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Biochemical and Molecular Analyses of Bacillus spp in Infant Fecal Samples in Internally Displaced Persons (IDP) Camps in Guma Local Government Area, Benue State, Nigeria

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Abstract: **Deoxyribonucleic acid (DNA) sequencing technologies have revolutionized the study of the gut microbiota, allowing for detailed characterization of microbial communities. Techniques such as 16S rRNA gene sequencing enable researchers to identify and quantify bacterial populations in fecal samples with high precision. In this study, biochemical and molecular analyses of Bacillus spp in infant fecal samples in selected IDP camps in Guma local government area, Benue State, North central Nigeria was carried out. Stool samples were randomly collected from infants aged 7 to 12 months using sterile, leak-proof containers. Participants were each provided with an ice bag containing an emesis basin (Ref. 104AA200, PRIM S.A Spain), a 50-mL sterile sampling bottle (Ref. 409526.1, Deltalab, Spain), and a sterile spatula (Ref. 441142.2, Deltalab, Spain). A total of two hundred (200) stool samples were collected from ten sampling locations, 20 samples per location in the three IDP camps. Each sample was sealed and appropriately labeled and transported to the Microbiology Laboratory, Department of Microbiology, Joseph Sawuan Tarka University Makurdi for bacteriological evaluation. DNA was extracted from a sample using a modified protocol. Polymerase Chain Reaction (PCR) was performed in a thermocycler (model). The sequence library was prepared using the Oxford nanopore (ONT) SQK-RBK114-24 protocol. Biochemical tests presented confirmed the rod-shaped isolates as *Bacillus* spp. The isolates tested positive to catalase, citrate, nitrate reduction, mannitol, glucose and gram reaction while taking its normal rod shape structure. Seven (7) samples analyzed genotypically using PCR were confirmed as *Bacillus* spp. Results revealed the presence of three species of *Bacillus; Bacillus siamensis, Bacillus subtilis* and *Bacillus velezensis* in the seven samples. *Bacillus siamensis* strain Bk6y1 and *Bacillus velezensis* strain GBWR73 were strains of *B. siamensis* and *velezensis* respectively. They were obtained from samples at Daudu camp 1 and Uikpiam camp respectively. *Bacillus subtilis* dominated the samples (5 out of 7) with different strains at Daudu camp 1 and 2. The strains were: *B. subtilis* strain CS-P-4, *B. subtilis* strain BS34A, *B. subtilis* subsp. subtilis AZFS, *B. subtilis* strain HSB2 and *B. subtilis* strain PSBC28. Sequence results of the 16sRNA revealed the isolates as three species of *Bacillus.* They were *Bacillus siamensis, Bacillus subtilis* and *Bacillus velezensis*. *Bacillus subtilis* dominated the samples with different strains including *B. subtilis* strain CS-P-4, *B. subtilis* strain BS34A, *B. subtilis* subsp. subtilis AZFS, *B. subtilis* strain HSB2 and *B. subtilis* strain PSBC28.**

Keywords: **Deoxyribonucleic acid (DNA), Microbiology Laboratory, Polymerase Chain Reaction (PCR).**

1. INTRODUCTION

Firmicutes are a major phylum of bacteria within the gut microbiota and are essential for maintaining a healthy gut environment. They are involved in the fermentation of indigestible polysaccharides, producing SCFAs such as butyrate, which serve as a primary energy source for colonocytes and help maintain the integrity of the gut lining (Louis *et al.*, 2010; Flint *et al.*, 2012). The balance between Firmicutes and other bacterial phyla, such as Bacteroidetes, is critical for gut

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homeostasis and metabolic health. Disruptions in this balance have been linked to various health conditions, including obesity, inflammatory bowel disease, and metabolic syndrome (Turnbaugh *et al.*, 2009). Understanding the factors that influence the abundance and activity of Firmicutes is thus vital for promoting gut health.

Infants have a developing gut microbiome that is highly susceptible to environmental and dietary influences (Penders *et al.*, 2006). Geophagy, which is practiced in many cultures as a means of nutritional supplementation or to alleviate gastrointestinal discomfort, could potentially affect the gut microbiota of infants. The consumption of soil and clay materials may introduce various minerals and elements that can impact microbial growth and activity. For example, clays have been shown to have binding properties that can modulate gut flora by adsorbing toxins and providing a substrate for beneficial bacteria (Dominy *et al.*, 2004). However, the specific effects of geophagy on the populations of Firmicutes in the infant gut microbiota remain underexplored.

While the direct impact of geophagy on Firmicutes populations in infants is not well-documented, studies on related topics provide some insights. For instance, research on dietary interventions has shown that certain fibers and prebiotics can selectively stimulate the growth of Firmicutes (Scott *et al.*, 2015). Similarly, the introduction of specific minerals and substrates through geophagic materials could potentially favor the growth of Firmicutes in the gut. Additionally, the detoxifying properties of clays consumed during geophagy might reduce gut inflammation, creating a more favorable environment for Firmicutes to thrive (Dominy *et al.*, 2004). However, empirical studies focusing specifically on geophagy and Firmicutes are needed to confirm these hypotheses and elucidate the mechanisms involved. The advent of DNA sequencing technologies has revolutionized the study of the gut microbiota, allowing for detailed characterization of microbial communities (Huttenhower *et al.*, 2012). Techniques such as 16S rRNA gene sequencing enable researchers to identify and quantify bacterial populations in fecal samples with high precision. This method involves amplifying and sequencing a specific region of the bacterial 16S rRNA gene, which contains hypervariable regions that are unique to different bacterial taxa. By comparing these sequences to reference databases, researchers can profile the microbial composition of fecal samples and assess changes in response to dietary or environmental interventions, such as geophagy (Claesson *et al.*, 2010).

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Guma Benue State in the North central Nigeria. Guma experiences a tropical climate with prominent wet and dry seasons and an average annual rainfall of 1290mm (Akintola, 1986), temperature in Guma varies from a daily maximum of 400c and a minimum of 22.50c with latitude 70 44' 01" N and longitude 80 31'17" E of the equator. Three soil types (alluvial, clayey loam, and sandy) predominate, with a total land mass of 3,993.3 km^2 and divided by the River Benue into North and South Banks, respectively (Ihula *et al.*, 2025). Figure 1 shows the map of the study area.

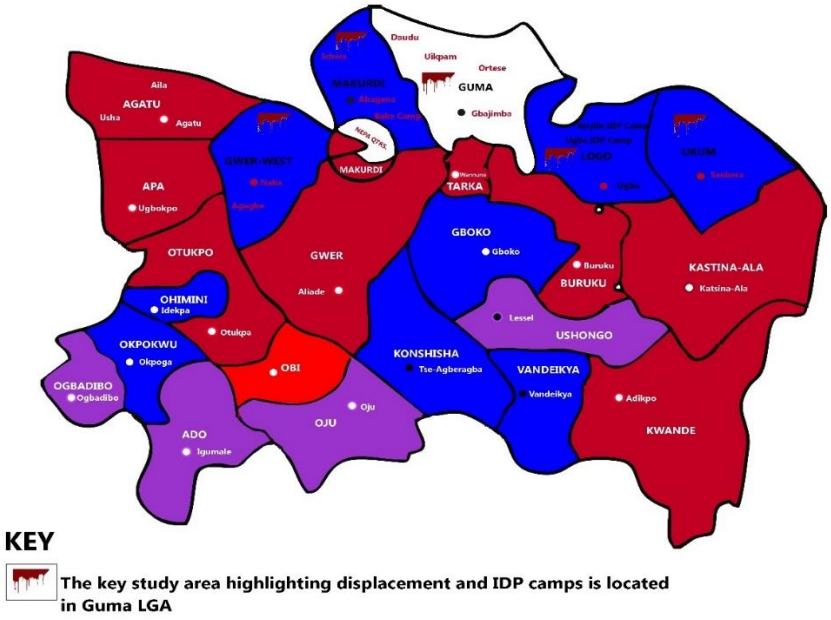

Figure 1: Map of Benue State showing the Study Area

2.2 Stool Samples Collection

Stool samples were aseptically collected randomly, from infants aged 7 to 12 months using sterile, leak-proof containers in line with Ihula *et al.* (2025). Participants were each provided with an ice bag containing an emesis basin (Ref. 104AA200, PRIM S.A Spain), a 50-mL sterile sampling bottle (Ref. 409526.1, Deltalab, Spain), and a sterile spatula (Ref. 441142.2, Deltalab, Spain) (Vandeputte *et al.*, 2017). A total of two hundred (200) stool samples were collected from ten sampling locations, 20 samples per location in the three IDP camps.

Each sample was appropriately labeled and transported to the Microbiology Laboratory, Department of Microbiology, Joseph Sawuan Tarka University Makurdi for bacteriological analysis. Samples were stored under refrigerated conditions (2 to 8°C) to preserve microbial viability and prevent overgrowth by contaminants. Processing took place within 2 to 4 hours of collection to ensure accurate and reliable bacterial recovery (Cheesbrough, 2000). The samples were coded as given below:

DU1A = Daudu Camp 1

DU1B = Daudu Camp 1

DU1C = Daudu Camp 1

DU1D = Daudu Camp 1

DU1E = Daudu Camp 1

DU1F = Dauda Camp 1

DU1G = Daudu Camp 1

DU2A = Daudu Camp 2

DU2B = Daudu Camp 2

UP= Uikpiam Camp

2.3 Bacteriological Analyses of Samples

i. Primary culturing on nutrient agar

Approximately 1 g of each stool sample was diluted in sterile normal saline (0.85% NaCl) and streaked onto freshly prepared nutrient agar plates using the streak plate technique to obtain isolated colonies. The plates were incubated at 37°C for 24 hours under aerobic conditions. Nutrient agar supports the growth of a broad range of non-fastidious bacteria, including many members of the phylum Firmicutes.

ii. Secondary culturing on blood agar

Well-isolated colonies from the nutrient agar plates were sub-cultured onto 5% sheep blood agar plates using a sterile wire loop and the streak method. Blood agar facilitates the differentiation of bacteria based on their hemolytic properties. The plates were incubated at 37°C for 24 hours under aerobic conditions. Colonies exhibiting reddish-brown pigmentation and hemolytic activity were selected as presumptive Firmicutes.

iii. Preservation of isolates

Pure colonies were transferred onto nutrient agar slants for preservation and stored at 4°C for subsequent biochemical and morphological characterization (Prescott *et al.*, 2008).

iv. Gram staining of isolates

Gram staining was carried out to differentiate bacterial isolates into Gram-positive or Gram-negative groups based on their cell wall composition, aiding in the classification of Firmicutes, which are typically Gram-positive. A smear of each bacterial isolate was prepared on a clean glass slide, heat-fixed, and stained with crystal violet for 1 minute. The slide was rinsed and then flooded with Gram's iodine for 1 minute to form a crystal violet–iodine complex. It was decolorized with 95% ethanol for 15 to 20 seconds and immediately rinsed with water. The slide was counterstained with safranin for 30 seconds, rinsed, air-dried, and examined under an oil immersion lens (×100 objective) (Cheesbrough, 2000).

2.3.1 Biochemical characterization of isolates

Biochemical tests were conducted to confirm the identity of Gram-positive, rod-shaped bacteria suspected to be Firmicutes. All tests were performed using standardized media and reagents (Cheesbrough, 2000; Cappuccino and Welsh, 2019). The agar slant cultures were used to perform some Biochemical tests which included; Catalase test, Citrate utilization test, Nitrate reduction test, Fructose test and Glucose test, for the identification of Firmicutes. To detect the presence of the catalase enzyme, which decomposes hydrogen peroxide into water and oxygen. A small portion of a fresh bacterial colony was transferred to a clean slide. A drop of 3% hydrogen peroxide was added. Immediate bubbling indicated a positive result. To assess the ability of the isolate to utilize citrate as its sole carbon source, the organism was streaked onto Simmon's citrate agar slant. Incubation was carried out at 37°C for 24 to 48 hours. To determine the ability of the isolate to reduce nitrate to nitrite or nitrogen gas, the organism was inoculated into nitrate broth and incubated at 37°C for 24 hours. sulfanilic acid and α-naphthylamine were added to detect nitrite. To assess glucose fermentation and potential gas production, the organism was inoculated into glucose broth containing phenol red indicator and an inverted Durham tube. Incubation was carried out at 37°C for 24 to 48 hours.

2.3.2 Molecular Characterization of Bacterial Isolate

i. **DNA extraction**

DNA was extracted from a sample using a modified protocol. Briefly, 1000 ul of bacterial brot sample was taken into a 1.5ml Eppendorf tube, and centrifuge for 10000 rpm for 5min supernatant was discarded. 200ul of PBS was added to the cell pellet, 250ul of lysozyme solution was added, and the mixture was vortexed and incubated at 37°C for 30 minutes. 250ul of SDS solution was added, and the mixture was vortexed and incubated at room temperature for 5 minutes, then on ice for 2 minutes, and finally at 65°C for 20 minutes. The sample was placed on ice for 2 minutes, and 250ul of 3M Sodium Acetate was added. The mixture was vortexed and centrifuged at 7000 rpm for 10 minutes. 500ul of the supernatant was transferred to a new 1.5-ml microcentrifuge tube, and 40ul of Glycogen was added. 2 volumes (~1000ul) of isopropanol were added, and the mixture was inverted slowly 5 times and incubated at room temperature for 10 minutes. The sample was centrifuged at 10,000 rpm for 10 minutes, and the supernatant was discarded. The pellet was washed with 500ul of 70% ethanol and centrifuged at 6000rpm for 2 minutes, repeating the wash step. The pellet was air-dried for 10 minutes and resuspended in 50ul of TE buffer.

ii. Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was performed in a thermocycler (model). SOLIS BIODYNE FIREPol Master Mix ready-to-load reagent was used for the PCR reaction. It consisted of the following components: 5 x FIREPol® Master Mix Ready to Load; Forward primer (10 pmol/µl); Reverse primer (10 pmol/µl); Template DNA and H_2O Up to 20 µl. The following primers were employed in the DNA amplification: 24F primer (AGAGTTTGATCCTGGCTCAG) and 1492R **(**TACGGYTACCTTGTTACGACTT) (Schindelin *et al.,* 2012). The RAPD-PCR thermal profile involved initial denaturation temperature of 95ºC for 5 minutes in I cycle, followed by 25 cycles of denaturation at 40s, annealing step at 42ºC for 60s and elongation step at 72ºC for 2 minutes. Final elongation was held at 5 minutes

iii. **Gel electrophoresis**

A 50ml solution of 1x TBE was prepared, and 0.75g of agarose gel was added. The mixture was heated in the microwave on high for 1 minute to melt the gel. Ethidium bromide was added to a final concentration of 1:20,000 (3ul). The solution was allowed to cool before being poured into a casting tray. Once set, the gel was transferred to a gel tank and covered with 1x TBE buffer. Amplification products (15ul aliquots) were loaded into the wells and the gel was run at 100V for 30 minutes. The resulting bands were visualized under UV light using a gel documentation system.

2.3.3 Oxford Nanopore Sequencing

i. Library Preparation

The sequence library was prepared using the Oxford nanopore (ONT) SQK-RBK114-24 protocol. Genomic DNA (gDNA) was first prepared in nuclease-free water. For samples exceeding four in number, 50 ng of gDNA was used, while for four or fewer samples, 200 ng of gDNA was transferred into 0.2 ml thin-walled PCR tubes. The volume was adjusted to 10 µl using nuclease-free water. For the barcoding reaction, 10 µl of template DNA (50 ng) was combined with 1 µl of Rapid Barcode (RB01-24 or RB01-96). The mixture was incubated at 30°C for 2 minutes, followed by 80°C for 2 minutes. The

barcoded samples were then pooled into a clean 1.5 ml Eppendorf DNA LoBind tube. An equal volume of AMPure XP Beads (AXP) was added to the pooled sample, and the mixture was gently flicked to ensure thorough mixing. The mixture was incubated on a Hula mixer for 5 minutes at room temperature. The sample was centrifuged after incubation, and the supernatant was carefully removed. The beads were washed twice with 1.5 ml of freshly prepared 80% ethanol to remove impurities. The beads were then resuspended in 15 µl of Elution Buffer (EB) and incubated for 10 minutes at room temperature to elute the DNA. For library preparation, 1 µl of diluted Rapid Adapter (RA) was added to the barcoded DNA, and the mixture was incubated for 5 minutes at room temperature.

ii. Flow cell priming

The flow cell (FLO-MIN114) was primed with a mixture of Flow Cell Flush (FCF), Bovine Serum Albumin (BSA), and Flow Cell Tether (FCT). The prepared library was loaded into the flow cell via the SpotON sample port. Sequencing was initiated on the MINION Mk1B device using the MINKNOW software with the following settings: a run limit of 30 minutes, active channel selection enabled, pore scan frequency set to 1.5 hours, reserved pores enabled, minimum read length of 200 bp, sequence quality score of 9 (QScore) was selected, read splitting enabled, base calling using the fast model at 400 bps, modified base calling disabled, and barcode trimming disabled.

iii. Base calling and blasting

For sequence base calling and demultiplexing, the Nanopore Dorado base caller software was utilized. The high-accuracy setting was selected for base calling, and the sequence data was stored in fastQ format. Following base calling, Dorado was used to demultiplex the samples into individual barcodes using the Kit code SQK-RBK114-24. (Oxford Nanopore Technologies in their 2023 documentation available on GitHub). The fastQ reads were submitted to NCBI blast to determine the species and strain of the isolates.

3. RESULTS

3.1 Biochemical and Molecular Confirmation of *Bacillus* Species

Biochemical tests presented in table 1 confirmed the rod-shaped isolates as *Bacillus* spp. The isolates tested positive to catalase, citrate, nitrate reduction, mannitol, glucose and gram reaction while taking its normal rod shape structure (table 1). Seven (7) selected samples analyzed genotypically using PCR were confirmed as *Bacillus* spp. as shown in the amplified 100 base pair (bp) bands in gel image of samples 1-7 (Plate 1).

Table 2 gives the species and strain identity of the genotyped samples using nanopore sequencing. Results revealed the presence of three species of *Bacillus* in the seven analyzed samples. They are *Bacillus siamensis*, *Bacillus subtilis* and *Bacillus velezensis*. *Bacillus siamensis* strain Bk6y1 and *Bacillus velezensis* strain GBWR73 were strains of *B. siamensis* and *velezensis* respectively. They were obtained from samples at Daudu camp 1 and Uikpiam camp respectively. *Bacillus subtilis* dominated the samples (5 out of 7) with different strains at Daudu camp 1 and 2. The strains were: *B. subtilis* strain CS-P-4, *B. subtilis* strain BS34A, *B. subtilis* subsp. subtilis AZFS, *B. subtilis* strain HSB2 and *B. subtilis* strain PSBC28.

Table 1: Biochemical Characteristics of *Bacillus* species

Test	Outcome
Catalase	+
Citrate	+
Nitrate Reduction	+
Mannitol	+
Glucose	+
Gram Reaction	+
Shape of cell	rods

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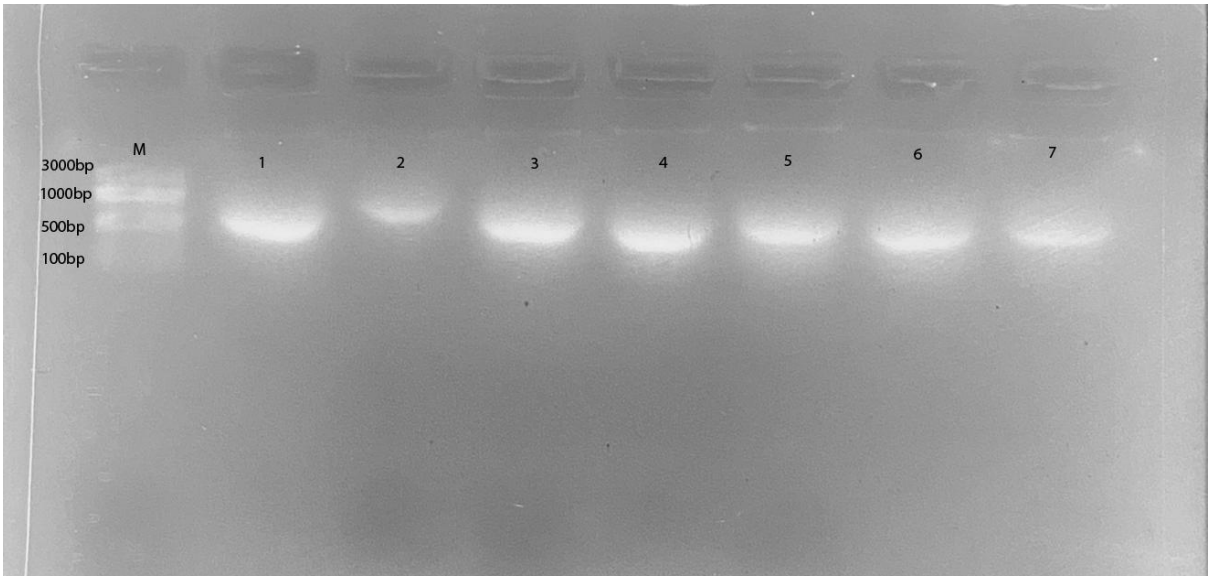

Plate 1: Agarose Gel Electrophoresis (1%) of PCR Products

Legend

1= DU1A = Daudu Camp 1

2= DU1B = Daudu Camp 1

3= DU1C = Daudu Camp 1

4= DU1D = Daudu Camp 1

5= DU2A = Daudu Camp 2

6= DU2B = Daudu Camp 2

7= UP= Uikpiam Camp

M = 100bp ladder

Table 2: Gene Sequencing Results of 16S rRNA of Isolates

S/N	Location Code	IDP Camp	Identified species	Strain identity
1	DU1A	Daudu Camp 1	*Bacillus siamensis*	*Bacillus siamensis* strain Bk6y1
2	DU1B	Daudu Camp 1	*Bacillus subtilis*	*Bacillus subtilis* strain CS-P-4
3	DU1C	Daudu Camp 1	*Bacillus subtilis*	*Bacillus subtilis* strain BS34A
4	DU1D	Daudu Camp 1	*Bacillus subtilis*	*Bacillus subtilis* subsp. subtilis AZFS3
5	DU2A	Daudu Camp 2	*Bacillus subtilis*	*Bacillus subtilis* strain HSB2
6	DU2B	Daudu Camp 2	*Bacillus subtilis*	*Bacillus subtilis* strain PSBC28
7	UP	Uikpiam Camp	*Bacillus velezensis*	*Bacillus velezensis* strain GBWR73

4. DISCUSSION

The positive pattern of biochemical tests across multiple tests suggests heterogeneity among the isolates either different *Bacillus* species are present, or There is intra-species variability among strains from different environments. The ability to variably use substrates (citrate, nitrate, sugars) may reflect adaptation to diverse environmental niches, such as soil, gut, or food. Biochemical characteristics of *Bacillus* spp**.** summarizes key results from standard microbiological tests used for bacterial identification and characterization. Both cultural and biochemical results were complimentary in revealing the identity of *Bacillus* species at the genus level suggesting the need for a more convincing method of revalidation at species and strains levels.

Post PCR analysis carried out using 16S rDNA sequencing revealed that *B. subtilis* was the predominant species. This aerobic spore-forming organism, is lately evoking considerable research interest due its involvement in cases of geophagy. Sequence results of the 16sRNA revealed the isolates as three species of *Bacillus.* They were *Bacillus siamensis, Bacillus subtilis* and *Bacillus velezensis*. Among them, *Bacillus siamensis* strain Bk6y1 and *Bacillus velezensis* strain GBWR73 were minor strains belonging to *B. siamensis* and *velezensis* respectively. *Bacillus subtilis* dominated the samples with different strains including *B. subtilis* strain CS-P-4, *B. subtilis* strain BS34A, *B. subtilis* subsp. subtilis AZFS, *B. subtilis* strain HSB2 and *B. subtilis* strain PSBC28.

The advent of DNA sequencing technologies has revolutionized the study of the gut microbiota, allowing for detailed characterization of microbial communities (Huttenhower *et al.*, 2012). Techniques such as 16S rRNA gene sequencing enable researchers to identify and quantify bacterial populations in fecal samples with high precision. This method involves amplifying and sequencing a specific region of the bacterial 16S rRNA gene, which contains hypervariable regions that are unique to different bacterial taxa. By comparing these sequences to reference databases, researchers can profile the microbial composition of fecal samples and assess changes in response to dietary or environmental interventions, such as geophagy (Claesson *et al.*, 2010).

Both primers are cited from the same reference by Weisburg *et al*. (1991), indicating they have been previously validated and widely accepted for molecular studies involving the 16S rRNA gene. Using these primers allows for the amplification of nearly the full-length 16S rRNA gene enhances the accuracy of bacterial identification, especially in studies focusing on microbial diversity, taxonomy, or environmental microbiology. In the context of this study (e.g., investigating geophagy effects on infant fecal bacteria), these primers enable the identification and characterization of the bacterial communities' present, providing insights into potential health implications. The combination of specific primers and nanopore sequencing in the study provide a strong molecular foundation for accurately identifying and analyzing bacterial communities in infant fecal samples to species and strain levels. This is essential for assessing the impact of geophagy on gut microbiota, which may have broader implications for child health and nutritional interventions in affected communities.

5. CONCLUSION

Sequence results of the 16sRNA revealed the isolates as three species of *Bacillus.* They were *Bacillus siamensis, Bacillus subtilis* and *Bacillus velezensis*. *Bacillus subtilis* dominated the samples with different strains including *B. subtilis* strain CS-P-4, *B. subtilis* strain BS34A, *B. subtilis* subsp. subtilis AZFS, *B. subtilis* strain HSB2 and *B. subtilis* strain PSBC28.

REFERENCES

[1] Claesson, M. J., Cusack, S., O'Sullivan, O., Greene-Diniz, R., de Weerd, H. A., Flannery, E., Marchesi, J. R., Falush, D., Dinan, T., Fitzgerald, G., Stanton, C., van Sinderen, D.,O'Connor, M., Harnett, F., O'Connor, K., Henry, C., O'Mahony, D. Fitzgerald, A. P., Shanahan, F., Twomey, C., Hill, C., Ross, R. P., O'Toole, P. W., and Cotter, P. D. (2010). Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proceedings of the National Academy of Sciences, 108(1),4586–4591.

[2] Dominy, N. J., Davoust, E. and Minekus, M. (2004). Adaptive function of soil consumption:An in vitro study modeling the human stomach and small intestine. Journal of Experimental Biology, 207(19), 319–324.

[3] Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., and Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. Gut Microbes, 3(4), 289–306.

[4] Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T.,Creasy, H. H., Earl, A. M., FitzGerald, M. G., Fulton, R. S., Giglio, M. G.,Hallsworth-Pepin, K., Lobos, E. A. and Madupu, R., (2012). Structure, function and diversity of the healthy human microbiome. Nature, 486(7402), 207–214.

[5] Ihula D. H., Iheukwumere C. C., Ugbogu M., and Olasan O. J. (2025). Bacteriological Analysis of Infant Fecal Samples in Internally Displaced Persons (IDP) Camps in Guma Local Government Area, Benue State, Nigeria. *International Journal of Healthcare Sciences* 13,1, pp 547-553

[6] Louis, P., Hold, G. L., and Flint, H. J. (2010). The role of the gut microbiota in energy metabolism and metabolic disease. Current Pharmaceutical Design, 16(18), 1978–1986.

[7] Louis, P., Young, P., Holtrop, G., and Flint, H. J. (2010). Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. Environmental Microbiology, 12(2), 304–314.

[8] Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. A., Affourtit, J. P., Egholm, M., Henrissat, B.,Heath, A. C., Knight, R., and Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. Nature, 457(7228), 480–484.

[9] Penders, J., Thijs, C., Vink, C., Stelma, F. F., Snijders, B., Kummeling, I., van den Brandt, P. A., and Stobberingh, E. E. (2006). Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics, 118(2), 511–521.

[10] Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173(2), 697–703.