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The Molecular Characterization of Rhizobacteria Isolates from Saki, Nigeria

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ABSTRACT

Plant growth promoting Rhizobacteria (PGPR) are important in the agricultural sector. Although different microorganism live in the soil but thrive in PGPR rhizosphere zones, improve the production and protection to them from diseases by production of metabolites, volatile compounds and phytohormones and Induced Systemic Resistance (ISR). This study was aimed at isolating and characterizing molecularly; the bacteria from the rhizosphere of pepper (*Capsicum annuum*), vegetable (*Spinacia oleracea*), rice (*Oryza sativa*) grown in Saki. The rhizosphere sample of pepper, rice and vegetable were collected between the hours of 1:40-2:00pm. The colonial characteristics, Gram staining techniques, biochemical tests were carried out on the isolates which were also tested against eight antibiotics (Ceftriaxone, Gentamicin, Ceftazidime, Ofloxacin, Augmentin, Cefuroxime, Erythromycin, and Cloxacillin) by using disc diffusion method. PCR techniques and subsequent use of sanger method was used for sequencing. DNA extraction was obtained using the lysozyme-SDS-phenol chloroform method in the prepared kits by Jena Bioscience. Amplification of the 16S rRNA gene was performed with the use of T1-Thermocycler PCR machine with 1492R primers pA (5′-TAC GGYBTAC CTT GTT ACG ACT T3′) and 27F primers pH (5′-AGAGTTTGATCMTGGCTCAG3'). This Primer was used for PCR amplification of 16S rRNA gene. All bacterial isolates were catalase (+) and indole (-). Antibiotics screening showed that all isolates resist most of the antibiotics except Ofloxacin and ciprofloxacin while sample 1, 2, 5 and 6was susceptible to Gentamicin. Six bacterial isolates obtained were characterized on molecular basis of 16S rRNA sequencing. The identified isolates were: *Bacillus thuringensis B. weidmanii, B. cereus*.

Keywords: PGPR; ISR; Rhizobacteria; Biopesticides; *Oryza sativa*; 16S rRNA.

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I. Introduction

Interactions happens between microorganisms from beneficial symbiotic relationships to detrimental pathogenic relationship in the rhizosphere [1]. The rhizobacteria that enhance beneficial effects on plant growth and development are known as plant growth promoting rhizobacteria (PGPR) [2]. PGPR's enhance plant growth via their ability to produce growth regulators or solubilize mineral phosphates and varieties of nutrients or fix atmospheric nitrogen or antagonistic action against phytopathogenic microbes by the secretion of antibiotics, endospore, siderophores, and cyanide [3]-[5]. The experimental evidences suggest that plant growth stimulation is the net result of multiple mechanisms that may be activated simultaneously [6]. Rice consumption has been increasing over the years due to the increasing population. Unfortunately, as a result of decrease in soil fertility, poor management of soil resources, build-up of pathogens and accumulation of phytotoxic substances rice productivity has decrease as expected and demanded [7]-[9]. Farmers relies a lot on agrochemicals to maintain the crop productivity [10], [11]. Nevertheless, extravagant use of agrochemicals in crop fields increase nitrate, nitrite, ammonium and phosphate and other reactive chemical species in groundwater and surface water bodies, which leads to serious environmental and health hazards [12]-[14]. Vegetable environment is strictly threatened by non-organic and organic factors. Among which crop loss due to organic factors especially due diseases is huge and estimated as 50–80 % from the heavily infected fields [15]-[17]. Farmers are greatly concerned for their crop, and for a quick remedy they use excessive amount of chemicals to control the diseases which in turn leads to detrimental effects on environment and microbiota organisms including animals and human beings [18]. The rhizosphere of vegetable crops has widely been studied which has been examine to a minor [19]-[22]. According to [23] the inoculation of *B. cereus* L90 interferes with the suppression of stress conditions to the biological characteristics of plant rhizosphere. *Bacillus species* are aerobic or facultative anaerobic bacteria closely related species *B. cereus, B. thuringiensis* are Gram-positive spore forming rod-shaped characterized by multiple morphological state, the vegetative cell (from 1.0 to 1.2 μm in width and from 3.0 to 5.0 μm in length), motile or non-motile, and the endospore (non-swelling the sporangium). The genus is characterized by the presence of endospores, which help in resistant to many stressful environment conditions (heat, cold, radiation, dehydration and disinfectants) and secreted either in the

presence or in the absence of air. According to [24], the presence of *B. thuringiensis* strain is capable to produce a capsule. The *Bacillus species* have unique functions and features in plant rhizospheres such as phytostimulation, bio-fertilization and bio-protection.

Bacilli rhizobacteria are phytostimulators by the production of phytohormones which are gibberellic acid (GA) and indole-3-acetic acid (IAA) the direct PGPR mechanisms characterized by *Bacilli rhizobacteria* [25]. Although *Bacilli rhizobacteria* produce IAA but few have been known on their ability to secrete abscisic acid (ABA). The Phytohormones biosynthesis by *Bacilli rhizobacteria* directly connects to nutrient availability and subsequent growth promotion in different plants [26].

B. cereus was stated to be having the potential of controlling many rice phytogenic fungi. *Bacillus cereus* has also been revealed to be efficient in bio-protection against several fungal pathogens in the rhizosphere [27]. Evidence revealed that *Bacilli cereus* and *B. thuringiensis* as rhizobacteria have the ability of stimulating plant growth either directly or indirectly via multifarious [28] including induced systematic resistance (ISR), antibiotics, hydrolytic enzymes, siderophores and nitrogen (N_2) fixation, nutrient solubilization and biosynthesis of phytohormones in plants to their pathogens [29].

An English biochemist Frederick Sanger and his colleagues in 1977 who developed a chain termination method for sequencing which is Sanger sequencing. This method is made for determining the sequence of nucleotide bases in a piece of DNA (commonly less than 1,000 bp in length). Sanger sequencing with 99.99% base accuracy is regarded as the "gold standard" for validating DNA sequences, including those already sequenced via next-generation sequencing (NGS). Sanger sequencing was used in the Human Genome Project to determine the sequences of relatively small fragments of human DNA (900 bp or less).

II. Materials and Methods

Collection of soil samples from the rhizosphere of vegetable, rice, and pepper (10-15 cm depth) was done aseptically, each sample container was labeled accordingly. The rhizosphere soil samples were collected from the root depth, aseptically collected into a petri dish for the isolation of rhizosphere bacteria. For isolation of bacteria, 1 g of rhizospheric soil sample from each site was serially diluted in 9 ml of distill water to10^5. The diluted suspensions (1 ml) were spread on pre-poured nutrient agar medium and incubated at 28±2 °C for twenty four hours. The isolated colonies that grew on nutrient agar were subcultured into differential and selective media. The isolates were tested against eight antibiotics (Ceftriaxone, Gentamicin, Ceftazidime, Ofloxacin, Augmentin, Cefuroxime, Erythromycin, and Cloxacillin) by using disc diffusion method.

A. Biochemical Characterization

The isolates were characterized by some specific morphological and biochemical features. The morphological characterization was carried out by viewing the isolated colonies under a compound microscope for colony color, forms, elevation, and margin. Also, cell shape, size, endospore presence and Gram's reaction was noted. The difference biochemical characterizations are indole production, citrate utilization test, oxidase test, catalase production and starch hydrolysis were carried out as per Bergey's Manual of Determinative Bacteriology [31].

B. DNA Isolation

Bacteria DNA Preparation Kit designed by JENA bioscience for easy and fast isolation of genomic DNA from both Gram-positive and Gram-negative bacteria samples. The solution based system reduces DNA fragmentation that may be problematic in the spin-column or filtration based techniques, because phenol or chloroform cannot not be used.

Solution based genomic DNA purification kits guarantee minimal DNA fragmentation and yield DNA sized up to 150 kb. Expected yield of genomic DNA varies from sample to sample dependent on the amount, quality and type of material involves. At least an amount of approximately 40 µg purified DNA per preparation can be expected.

C. Cell Lysis for Gram-Positive Bacteria

1 ml of cultured cells was transferred into a 1.5 ml micro tube, to get the cells it was centrifuge at 15,000 g for 1 min and the supernatant was discarded. The cell pellet was re-suspended in 300 µl of cell resuspension solution. Add 2 µl of Lysozyme solution then mixed well by inverting the tubes and was incubated at 37 °C for 60 minutes by occasionally inverting. It was centrifuged at 15,000 g for 1 min and the supernatant was discarded then the pellet was re-suspended in 300 µl of Cell Lysis Solution.

D. Cell Lysis for Gram-Negative Bacteria

For Gram negative bacteria 1 ml of cultured cells was transferred into a 1.5 ml microtubes in order to harvest the cells and was centrifuged at 15,000 g for 1 minutes before the supernatant was discarded. The pellet was re-suspended in 300 µl of cell lysis solution.

RNase Treatment was done by the addition of 1.5 µl of RNAase Solution and mixed by inverting the tubes. It was incubated at 37 °C for 15-30 min and cool on ice for 1 min.

Protein Precipitation: 100 µl of Protein Precipitation Solution was added and vortex vigorously for 20-30 sec then centrifuge at 15,000 g for 5 min.

DNA Precipitation: The supernatant was transferred to a clean 1.5 ml micro tube containing 300 µl Isopropanol >99% then mixed by inverting gently for 1 minute. It was centrifuged at 15,000 g for the same duration (DNA was visible as a small white pellet). The supernatant was discarded and the tube was drained briefly on clean absorbent paper. About 500 µl of washing buffer was added with the tube inverted for several times to wash the DNA pellet. Then this was centrifuged at 15,000 g for 1 minute and the ethanol was discarded carefully. It was air dried at room temperature for 10-15 minutes.

5 DNA Hydration: Addition of 50-100 µl of DNA hydration solution into the dried DNA pellet and hydrated by incubating at 65 °C for 60 minutes. Then the DNA Stored at 4 °C. For long time storage, it was stored at -20 °C or -80 °C. Stored at -20 °C and used as template DNA in PCR to amplify the 16S rRNA for phylogenetic analysis.

E. PCR Amplification and Sequencing for Total Genomic DNA

Isolates was carried out on the basis of 16S rRNA sequencing. For this, the isolates were sent to Humanizing Genomics MACROGEN Laboratory, United States of America. As per the details shared the 16RRNA sequence was obtained using Sanger sequencing method. Amplification of the 16S rRNA gene was performed using a T1-Thermocycler PCR machine with 1492R primer spA (5′-TAC GGYTAC CTT GTT ACG ACT T-3′) and 27F primers pH (5′-AGA GTT TGA TCM TGG CTC AG-3′).

According to Sanger sequencing method, a DNA primer complementary to the template DNA (the DNA to be sequenced) is used to be a starting point for DNA synthesis. In the availability of the four deoxy nucleotide triphosphates (dNTPs: A, G, C, and T), the polymerase stretched the primer by the addition of the complementary dNTP to the template DNA strand. So as to know which nucleotide is associated into the chain of nucleotides, four dideoxynucleosides triphosphates (ddNTP: ddATP, ddGTP, ddCTP, and ddTTP) labeled with a distinct fluorescent dye are used to terminate the synthesis reaction. Compared to dNTPs, ddNTP has an oxygen atom dismissed from the ribonucleotide, hence cannot form a link with the next nucleotide. Following synthesis, the reaction products are loaded into four lanes of a single gel based on the diverse chain-terminating nucleotide and subjected to gel electrophoresis. According to their sizes, the sequences of the DNA is then determined [32]. The nucleotide sequences of 16S rRNA gene were analyzed using BLAST online at *http://blast.ncbi.nlm.nih.gov/Blast.cgi.* The phylogenetic tree was constructed on the aligned data set using the neighbor-joining method *(SaitouandNei1987)* implemented in the program using MEGA4.0 software [33].

III. Results And Discussion

The molecular characterization of Rhizobacteria isolates was carried out on the basis of 16S rRNA sequencing, the results of BLAST showed 100% similarity between the nucleotide sequences of 16S rRNA gene of the obtained isolates which included: *B. cereus, B. thuringensis and B. wiedmannii* with the nucleotide sequences of the 16SrRNA gene in GenBank. Online alignment was done at http://expasy.org/tools/ on_each isolate which had a kinship with several bacteria which had 99% similarity of 16S rRNA gene.

Isolate 1: Query ID MW362295.1
Description
Bacillus thuringiensis strain CBC 123 16S ribosomal RNA gene, partial sequence...
Molecule type nucleic acid Query Length 1483
Bacillus thuringiensis strain Bt11 16S ribosomal RNA gene, partial sequence
Subject Sequence ID: MT292101.1 Length: 1539 Number of Matches: 1

Alignment statistics for match #1

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Sbjct 33 GATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTGAGAGCTTGCT 92

Query 61 CTCAAGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG 120
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Sbjct 93 CTCAAGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG 152

Query 121 ATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATT 180
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Query 181 GAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAG 240
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Sbjct 213 GAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAG 272

Query 241 GTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG 300
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Sbjct 273 GTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG 332

Query 301 GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGAC 360
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Query 361 GAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGT 420
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Query 421 TGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAA 480
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Query 541 AATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGC 600
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Sbjct 693 CCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT 752

Query 721 CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT 780
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Query 841 GAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGG 900
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Query 1081 CGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGAC 1140
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Query 1141 TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACC 1200
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Sbjct 1293 AATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGG 1352

Query 1321 AATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA 1380
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Query 1441 CCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAA 1483
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Sbjct 1473 CCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAA 1515
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Query Length
1478
Subject Sequence ID: CP053954.1Length: 5381410 Number of Matches: 14

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Query 1081 AAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAA 1140
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Sbjct 2714805 GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT 2714864

Query 1201 ATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGT 1260
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Sbjct 2714865 ATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGT 2714924

Query 1261 GGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA 1320
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Sbjct 2714925 GGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA 2714984

Query 1321 AGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 1380
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Sbjct 2714985 AGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 2715044

Query 1381 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT 1440
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Sbjct 2715045 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT 2715104
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Query 1441 TTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGG 1478
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Sbjct 2715105 TTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGG 2715142
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Isolate 3 Query ID MW362293.1
Description
Bacillus cereus strain ABC 1234 16S ribosomal RNA gene, partial sequence ...
Query Length
1483
Bacillus cereus strain HFBP18 16S ribosomal RNA gene, partial sequence
Sequence ID: MT538265.1Length: 1535Number of Matches: 1

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Query 1 GATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCT 60
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Sbjct 21 GATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCT 80

Query 61 CTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG 120
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 81 CTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG 140

Query 121 ATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATT 180
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Sbjct 141 ATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATT 200

Query 181 GAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAG 240
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Sbjct 201 GAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAG 260

Query 241 GTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG 300
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Sbjct 261 GTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG 320

Query 301 GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGAC 360
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Sbjct 321 GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGAC 380

Query 361 GAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGT 420
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 381 GAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGT 440

Query 421 TGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAA 480
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Sbjct 441 TGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAA 500

Query 481 AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGG 540
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Sbjct 501 AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGG 560

Query 541 AATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGC 600
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Sbjct 561 AATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGC 620

Query 601 TCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATT 660
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Sbjct 621 TCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATT 680

Query 661 CCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT 720
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Sbjct 681 CCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT 740

Query 721 CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT 780
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Sbjct 741 CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT 800

Query 781 GGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCT 840
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Sbjct 801 GGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCT 860

Query 841 GAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGG 900
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Sbjct 861 GAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGG 920

Query 901 AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGA 960
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Sbjct 921 AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGA 980

Query 961 ACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGC 1020
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Sbjct 981 ACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGC 1040

Query 1021 AGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC 1080
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Query 1081 CGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGAC 1140
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Sbjct 1101 CGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGAC 1160

Query 1141 TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACC 1200
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Query 1201 TGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCT 1260
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Query 1321 AATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA 1380
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Sbjct 1341 AATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA 1400

Query 1381 CCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTTTGGAG 1440
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Sbjct 1401 CCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTTTGGAG 1460

Query 1441 CCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAA 1483
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Sbjct 1461 CCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAA 1503
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Isolate 4 Query ID MW362292.1
Description
Bacillus wiedmannii strain ABC 123 16S ribosomal RNA gene, partial sequence...
Query Length
1493
Bacillus wiedmannii strain SX13.1LB 16S ribosomal RNA gene, partial sequence
Sequence ID: MT052668.1Length: 1540Number of Matches: 1

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Query 1 GCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAG 60
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Sbjct 15 GCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAG 74

Query 61 CTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAG 120
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Sbjct 75 CTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAG 134

Query 121 ACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTC 180
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Sbjct 135 ACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTC 194

Query 181 GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT 240
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Sbjct 195 GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT 254

Query 241 GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA 300
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Sbjct 255 GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA 314

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Sbjct 315 CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA 374

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Query 421 CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAA 480
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Sbjct 435 CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAA 494

Query 481 CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT 540
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Sbjct 495 CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT 554

Query 541 ATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCC 600
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Sbjct 555 ATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCC 614

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Query 661 GGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGC 720
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Sbjct 675 GGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGC 734

Query 721 GACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGA 780
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Sbjct 735 GACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGA 794

Query 781 TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTT 840
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Sbjct 795 TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTT 854

Query 841 AGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACT 900
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Sbjct 855 AGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACT 914

Query 901 CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG 960
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Sbjct 915 CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG 974

Query 961 CGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTC 1020
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Sbjct 975 CGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTC 1034

Query 1021 GGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT 1080
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Sbjct 1035 GGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT 1094

Query 1081 AAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAA 1140
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Sbjct 1095 AAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAA 1154

Query 1141 GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT 1200
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Sbjct 1155 GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT 1214

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Sbjct 1215 ATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGT 1274

Query 1261 GGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA 1320
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Sbjct 1275 GGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA 1334

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Sbjct 1335 AGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 1394

Query 1381 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT 1440
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Sbjct 1395 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT 1454

Query 1441 ATGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAG 1493
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Sbjct 1455 ATGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAG 1507
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Isolate 5 Query ID MW362291.1
Description
Bacillus cereus strain ABC 12 16S ribosomal RNA gene, partial sequence ...
Query Length
1489
Bacillus cereus strain HFBP18 16S ribosomal RNA gene, partial sequence
Sequence ID: MT538265.1Length: 1535Number of Matches: 1

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Query 1 GCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAG 60
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Sbjct 15 GCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAG 74

Query 61 CTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAG 120
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Sbjct 75 CTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAG 134

Query 121 ACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTC 180
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Sbjct 135 ACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTC 194

Query 181 GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT 240
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Sbjct 195 GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT 254

Query 241 GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA 300
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Sbjct 315 CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA 374

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Sbjct 435 CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAA 494

Query 481 CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT 540
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Query 661 GGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGC 720
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Sbjct 675 GGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGC 734

Query 721 GACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGA 780
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Sbjct 735 GACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGA 794

Query 781 TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTT 840
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Sbjct 795 TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTT 854

Query 841 AGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACT 900
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Query 901 CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG 960
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Sbjct 915 CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG 974

Query 961 CGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTC 1020
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Sbjct 975 CGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTC 1034

Query 1021 GGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT 1080
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Sbjct 1035 GGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT 1094

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Sbjct 1095 AAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAA 1154

Query 1141 GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT 1200
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Sbjct 1155 GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT 1214

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Sbjct 1215 ATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGT 1274

Query 1261 GGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA 1320
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Sbjct 1275 GGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA 1334

Query 1321 AGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 1380
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Sbjct 1335 AGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 1394

Query 1381 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT 1440
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Sbjct 1395 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT 1454

Query 1441 TTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAA 1489
 ||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct 1455 TTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAA 1503
```

Isolate 6 Query ID MW362290.1
Description
Bacillus cereus strain ABC 11 16S ribosomal RNA gene, partial sequence ...
Query Length
1493
Bacillus cereus strain HFBP18 16S ribosomal RNA gene, partial sequence
Sequence ID: MT538265.1Length: 1535Number of Matches: 1

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Query 1 GCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAG 60
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Sbjct 15 GCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAG 74

Query 61 CTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAG 120
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Sbjct 75 CTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAG 134

Query 121 ACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTC 180
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 135 ACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTC 194

Query 181 GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT 240
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 195 GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT 254

Query 241 GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA 300
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 255 GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA 314

Query 301 CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA 360
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 315 CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA 374

Query 361 ATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAA 420
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 375 ATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAA 434

Query 421 CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAA 480
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 435 CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAA 494

Query 481 CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT 540
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Sbjct 495 CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT 554

Query 541 ATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCC 600
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 555 ATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCC 614

Query 601 CACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGT 660
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Query 661 GGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGC 720
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Sbjct 675 GGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGC 734

Query 721 GACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGA 780
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Sbjct 735 GACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGA 794

Query 781 TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTT 840
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Sbjct 795 TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTT 854

Query 841 AGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACT 900
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Sbjct 855 AGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACT 914

Query 901 CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG 960
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Sbjct 915 CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG 974

Query 961 CGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTC 1020
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Sbjct 975 CGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTC 1034
Query 1021 GGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT 1080
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1035 GGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT 1094

Query 1081 AAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAA 1140
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 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1095 AAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAA 1154

Query 1141 GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT 1200
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1155 GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT 1214

Query 1201 ATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGT 1260
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1215 ATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGT 1274

Query 1261 GGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA 1320
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1275 GGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA 1334

Query 1321 AGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 1380
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Sbjct 1335 AGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 1394

Query 1381 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT 1440
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Sbjct 1395 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT 1454

Query 1441 TTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAG 1493
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1455 TTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAG 1507
```

The Query Cover (QC) for six isolates of bacteria had a value in the range of 100% (Table I). The E-value of 0.0 indicated perfect number of alignments with a score equal to or higher than expected to occur in the database by chance. Therefore, the lower the E-value was, the more significant the score and the better quality of BLAST alignment search were. In this study, the 16S rRNA nuclear gene had a length of about less than 1535 base pairs (bp). Then the search for similarities with the limited query sequence was performed. According to Claverie and Notredame (2003), DNA sequences have a high similarity if the Query Cover value approaches 100% and the E-value approaches 0.0. Based on the Query Cover (QC), the E-Value of 0.0, and similarity.

TABLE I: SUMMARY ALIGNMENT RESULTS OF THE SIX ISOLATES

Accession number		Lengths	No of matches	E- Value	Query cover
Query	MW362290.1	1493	1	0.0	100%
Subject	MT538265.1	1507			
Query	MW362291.1	1489	1	0.0	100%
Subject	MT538265.1	1503			
Query	MW362292.1	1493	1	0.0	100%
Subject	MT052668.1	1507			
Query	MW362293.1	1483	1	0.0	100%
Subject	MT538265.1	1503			
Query	MW362294.1	1483	14	0.0	100%
Subject	CP053954.1	1478			
Query	MW362295.1	1483	1	0.0	100%
Subject	MT292101.1	1515			

16S rRNA gene has a characteristic size of about 500 bases until 1550 bp. For the 16S rRNA used for sequencing measuring 1535 bp. The use of 16S rRNA is often used in prokaryotic organisms rather than 23S rRNA because of its higher variation. In Eukaryote it uses 18S rRNA for identification. Therefore, uses 16S rRNA is of good identification in bacteria. A phylogenetic relationship based on 16S rRNA nuclear gene. The construction of phylogenic tree (Fig. 1) described the phylogenetic relationship of the 6 species found, namely were three *Bacillus cereus* strain and *Bacillus weidmanii* strain and *B. thuringiensis.*

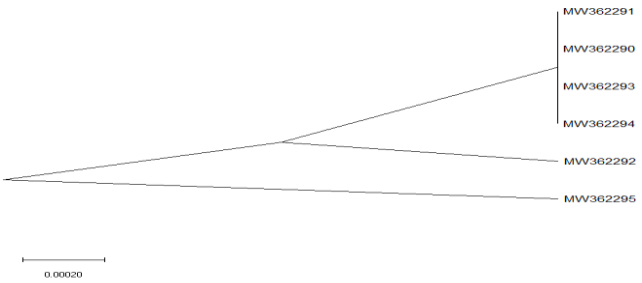

Fig. 1. Phylogenetic tree of the 6 isolates.

Mahwish *et al.* reported that the genera *Bacillus* and *Pseudomonas* were the most dominant and most commonly found in various plant studies According to Cakmakci et *al.* [34], similar results were presented by [35] who reported an increase in nutrient absorption efficiency by PGPRs inoculation which resulted in increased root growth and hence efficient absorption of nutrients by plants. Plants will be more resistant to drought, salinity, and toxins derived from metals and metals. PGPR also maintain plants against pathogens and pests.

IV. CONCLUSION

The study showed a remarkable identity between all the isolates (query) and the subjects with very considerable number of matches and the number of nucleotides sequences. The prompt assignment of accession numbers to the isolates showed the validity and reliability of the protocols used ranging from the conventional identification procedures and the molecular analysis. MW362295 (Bt) is distantly related to others in the evolutionary trend.

ACKNOWLEDGMENTS

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1. Adeoti O.M, Olaleye O.M., Adeoye K.A, Adesina D. A, Olaoye O.J., Adeoye B.A (2020). Molecular typing of 16S rRNA of

Azadirachta indica and O*cimum gratissimum* Resistant *Pseudomonas spp.* from Sabe in Oke-Ogun, Nigeria. Archive of Clinical Microbiology. Archives of Clinical Microbiology. 10.36648/1989-8436.11.4.117.

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4. Adeoti O. M, Bello A. H, Adeoye K., Olaoye O. J (2021). Molecular typing of 16sRNA of *Bacillus species* isolated from soil and cow dung from farm settlement
5. Adeoti O.M., Aderoju D.A, Adeoye K.A (2021). Physical and Chemical Assessment of Charcoalized Soil on the Field Performance of Cowpea (*Vigna esculantus*)
6. Adeoti O.M., Aderoju D.A, Adeoye K.A (2021). Effects of Charcoalized Soil on the Field Performance of Maize (*Zea mays*) and Cowpea (*Vigna esculantus*)
7. Adeoti O.M., Adula B.B, Adesina D.A., Adedokun E.O., Olaoye O.J (2021). Multi components Putative Oral vaccine against *Plasmodium falciparum*
8. Adeoti O.M, Agoro A.A, Adeoye K, Adesina D.A, Olufemi S.O (2021). 16S rRNA profiling of PGPR bacteria farmland isolates in Saki
9. Adeoti O.M., Sodiq Z, Olufemi S.O., Komolafe K.A (2021). Effects of chemical mutagen (Sodium Azide) on Onions grown in organic and Inorganic fertilized soil (Ecology free)
10. Adeoti O. M, Sanusi T. A, Adesina D. A., Komolafe K.A., Olaoye O. J. (2021). An *In Silico* Design of Oral vaccine Against *Salmonella typhi*. Open Journal of Case Report 2: Volume 127, Issue 2. Pg. 1-5
11. Adeoti O. M., Bello A. H., Adeoye K. A, Olaoye O. J (2021). Antibacterial Potency of the Coat of *Citrus sinensis* (Orange) On Bacillus Species (Microbes)
12. Adeoti O.M, Adeoye K.A (2021). Comparative Histopathological Effects of *Alchornea cordifolia* on Liver and Kidney of *E. coli*-induced Rats.
13. Adeoti O.M., Usman A.T., Adeoye K.A, Olaoye O.J. (2021) The Microbiological profiles of *Bacillus spp* isolated from Command peasant farm in Saki (highlight in science)
14. Adeoti O.M., Aderibigbe T.S. (2021). The intercontinental 'omics' mining of antibiotic Resistant Genes of *Mycobacterium tuberculosis (Live DNA)*
15. Adeoti O.M, Akinsooto A.V. (2021). Prediction of multi-epitopic region of a putative Oral Vaccine against Hepatitis C Virus
16. Adeoti O.M., Aderinto A.O., Adesanya V.O., Olaoye O.J. (2021). A bioinformatics approach to designing a multi-valent vaccine against *E. histolytica.* Biomed Research Journal (ISSN: 2578-8892)
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Mr. Adeoti Olatunde is member of learned societies with some of his selected membership and distinguished academic laurels herein stated.

Awards and Fellowship

- Scientific Engagement Grant: An award from Public Health foundation of India 2016.
- Member, Oyo State Education Trust Fund (OYOETF) 2017-2021.
- Editorial Board Member of Biomedical Sciences of Science Publishing Group, New York, NY 10018, United State of America 2018.
- The Founder, Face Out Malaria and AIDS Foundation (FOMA), Non-Governmental Organization which is a non-governmental organization from, 2005 to date.

Usman Ashiat Temitope, a graduate student was born in Ibadan Oyo State, Nigeria on June 21st 1997. She attended Araromi Community primary school from 2002–2008 for basic education then Aperin Oniyere Commercial Grammar school between 2008-2014 for her Secondary education. She is a graduate student of the Oke Ogun Polytechnics Saki Oyo State and now a holder of equivalent of first degree in the Department of Science Laboratory Technology (Microbiology option). She has peer authored books and six journals articles. She is a trained molecular biologist and an astute scholar of molecular epidemiology. Currently she is working in a molecular team that is deciphering whole genome sequencing of common pathogens. She has attended several workshops on vaccine development by the use of bio-informatics approach. She is a diligent trainee in molecular epidemiology of *Mycobacterium tuberculosis.* She loves singing and friends' making. She is a devotee of community services and advocacy. She is endowed with highly enterprising skills and has a promising dream to be one of the youngest professors of molecular epidemiology in just no distant future. Above all, she is a God-fearing, generous and kind-hearted.