body Temperature *(mean, SD, °C)*	36.3 (0.7)	36.2 (0.6)	0.046
Blood Pressure *(mean, SD, mm Hg)*			
Systolic	118.1 (12)	116.9 (9.8)	0.73
Diastolic	72.7 (7.4)	73.2 (8.2)	0.58

Table 2. Change in TNSS, PNIF values, eosinophils, and IgE from baseline in the control and investigational groups on days 7, 14, and 21

Measurement	Control Group *(n = 33 to 35)*			Investigational Group *(n = 30 to 34)*			*p*-value		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
TNSS *(change, SD)*									
Nasal Obstruction	-0.6 (0.7)	-0.6 (0.8)	-0.7 (0.9)	-0.3 (0.8)	-0.2 (0.8)	-0.3 (1)	0.17	0.032	0.08
Itching/Sneezing	-0.4 (0.8)	-0.7 (0.9)	-0.6 (0.9)	-0.2 (0.7)	-0.4 (0.8)	-0.5 (1.1)	0.67	0.11	0.71
Secretion/Runny Nose	-0.5 (0.9)	-0.7 (0.8)	-0.7 (0.8)	-0.1 (0.8)	-0.4 (0.9)	-0.3 (0.8)	0.22	0.10	0.053
Overall	-1.4 (2)	-2.1 (1.9)	-2 (2)	-0.6 (1.8)	-1.1 (2)	-1.1 (2.6)	0.19	0.016	0.079
PNIF *(change, SD, in L/min)*	18.1 (38)	29 (41.5)	17.8 (24.2)	6.7 (42.6)	4.4 (42.1)	8.7 (45.2)	0.38	0.045	0.14
Eosinophils *(mean, SD, ×10^9/L)*	0.003 (0.05)	--	-0.006 (0.07)	0.004 (0.04)	--	-0.02 (0.07)	0.99	--	0.73
IgE *(change, SD, IU/mL)*	2.2 (32.8)	--	-17.3 (134.2)	1.4 (17.5)	--	4.6 (31.6)	0.62	--	0.80

The overall TNSS score for the investigational group improved significantly, decreasing by 1.1 through day 14 (*p*-value = 0.002) (See **Figure 1** and **Table 3**). The overall change was also significant for the runny nose (P = 0.022) and itching/sneezing (P = 0.002) TNSS sub-scores. Analysis with the Wilcoxon signed-rank test found all three changes above to be significantly different from baseline at the 14-day mark (*p*-values ranging from 0.004 to 0.013), with one change (i.e., overall) being significant at day 7 (P = 0.033), and two changes (i.e., overall and itching/sneezing) being significant at 21 days (P = 0.017 and 0.025). TNSS also improved significantly in the control group overall and for all three sub-scores (P < 0.00001 for all four changes). When comparing TNSS changes week-over-week against baseline, no significant changes were observed between days 14 to 21 for either the control group (*p*-values ranging from 0.14 to 1.00) or the investigational group (*p*-values ranging from 0.27 to 0.67).

Although PNIF values improved for both the investigational and control groups, the change from baseline was significant in the control group (P = 0.00002) but not in the investigational group (P = 0.61). Similar to TNSS changes, PNIF values did not experience a significant change between days 14 to 21 for either the control group (P = 0.40) or the investigational group (P = 0.86).

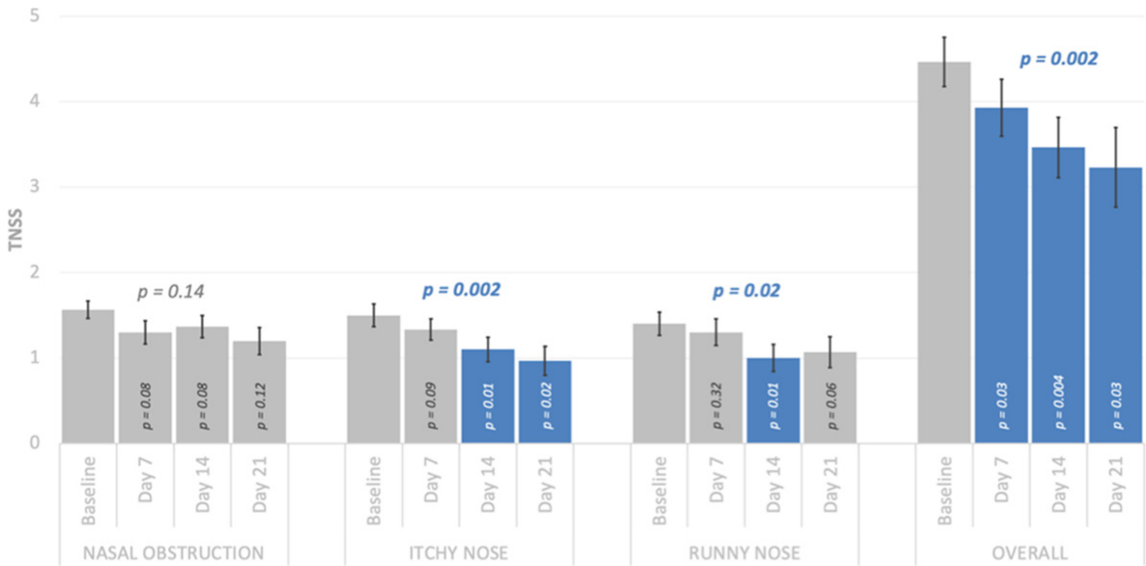

Figure 1. Comparison of TNSS from baseline to 7, 14, and 21 after the start of treatment. Bar represents the mean; whiskers represent positive and negative standard error. Blue text and bars represent statistical significance at the 95% level. The overall *p*-value was calculated using RM-ANOVA, while the change from baseline for each week was calculated using the Wilcoxon signed-rank test.

Table 3. Change in TNSS, PNIF values, eosinophils, and IgE from baseline to day 7/14/21 in the investigational group

Measurement	*p*-value			
	Overall*	Change from Baseline to Day...		
		7**	14**	21**
TNSS *(change, SD)*				
Nasal Obstruction	0.14	0.08	0.08	0.12
Itching/Sneezing	0.002	0.09	0.013	0.017
Secretion/Runny Nose	0.022	0.32	0.013	0.06
Overall	0.002	0.033	0.004	0.025
PNIF *(change, SD, in L/min)*	0.61	0.27	0.46	0.12
Eosinophils *(mean, SD, ×10^9/L)*	0.18	0.89	--	0.18
IgE *(change, SD, IU/mL)*	0.58	0.43	--	0.83

*repeated measures ANOVA; **Wilcoxon signed-rank test.

IgE and eosinophils levels both changed throughout the treatment period but never at a level deemed statistically significant.

16S profiling of saliva microbiome indicates AllerPops promotes oral probiotics

The amplicon was sequenced on Illumina paired-end platform to generate 250 bp paired-end raw reads (Raw PE) and then merged and pre-treated to obtain Clean Tags. The chimeric sequences in Clean Tags were detected and removed to obtain the Effective Tags, which can be used for subsequent analysis. Each sample had a minimum of 100,000 sequences.

Operational Taxonomic Units (OTUs) were obtained by clustering with 97% identity on the Effective Tags of all samples to study the microbial community composition in each sample. Then the level of the kingdom, phyla, class, order, family, genus, and species was identified. Each saliva sample has 215-658 observed species, averaging 341 (Table S1).

Clustering analysis was used to construct a cluster tree to study the similarity among different samples. The Unweighted Pair-group Method with Arithmetic Mean (UPGMA) is a hierarchical clustering method widely used in ecology to classify samples [30]. Nine participants (eight from the experimental group and one from the control group, Figure S2) have their baseline and final samples clustered next to each other, which indicates that the microbiome in the paired samples had minimal changes during the intervention. However, their TNSS score improvements are similar to other subjects (1.77 verse 1.73). This result

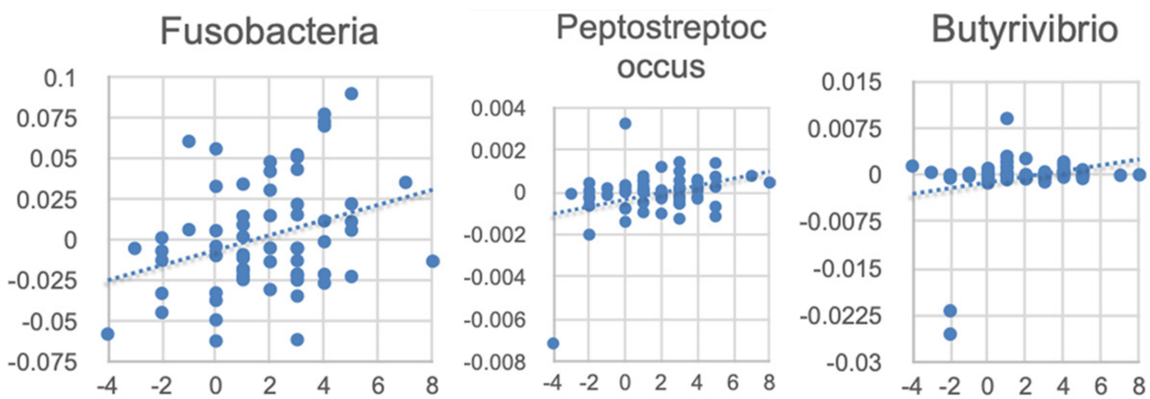

Figure 2. Positive correlation between TNSS score changes (x axle) and relative abundance changes (Y axle) of *Fusobacteria* (R = 0.32, P = 0.009), *Butyrivibrio* (R = 0.25, P = 0.044), and *Peptostreptococcus* (R = 0.34, P = 0.005). Blue, slow dissolving group; Red, fast ingestion group.

indicates that changes not detected by this method may contribute the clinical improvement.

The t-test to compare the relative abundance between treatment groups detected in the control group genus *Rothia* decreased after the intervention compared to baseline (from 4.0% to 2.6%, P = 0.024), and genus *unclassified_Clostridia_UCG-014* increased (from 0.1% to 0.2%, P = 0.049). The physiological significance of the change is unknown. Other differences between the two trial groups are likely unrelated to the treatment.

In addition to the standard analysis by Novogene, we performed a correlation test between changes in TNSS score and changes in relative abundance at the genus level. The abundance of three genera is positively correlated with the clinical symptom change (**Figure 2**) and determined to be statistically significant. The three genera are *Fusobacteria* (R = 0.32, P = 0.009), *Butyrivibrio* (R = 0.25, P = 0.044), and *Peptostreptococcus* (R = 0.34, P = 0.005). All three genera can produce short-chain fatty acids [31-33], a group of chemicals that can calm the immune system [34].

On average, the relative abundance of *Fusobacteria* increased by 0.17%, which was not statistically significant. In addition, one outlier for *Peptostreptococcus* and two for *Butyrivibrio* were recorded, with significantly reduced relative abundances. These outliers likely resulted from events unrelated to this trial. The two bacteria's relative abundance also increased on average for all other participants. Changes in the relative abundance of *Streptococcus* and *Veillonella* previously identified in our pilot study are insignificant in this trial data set.

Discussion

AllerPops prebiotic supplements relieve allergies within a week

The primary outcome of this clinical trial was to determine the effectiveness of AllerPops in relieving nasal symptoms at the day seven mark and to assess the impact of two different administration methods on the level of relief. Individuals receiving AllerPops as a potential treatment for allergic rhinitis subjectively experienced improvements in nasal symptoms, compared to their baseline value, in both their overall TNSS score and all three TNSS sub-scores.

This improvement occurred in both the control and investigational groups. The improvement in the overall score in the investigational group was statistically significant (P = 0.025), with the change in nasal obstruction showing the most improvement, decreasing by 0.3. Similarly, PNIF values showed an improvement of 28.8% or 6.7 L/min (P = 0.27) over the first seven days of treatment.

AllerPops provide sustained allergy relief

A secondary analysis was completed to assess the impact of treatment on symptoms beyond the seven-day mark and into weeks two and three. There was a significant (P = 0.002) positive effect of AllerPops on participant TNSS overall score, as well as for the itching (P = 0.002) and runny nose (P = 0.022) sub-scores. Results, broken down week-by-week, showed that a 14-day intervention period appeared superior, with 75% of sub-scores showing a significant change compared to baseline, and all four scores showing the most significant improvement compared to the 7-day and 21-day intervention periods (See **Figure 1**).

No significant changes were seen after day 14 in the investigational or control group for any TNSS score. As participants did not consume any product after day 13, this confirms that the initial symptom improvement was strictly due to AllerPops, regardless of how they were consumed. In addition, this finding strongly suggests that both sets of improvements persisted for at least one week after treatment.

In contrast, for PNIF values, while descriptive statistics revealed that participants did improve when slowly consuming AllerPops (i.e., left to melt in the mouth rather than swallowing), this difference was not significant (P = 0.61). For PNIF improvement, the maximum effect happened on day 21, with PNIF values improving by a mean of 8.7 L/min. Also, contrary to TNSS, the slightest change in PNIF values happened on day 14. For the group quickly consuming AllerPops, the change in PNIF values from baseline to day 21 was significant (P = 0.00002), with the change from days 14 to 21 not being significant (P = 0.40). This provides further evidence that improvement was due to AllerPops, regardless of how it was consumed, and that this improvement was sustained for at least one week after the product was last ingested.

However, AllerPops did not reach its full potential in the experiment group due to regulatory restraints. Most significantly, we could not introduce oral hygiene changes in the trial. The frequency of oral hygiene before and while taking AllerPops may have slowed improvement and negatively impacted the oral probiotics for some participants.

In addition, we could not formulate a placebo product that does not change oral microbiota because the potential ingredients, such as starch and sweeteners, can all be used by some bacteria, which may have led to unexpected results. For that reason, we compared two different application methods instead. We can design future studies with proper placebo control, as we have developed new products in other forms, such as prebiotic toothpaste.

AllerPops' allergy relief correlated with changes in saliva microbiome

The microbiome analyses of saliva samples collected from participants before, during, and after the intervention yield both consistencies and inconsistencies with our pilot study. The inconsistency is that we did not find that two genera, *Streptococcus* and *Veillonella*, became more abundant after using AllerPops. Instead, we found that the variations of three other genera, *Fusobacteria*, *Butyrivibrio*, and *Peptostreptococcus,* positively correlate with improving clinical symptom scores. These three genera can all produce SCFAs, pacifying the immune system [34]. These results are consistent with our previously proposed etiological theory on allergic rhinitis. Oral probiotic deficiency, or the lack of probiotics that produce metabolites, such as SCFA, causes the immune system to become hypersensitive, leading to allergic inflammation [25].

The fact that all three bacterial genera that correlated with the shift of clinical symptoms are SCFA producers further confirms the hypothesis. There are 13 known SCFA producers among the 49 most common genera analyzed. The chance that all three associated genera are SCFA producers is 1.55% in this analysis. Insufficient SCFA is likely the major, if not the only, microbe-related mechanism leading to allergies.

The new results indicate that the human immune system and microbiota interaction is very complicated. Multiple bacterial genera participate in the process of calming down the immune system. We previously regarded these bacteria as negative triggers for the human immune system. It is also possible that these bacteria are simply the balancing force against positive triggers, likely MAMPs (microbial-associated molecular pattern) from commensal bacteria. In this and previous studies, we identified five genera as balancing forces of the immune system. Other common oral bacteria, such as genera *Porphyromonas*, *Prevotella*, and so on [31, 35], which can also produce SCFAs likely contribute to the negative control of the immune system.

Each of the thirteen oral SCFA producers may contribute only a tiny portion of the negative control to the immune system in and around the mouth, airway and upper digestion tract. This is likely part of the explanation for why previous studies have not consistently associated any of the bacteria with allergies [36]. The situation is similar in the gut with many SCFA-producing bacteria. Many studies recognize the importance of these groups of gut bacteria in health and preventing diseases [37]. A recent study identified a reduced relative abundance of *Prevotella* and *Veillonella* in the mouth of people with peanut allergies, which is consistent with our observation and supports the hypothesis that food allergies and allergic rhinitis may have a common root cause [38].

These three bacterial genera, *Fusobacteria*, *Butyrivibrio*, and *Peptostreptococcus,* are part of the oral microbiome in all participants and almost all 205 samples except two, likely due to sampling bias of insufficient genera *Butyrivibrio*. Most of these bacteria are anaerobic, can live in the pocket between the teeth and gums, and have been identified in sites of periodontal disease [39].

On the one hand, these bacteria at sufficient levels can prevent allergies. Conversely, they may worsen things when chronic gum infections occur. Proper oral hygiene is likely the key to keeping these bacteria in check and staying on the good side of allergy prevention with minimal harm to the teeth and gum. Further studies are needed to redefine the boundary of the best practice for oral hygiene for professionals and the general public.

A balance of positive and negative triggers to the immune system is necessary to achieve a healthy state. As Snyder et al.'s germ-free mice experiment demonstrated, a balanced condition can be one with no interaction on either side [40]. We discussed how the balance falters during diseases in our pilot study [25]. Briefly, on one side, acute infection, fever, and diarrhea remove probiotics so that the immune system can fight infectious agents without restraints. This suggests that one should not treat fever and diarrhea prematurely before infections fade. On the other side, chronic infections may arise from probiotic overgrowth.

Oral probiotic deficiency causes allergic rhinitis

We have identified several bacterial genera that likely contribute to initiating allergic rhinitis and have regarded them as probiotics for their capacity to produce SCFA and calm down the immune system. The first critical function of a microbiota associated with a host should be communicating "peace" with the host. Bacteria in this role should (1) not cause immediate harm to the host and (2) be able to send pacifying signals to the host's immune system. In humans, this communication is likely achieved through SFCA-producing bacteria. These bacteria include *Streptococcus*, *Veillonella*, *Fusobacteria*, *Porphyromonas*, and so on in the mouth and airway, fiber-degrading bacteria in the gut, and *Cutibacterium acnes* on the skin. However, it is vital to remember that these probiotics are not always good and may contribute to pathogenicity in certain disease conditions.

These SCFA-producing probiotics are negative immune system triggers, and removing and reapplying them can be relatively easy and quick. Theoretically, the effects of the negative trigger should not last long after removing it. Otherwise, the immune system's power cannot be released in time to protect the host. Many different cells in the human body can absorb and metabolize SCFAs, which can rapidly decrease their levels. Several observations support this proposition. (1) The epidemic of common allergies progresses reasonably quickly in a short time at a national level. (2) Oral biofilm disappears in less than a day under moderate fever. (3) The prebiotic compound promotes oral probiotics to produce fast and lasting allergy relief.

It was hypothesized that immune-mediated disorders such as allergic rhinitis are linked to reduced early microbial exposure, gut dysbiosis, and nasal and oral microbiota. AllerPops contains L-Arginine, which is capable of providing balanced nutritional support and restoration to the oral microbiota, alleviating AR symptoms. The results from this trial and our pilot study link the cause of allergic rhinitis to oral microbiota, the lack of good bacteria, or oral probiotic deficiency, causes allergic rhinitis. Additional testing should be performed to verify this theory on a larger scale.

Overall, this study found AllerPops safe and effective in individuals with allergic rhinitis, whether they swallowed the product or allowed it to melt in their mouth. Oral hygiene requirements in the treatment group and the method of consumption for the control group may have limited the impact and effectiveness of the investigational product.

Summary

Allergic rhinitis is commonly associated with an itchy, stuffy, or runny nose. Whether consumed quickly or slowly, AllerPops improved allergy symptoms significantly and airflow in the nasal airway. Furthermore, these improvements did not deteriorate one week after investigational product consumption was terminated. AllerPops's capability to promote oral probiotics enables sustained allergy relief.

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Disclosure of conflict of interest

Cliff Shunsheng Han is the founder and Owner of AllerPops Corp.

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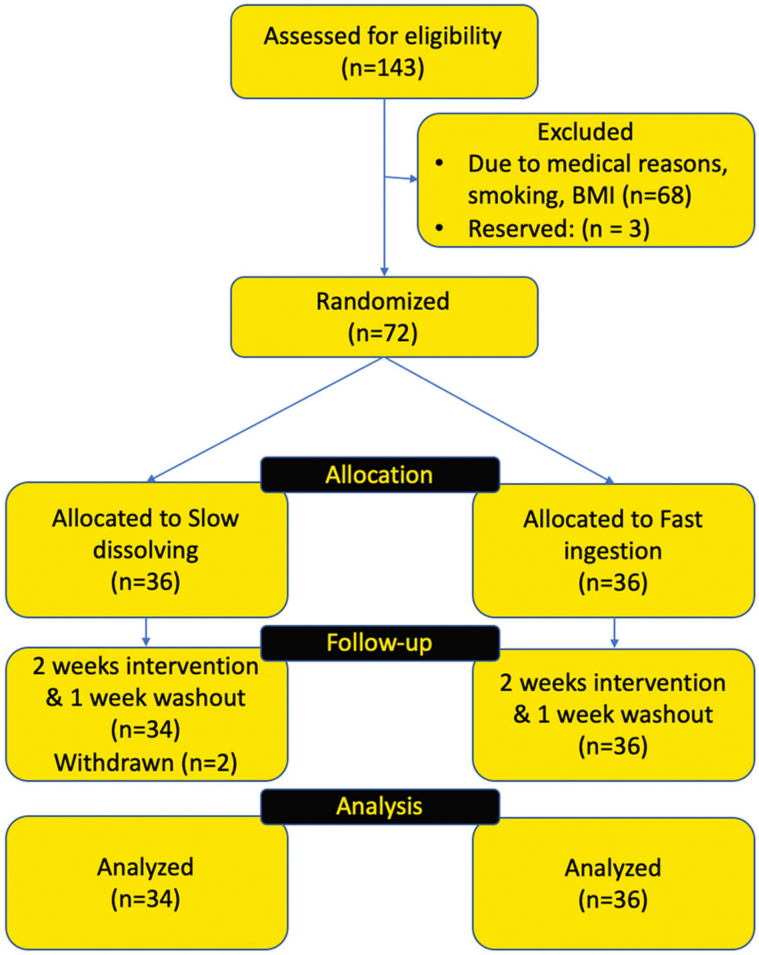

Figure S1. CONSORT diagram of the clinical trial.

Table S1. Alpha diversity indices

Sample	Observed species	Shannon	Simpson	Chao1	ACE	Goods coverage	PD_whole_tree
OAO.003.V2	397	5.438	0.955	505.778	492.534	0.999	36.192
OAO.003.V3	342	4.617	0.923	413	404.043	0.999	27.375
OAO.003.V5	330	5.028	0.943	399.837	412.543	0.999	24.984
WKE.001.V2	354	5.253	0.949	424.222	435.868	0.999	37.577
WKE.001.V3	284	4.92	0.936	365.476	335.915	0.999	21.935
WKE.001.V5	291	5.344	0.953	324.6	328.664	0.999	26.451
J.C.007.V2	266	4.521	0.917	301.25	308.305	0.999	19.727
J.C.007.V3	324	4.792	0.924	395.5	382.606	0.999	22.979
MRB.005.V2	315	4.455	0.904	348.6	348.092	0.999	22
MRB.005.V3	295	4.415	0.913	346.037	335.366	0.999	19.693
MRB.005.V5	308	4.718	0.932	385.656	381.933	0.999	22.3
M.S.006.V2	300	4.989	0.934	319.194	323.168	1	20.534
M.S.006.V3	658	4.918	0.914	821.456	856.781	0.998	50.155
M.S.006.V5	567	4.434	0.886	739.068	732.48	0.998	44.672
GBM.002.V2	519	4.644	0.914	651	661.355	0.998	40.206
GBM.002.V3	544	4.198	0.851	737.5	746.441	0.998	45.482

GBM.002.V5	484	4.622	0.921	637.3	655.692	0.998	41.478
JKL.013.V2	412	3.735	0.841	547	575.394	0.998	52.05
JKL.013.V3	469	4.621	0.91	576.408	591.117	0.998	43.569
PJC.020.V2	569	5.3	0.944	659.75	668.592	0.999	60.565
PJC.020.V3	516	4.942	0.921	641	628.939	0.998	38.131
PJC.020.V5	362	5.59	0.961	398.692	403.066	0.999	27.208
TMD.028.V2	735	4.753	0.848	876.117	880.454	0.998	99.532
TMD.028.V3	396	4.329	0.829	442.243	438.663	0.999	42.43
TMD.028.V5	430	5.532	0.948	481	486.639	0.999	59.962
A.T.023.V2	392	5.221	0.919	422.692	438.016	0.999	35.556
A.T.023.V3	388	5.555	0.953	484.871	463.245	0.999	43.316
A.T.023.V5	336	4.887	0.903	360.6	363.389	0.999	28.539
F.W.025.V2	288	4.309	0.909	321.786	317.945	0.999	24.153
F.W.025.V3	321	4.961	0.942	354.176	354.29	0.999	31.76
F.W.025.V5	369	5.097	0.941	407.5	411.078	0.999	32.026
D.H.022.V2	332	4.991	0.943	366.895	372.681	0.999	21.701
D.H.022.V3	342	4.948	0.931	409.097	399.452	0.999	29.052
G.S.047.V2	387	4.963	0.937	443.269	454.154	0.999	37.753
G.S.047.V3	343	4.308	0.871	404.737	403.428	0.999	29.916
G.S.047.V5	430	5.371	0.944	493.2	505.598	0.999	34.442
J.N.029.V2	364	5.095	0.944	425.568	424.372	0.999	25.991
J.N.029.V3	314	4.805	0.935	371.188	367.961	0.999	26.526
J.N.029.V5	388	5.294	0.946	426.077	425.39	0.999	39.171
C.M.021.V2	343	4.968	0.933	397.486	399.242	0.999	23.93
C.M.021.V3	327	5.075	0.943	364.276	360.218	0.999	21.828
C.M.021.V5	341	5.197	0.95	393.528	401.829	0.999	24.208
T.A.034.V2	352	5.181	0.934	419.536	402.126	0.999	41.797
T.A.034.V3	337	5.368	0.948	373.029	374.784	0.999	41.789
T.A.034.V5	375	5.609	0.958	446.079	441.294	0.999	33.865
BLS.035.V2	318	5.106	0.95	363.026	368.897	0.999	20.478
BLS.035.V3	311	5.01	0.944	370.429	372.441	0.999	21.004
BLS.035.V5	220	4.14	0.906	277.955	265.526	0.999	17.872
BSB.044.V2	330	5.234	0.955	410	387.478	0.999	22.818
BSB.044.V3	322	5.06	0.95	425.552	405.72	0.999	22.714
BSB.044.V5	292	5.014	0.944	323.41	337.657	0.999	20.763
C.N.045.V2	300	4.85	0.941	343.75	341.146	0.999	19.026
C.N.045.V5	286	4.799	0.937	315.562	318.37	0.999	18.176
T.V.031.V2	341	4.953	0.931	383.088	377.76	0.999	25.977
T.V.031.V3	311	4.821	0.93	351.182	349.987	0.999	22.955
T.V.031.V5	339	5.056	0.935	377.886	394.854	0.999	27.738
H.B.032.V2	323	4.778	0.925	353.154	358.395	0.999	29.403
H.B.032.V3	254	4.432	0.898	300.312	281.919	1	20.859
H.B.032.V5	206	4.316	0.897	239	230.191	1	16.539
W.K.014.V2	299	3.883	0.878	409.25	443.503	0.999	33.238
W.K.014.V3	307	4.396	0.887	354.517	353.77	0.999	19.197
W.K.014.V5	334	5.279	0.954	385.037	375.544	0.999	22.321
C.T.041.V2	326	4.302	0.899	383.303	379.337	0.999	26.35
C.T.041.V3	340	4.42	0.887	392.528	396.434	0.999	25.872
C.T.041.V5	369	4.295	0.883	410.109	418.554	0.999	50.205

T.S.053.V2	309	4.447	0.904	362.2	361.746	0.999	19.87
T.S.053.V3	330	5.042	0.937	380.324	387.337	0.999	22.966
T.S.053.V5	301	4.745	0.92	339.077	337.029	0.999	19.377
FMG.056.V2	323	4.965	0.942	386.103	381.138	0.999	22.29
FMG.056.V3	311	5.382	0.944	350.808	350.379	0.999	33.097
FMG.056.V5	282	4.876	0.938	348.3	327.733	0.999	20.903
F.F.051.V2	274	3.933	0.859	310.75	312.326	0.999	19.765
F.F.051.V3	280	4.19	0.899	333.2	328.486	0.999	19.285
F.F.051.V5	300	4.509	0.914	357.097	360.125	0.999	29.576
COD.050.V2	315	4.542	0.909	345.028	347.713	0.999	25.618
COD.050.V3	389	4.629	0.892	489.636	463.151	0.999	36.574
COD.050.V5	280	4.134	0.893	337.652	318.805	0.999	20.74
FWD.057.V2	258	4.386	0.914	307.345	310.23	0.999	18.445
FWD.057.V3	289	4.503	0.924	362.182	365.186	0.999	20.024
FWD.057.V5	248	4.451	0.926	305.115	304.965	0.999	16.137
JBS.058.V2	250	4.218	0.893	272.941	279.862	1	16.445
JBS.058.V3	244	4.113	0.883	278.138	278.3	0.999	17.368
JBS.058.V5	333	5.266	0.951	344.6	349.001	1	22.911
EPE.067.V2	249	3.313	0.761	268.345	272.72	1	18.432
EPE.067.V3	313	4.442	0.9	341.286	344.729	0.999	25.733
EPE.067.V5	302	4.881	0.934	352.324	360.009	0.999	33.051
T.I.060.V2	337	5.293	0.946	380.062	381.87	0.999	29.833
T.I.060.V3	345	5.38	0.953	379.071	387.964	0.999	25.019
T.I.060.V5	345	5.065	0.928	377.237	378.384	0.999	34.5
L.G.038.V2	307	4.837	0.929	344.935	347.584	0.999	21.138
L.G.038.V3	303	4.742	0.924	330.364	333.434	0.999	19.131
L.G.038.V5	282	4.72	0.929	319.273	310.416	1	19.198
M.S.069.V2	307	4.845	0.938	334.237	342.566	0.999	22.367
M.S.069.V3	339	4.947	0.942	376.125	385.024	0.999	21.406
M.S.069.V5	322	4.881	0.939	385.103	370.348	0.999	22.954
SJL.074.V2	318	4.921	0.935	344.559	346.778	0.999	20.866
SJL.074.V3	308	4.978	0.943	344.964	341.404	0.999	21.884
SJL.074.V5	349	4.728	0.913	414.086	412.636	0.999	25.59
S.P.061.V2	369	4.876	0.921	416.229	409.148	0.999	26.507
S.P.061.V3	349	5.048	0.931	389.286	380.44	0.999	24.148
S.P.061.V5	380	5.431	0.954	420.923	426.82	0.999	25.462
AAG.048.V2	369	4.46	0.892	370.813	376.702	1	33.78
AAG.048.V3	346	5.287	0.953	378.229	376.994	0.999	22.858
AAG.048.V5	256	4.255	0.891	292.964	294.861	0.999	25.279
DLL.073.V2	248	3.925	0.851	287.516	295.709	0.999	27.863
DLL.073.V3	312	4.751	0.927	367.618	373.404	0.999	20.461
DLL.073.V5	230	4.272	0.9	260.37	268.083	1	23.561
K.D.082.V2	273	4.566	0.923	304.935	308.853	0.999	23.33
K.D.082.V3	320	4.687	0.922	358.897	355.347	0.999	25.124
K.D.082.V5	312	4.384	0.9	349.243	354.711	0.999	26.114
C.T.036.V2	321	4.992	0.941	354.15	362.047	0.999	22.435
C.T.036.V3	311	4.906	0.94	343.81	354.875	0.999	23.692
C.T.036.V5	253	4.625	0.935	288.636	295.103	0.999	21.126
A.K.100.V2	284	4.313	0.913	360.25	341.266	0.999	22.527

A.K.100.V3	302	4.603	0.921	344.5	341.135	0.999	21.622
A.K.100.V5	293	4.446	0.915	371.964	360.466	0.999	20.91
E.P.076.V2	319	4.736	0.921	357.927	371.028	0.999	20.832
E.P.076.V3	328	4.987	0.925	362.138	360.007	0.999	24.492
E.P.076.V5	339	4.981	0.921	384.294	383.193	0.999	35.129
A.M.094.V2	333	4.63	0.895	366.108	369.685	0.999	23.512
A.M.094.V3	312	4.433	0.899	361.763	368.986	0.999	29.053
A.M.094.V5	343	4.193	0.855	387.688	402.395	0.999	29.52
A.M.104.V2	331	4.481	0.905	404.312	400.221	0.999	24.91
A.M.104.V3	338	4.624	0.913	362.07	370.844	0.999	22.702
A.M.104.V5	331	4.928	0.93	359.219	357.892	0.999	34.285
R.C.102.V2	358	5.102	0.943	409.383	420.928	0.999	53.637
R.C.102.V3	287	4.587	0.917	316.129	314.789	0.999	22.212
R.C.102.V5	313	4.828	0.928	357.333	363.147	0.999	30.836
MKR.064.V2	324	5.178	0.95	384.273	363.026	0.999	20.42
MKR.064.V3	296	4.852	0.937	333.935	333.948	0.999	25.457
MKR.064.V5	280	4.891	0.939	344.565	330.84	0.999	20.614
TSC.090.V2	329	5.024	0.937	370.438	366.65	0.999	20.525
TSC.090.V3	416	4.522	0.904	478.341	468.095	0.999	35.943
TSC.090.V5	356	5.092	0.939	401.818	413.192	0.999	38.791
NYT.070.V2	414	4.596	0.904	443.518	451.13	0.999	41.175
NYT.070.V3	215	4.314	0.907	231.24	237.708	1	29.123
NYT.070.V5	273	4.169	0.878	313.552	315.666	0.999	18.784
T.H.072.V2	377	5.361	0.952	477.037	442.07	0.999	51.75
T.H.072.V3	305	4.571	0.892	341.033	338.836	0.999	25.602
IJT.096.V2	306	4.715	0.919	366.273	348.228	0.999	27.172
IJT.096.V3	325	4.847	0.933	376.333	366.353	0.999	23.243
IJT.096.V5	294	4.655	0.933	353.032	352.654	0.999	26.458
R.L.097.V2	338	4.65	0.917	382.25	385.843	0.999	32.958
R.L.097.V3	311	4.637	0.904	349.636	352.695	0.999	20.494
R.L.097.V5	456	4.709	0.899	519.984	525.58	0.999	45.254
KTS.086.V2	272	4.136	0.891	310.281	310.983	0.999	24.788
KTS.086.V3	402	4.803	0.927	460.882	468.244	0.999	87.441
KTS.086.V5	294	4.639	0.93	309.283	319.744	1	23.926
B.H.093.V2	349	4.956	0.94	418.256	416.892	0.999	49.439
B.H.093.V3	298	4.528	0.907	339.438	337.729	0.999	42.133
B.H.093.V5	272	4.406	0.916	304.758	310.142	0.999	19.561
M.C.118.V2	427	4.551	0.891	472.56	473.414	0.999	45.161
M.C.118.V3	310	4.488	0.897	335.8	339.576	0.999	23.837
M.C.118.V5	318	4.187	0.884	377.094	373.218	0.999	25.692
C.V.116.V2	251	4.264	0.891	267.034	270.518	1	27.052
C.V.116.V3	233	4.175	0.886	270.625	270.127	0.999	21.075
C.V.116.V5	321	5.125	0.942	350.467	362.691	0.999	32.079
SHS.081.V2	240	4.278	0.907	283.062	292.388	0.999	24.59
SHS.081.V3	251	3.67	0.848	276.324	284.008	0.999	22.743
SHS.081.V5	294	4.855	0.933	332.333	326.747	0.999	35.238
D.N.124.V2	497	4.661	0.9	597.846	598.363	0.999	43.021
D.N.124.V3	308	5.013	0.938	318.324	323.15	1	28.231
D.N.124.V5	353	5.045	0.926	389.273	402.309	0.999	33.68

A.R.115.V2	481	4.501	0.902	548.9	563.153	0.999	38.062
A.R.115.V3	354	5.338	0.954	390.429	390.554	0.999	28.558
A.R.115.V5	372	5.062	0.943	469.9	460.435	0.999	34.753
A.K.114.V2	319	5.302	0.954	349	350.254	0.999	21.627
A.K.114.V3	306	5.136	0.955	344.278	346.355	0.999	26.768
A.K.114.V5	307	5.324	0.96	340.387	338.838	0.999	27.937
N.A.132.V2	312	4.719	0.911	354.774	355.699	0.999	33.485
N.A.132.V3	313	5.016	0.938	340.774	342.939	0.999	21.982
N.A.132.V5	605	5.247	0.933	697.959	699.9	0.999	52.087
T.Y.105.V2	282	4.898	0.929	310.636	306.985	1	19.968
T.Y.105.V3	294	4.377	0.891	319.8	328.474	0.999	22.524
T.Y.105.V5	272	4.281	0.879	296.667	296.921	1	25.455
S.A.092.V2	276	4.407	0.903	311.69	311.97	0.999	20.177
S.A.092.V3	318	5.179	0.947	350.047	364.427	0.999	21.025
S.A.092.V5	302	4.784	0.925	345.676	355.284	0.999	27.828
J.N.099.V2	503	5.461	0.954	586.081	598.959	0.999	42.663
J.N.099.V3	297	4.818	0.937	330.781	331.409	0.999	20.872
J.N.099.V5	310	5.309	0.95	327	331.412	1	27.938
NCA.119.V2	287	4.054	0.892	316.562	315.633	0.999	21.025
NCA.119.V3	346	4.874	0.937	391.917	387.629	0.999	24.647
NCA.119.V5	346	4.536	0.913	402	397.223	0.999	24.611
M.P.131.V2	284	5.114	0.949	301.233	314.161	1	19.506
M.P.131.V3	298	4.782	0.933	326.447	332.498	0.999	24.57
JJD.121.V2	522	5.17	0.932	601.217	601.751	0.999	35.044
JJD.121.V3	528	5.26	0.94	571.13	582.962	0.999	36.512
JJD.121.V5	490	4.653	0.896	661.651	612.218	0.999	33.852
AMM.134.V2	291	4.921	0.94	325.182	335.113	0.999	22.955
AMM.134.V3	290	4.653	0.929	330.286	329.289	0.999	21.931
AMM.134.V5	308	4.859	0.937	357.214	353.187	0.999	22.01
D.S.135.V2	334	4.752	0.916	420.059	413.516	0.999	24.218
D.S.135.V3	253	4.006	0.844	281.889	281.883	1	16.975
D.S.135.V5	270	4.801	0.94	309.808	305.912	0.999	21.764
SML.138.V2	500	4.852	0.927	603.109	590.12	0.999	33.248
SML.138.V3	605	5.069	0.912	668.462	678.739	0.999	37.032
SML.138.V5	473	4.884	0.926	555.787	570.139	0.999	31.604
J.Y.136.V2	475	4.848	0.929	570.509	565.913	0.999	38.185
J.Y.136.V3	305	3.909	0.879	349.8	358.781	0.999	28.321
J.Y.136.V5	453	4.696	0.915	514.873	515.593	0.999	30.09
SSO.143.V2	428	4.317	0.891	472.531	482.299	0.999	32.111
SSO.143.V3	438	4.043	0.853	513.362	522.751	0.999	33.396
SSO.143.V5	384	4.015	0.865	411.051	419.746	0.999	30.831

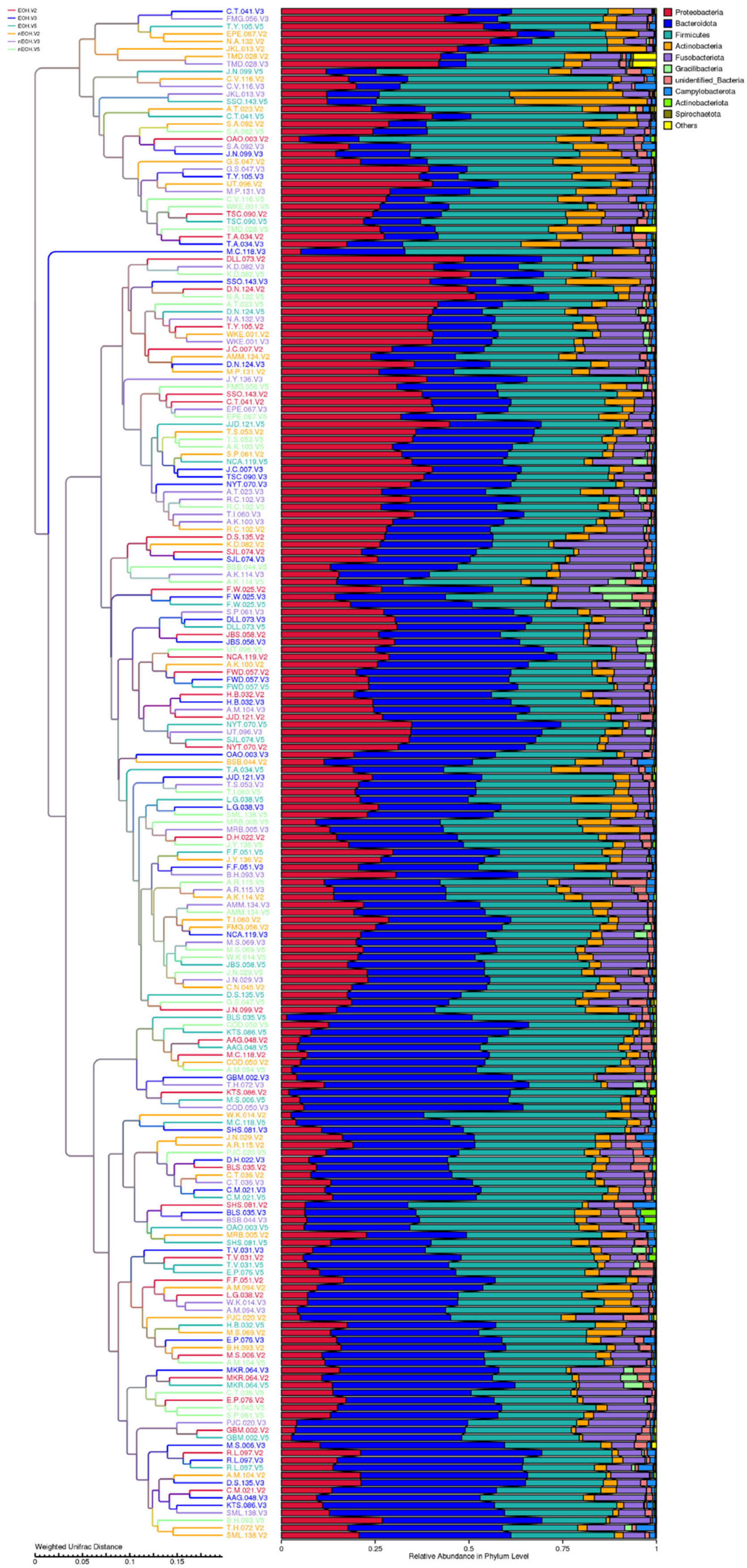

Figure S2. UPGMA cluster tree based on Weighted Unifrac distance.