Role of Nitrate reducing Bacteria found in the Oral Cavity of Adolescents with dietary nitrate consumption

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Abstract

- Studies demonstrated that a nitrate-rich diet for humans is associated with many beneficial physiological outcomes. Green leafy vegetables and processed meat are the predominant nitrate sources in human diet.
- We hypothesize that there will be a positive correlation between nitrate-rich food and the abundance of nitrate-reducing bacteria (NRB) in adolescents' oral cavities. Therefore, this study focused on determining oral microbiomes and their potential implications on cardiovascular health.
- We conducted a survey of high-school participants to collect background information on participants cardiovascular disease (CVD) history and eating habits, then completed the DNA extraction and isolation process with their saliva samples.
- We assessed the percent abundance of each microbial species identified at the genus and some at the species level, which revealed that the low abundance of NRBs in these samples may be due to many inconsistent factors. We observed a weak correlation between the dietary nitrate consumption and the percent abundance of NRBs.
- Larger set of salivary microbiomes generated from high-school adolescents along with their dietary nitrate intake will provide useful insights in the future to predict the role of NRBs in health recommendations.

Introduction

- ***Hypothesis:*** dietary nitrate intake can change the abundance of nitrate reducing bacteria in adolescences
- NRB located in the back of the tongue (the oral commensals), convert nitrate (NO_3^-) to nitrite (NO_2^-) [1] and then nitric oxide (NO) through nitrate oxide synthase pathway (NOS)[2]
- There is a positive correlation between the oral NRBs and exogenous nitrite production in human saliva[3].
- Salivary NO acts as an antimicrobial defense system for the mouth and is positively associated with vascular[4] and metabolic functions[3] to potentially prevent cardiovascular diseases (CVD)[5, 6].
- African Americans and Mexican Americans have most disproportionate rates of CVD[7].
- Highest coronary heart disease (CHD) rate is reported for black women[8].

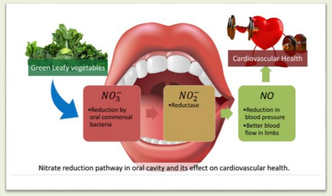


Figure 1. Nitrate reduction pathway.

Materials and Methods

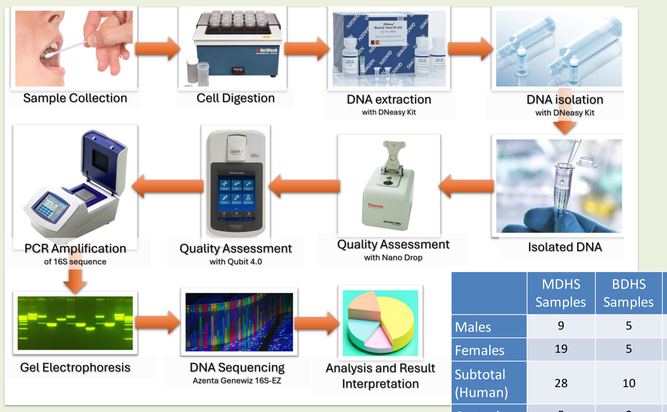


Figure 2. DNA Sample Collection and Processing.

	MDHS Samples	BDHS Samples	Total Number of Samples Collected
Males	9	5	14
Females	19	5	24
Subtotal (Human)	28	10	38
Control	5	3	8
Total Collected	33	13	46

Table 1. Demographics of the collected samples.

Results

Sample	Ethnicity	Sex	Family CVD	Greens	Freq. (1-4)	Qubit 4 (ng/µL)	Nanodrop (ng/µL)
MDHS11-1	Asian	F	Y	Lettuce	4	5.98	8.8
MDHS14-1	White	F	Y	Broccoli	2	7.90	14.4
BDHS07-1	Hispanic/Latino	F	Y	Broccoli	2	18.10	28.4
MDHS06-1	Asian	F	N	Cabbage	4	20.00	37.6
BDHS03-1	Asian	F	Y	Bok Choy	4	4.40	12.9
BDHS09-1	African American	M	N	Broccoli, BruSpr.	4	13.00	36.0
FS4.29-1	Asian	F	Y	Spinach	4	1.57	2.9
MDHS17-1	Asian	M	Y	Spinach, lettuce	2	3.74	19.1

Table 2. Samples were selected for 16S-EZ metagenomic sequencing with dietary and demographic information. Quantification of the extracted salivary DNA indicated.

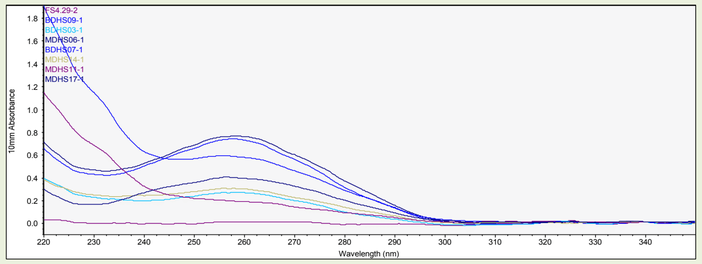


Figure 4. Spectrophotometer quantification graphs depicting the absorbance at 260 nm. The list of samples overlayed here are the samples selected for metagenomic sequencing.

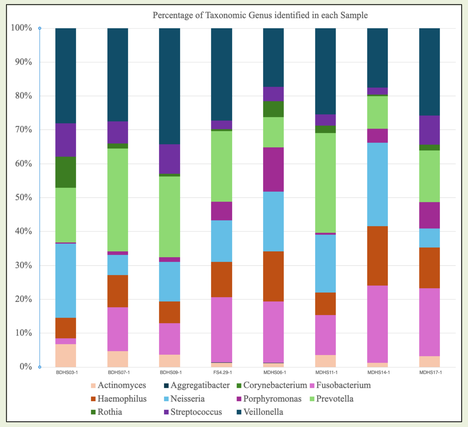
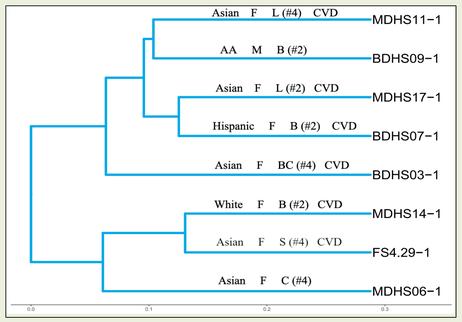


Figure 5. Displays the percentage of taxonomic genus identified in each sample.



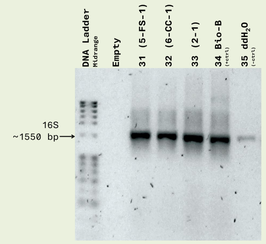


Figure 3. Representative 2% Agarose gel electrophoresis of the PCR products.

Sample	ace	chao1	shannon	simpson	goods_coverage
BDHS03-1	322.264	307.06	4.228	0.909	1
BDHS07-1	454.661	468.565	4.671	0.93	0.999
BDHS09-1	451.386	457.818	4.918	0.945	1
FS4.29-1	380.567	383.27	4.621	0.924	1
MDHS06-1	523.124	524.31	4.85	0.935	0.999
MDHS11-1	491.432	491.432	5.102	0.952	1
MDHS14-1	412.177	404.06	4.036	0.891	0.999
MDHS17-1	505.573	509.85	4.751	0.937	0.999

Table 3. Alpha Diversity table that demonstrates the range of diversity observed with each sample.

Figure 6. Phylogenetic tree that displays the clusters between samples based on abundance of genus species and the background data of each sample. AA, African American; F, Female; M, Male; CVD, Cardiovascular disease; B, Broccoli; BC, Bok choy; C, cabbage; L, Lettuce; S, Spinach; #, indicates the frequency of intake of these vegetables in a week.

Discussion

- **Figure 3** indicated the PCR-amplified, strong 16S rDNA band in the agarose gel electrophoresis.
- The Nanodrop Spectrophotometer graph indicated the quality of DNA concentration, a ratio of 260/280 nm above 1.8 resulted in a clean sample with few solvent contamination (**Fig. 4**).
- BDHS03-1 and MDHS14-1 have a higher percentage of the genus *Neisseria* (**Fig. 5**).
- Percentage of *Prevotella* greatly varies amongst all the samples (**Fig. 5**).
- Abundance of *Actinomyces* and *Rothia* remains mostly similar amongst all the samples, except slightly higher percentage observed in BDHS03-1 (**Fig. 5**).
- The low frequency of dietary nitrate intake as seen in MDHS14-1 and BDHS07-1 also correlated with varied abundance of NRBs in these samples (**Fig. 5**).
- The phylogenetic tree demonstrates the similarity coefficients between each sample, and the varied similarity between sample clusters of high frequency of dietary nitrate intake and low frequency of dietary nitrate intake demonstrate a weak correlation between the dietary nitrate consumption and similar NRBs (**Fig. 6**).

- The alpha diversity table **(Table 3)** from the shannon index demonstrates how sample MDHS11-1 has the highest diversity of NRB species of 5.102, possibly due to high frequency of dietary nitrate intake **(Table 1)**.
- Bioinformatical analyses flow-chart can be performed from the metagenomic sequencing to yield useful insights about the DNA collection from a typical sample in order to understand functional genomics **(Fig. 8)**.
- Greater DNA yield could be obtained through using tools such as a tongue scraper or plastic knife.
- Collecting and processing more samples may help establish a clearer relationship between NRB across ethnic, racial, and gender lines.

Figure 8. Analysis for metagenomics sequencing.

References

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