

Full Length Research Paper

Molecular identification of indigenous bacteria isolated from pesticides heavily contaminated soils

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This study was carried out to isolate and identify indigenous bacteria associated with pesticides heavily contaminated soil in Sudan. The samples were collected from pesticides contaminated soil in different locations. The predominant bacterial isolates were then purified by subculturing on selective media. Seven isolates were selected based on their capability to degrade pesticides and three of them having the highest performance in biodegradation of frequently reported pesticide contaminants were subject to identification by molecular tools using 16S rDNA gene sequence analysis. Amplification of 16S rDNA gene was done from genomic DNA and sequenced. Construction of phylogenetic tree was done after aligning of multiple sequences. The results reveal that the three bacterial isolates are *Bacillus cereus* ATCC 14579, *Bacillus subtilis* subsp. *inaquosorum* KCTC 13429 and *Bacillus safensis* FO-36b. This is the first record of pesticides biodegradation by *B. safensis* FO-36b which can be considered as a novel contribution in this field. Since these strains were found effective and are living naturally in an environment heavily contaminated with pesticides, therefore, they might have potential in removing pesticides from contaminated soils, especially soils of pesticide stores and dumping sites in developing countries.

Key words: Bacillus, gene sequencing, pesticides-polluted soil, Sudan.

INTRODUCTION

Pesticide residues in soil and plants are one of the major environmental issues, which affect directly or indirectly humans and animal's health and act as a potential threat to the environment. Microorganisms with high efficient biodegradation rates could be considered as potential bioremediation agents and could be used for

biotechnological applications. Biodegradation by naturally occurring microorganism to remove or detoxify pesticides (Huang et al., 2018), insecticides (Ishag et al., 2016, 2017), herbicides (Kanissery and Sims, 2011) and oil (Abbas et al., 2018) residues from contaminated soils is a useful and eco-friendly approach.

Many genera of different types of bacteria such as *Alcaligenes*, *Bacillus*, *Flavobacterium* and *Pseudomonas* had been reported to effectively degrade different types of pesticides (Ishag et al., 2016; Huang et al., 2018). Moreover, Trama et al. (2014) concluded that microorganisms that originated from contaminated environments usually are strong and powerful in bioremediation. The success of the pesticides bioremediation process is affected by the type of microorganism which affects the rate of bioremediation, environmental factors and nature (Lovecka et al., 2015; Dzionek et al., 2016; Abdelbagi et al., 2018).

The potential of local existing soil microorganisms in remediation of heavily contaminated soils in Sudan was reported by Abdelbagi et al. (2000, 2003). Further investigation was done by Ali (2005), who studied the biodegradation of endosulfan and γ -HCH and confirmed the efficiency of the local existing soil microorganisms in degradation process of tested pesticides. Subsequent works on endosulfan biodegradation were carried out by Elsaid et al. (2009, 2010a, b) who provided further evidence for the potential of local microorganisms in biodegradation of tested pesticides and they found that the degradation rate could be accelerated by activators such as farm manure and synthetic fertilizers (Elsaid et al., 2009). The biodegradation of pendimethalin, endosulfan, chlorpyrifos, malathion, and dimethoate utilizing local bacteria were investigated by Ishag et al. (2016, 2017) and Sharef et al. (2013). The latter reported that the pendimethalin could best be mineralized by *Pseudomonas aeruginosa*. All the previous works did not use molecular biotechnological tools method such as 16s rDNA gene (Aakra et al., 1999; Bosshard et al., 2006) and rather depends on biochemical identification, except Ishag et al. (2016; 2017) which utilized both biochemical and molecular biotechnological tools, however, these papers displayed the degradation pathways and the kinetics of the reaction. The molecular identification findings were considered in details in the current paper. Therefore, this study present the molecular identification (using 16s rDNA gene) data of some of the indigenous bacteria found in pesticides highly polluted soil. The significance of the method 16s rRNA gene includes the followings; it could identify bacteria at the species level and assist in differentiating between closely related bacterial species. Moreover, the 16s rRNA is part of the translation process, in all types of bacteria and therefore it represent a perfect universal target. Besides that it has a multi-copy gene, which increases the detection sensitivity. Furthermore, it consists of conserved and highly variable regions, which increases its detection specificity and also allows for the use of universal primer

In addition it evolves at relatively constant rates, which allows inferring phylogenetic relationships (Patel, 2001, Clarridge 2004, Woo et al., 2008).

MATERIALS AND METHODS

Soil samples collection method

Various pesticides contaminated storage soils (Alrahad, Barakat, and Hassahisa) were randomly sampled, using a soil auger of 10 cm length and 5 cm diameter. Soil samples were collected aseptically from upper layers (0 -30 cm) of the pesticide store where the maximum population of microorganisms is expected to exist. Five augers of soil samples were taken from each of the three stores and mingled carefully to produce composite sample (1 kg). Taken samples were wrapped in tapestry cloth sacks and labeled and transferred at once to pesticides center, University of Khartoum for isolation and identification.

Sample preparation

The samples were spread on the bench overnight to dry under room temperature. Samples were blended carefully and the aggregated particles and clods were broken manually under aseptic conditions to a reasonable and homogeneous size. Crushed samples were then wrapped in tapestry cloth sacks, labelled and sent directly to Plant Pathology Laboratory, University of Khartoum for Isolation and Identification.

Media preparation method

Nutrient agar (NA)

The medium was prepared by adding 5.0 g of peptone, 5.0g of NaCl, 1.5 g of beef extract, 1.5 g of yeast extract and 15.0 g of agar to a liter of distilled water, then the final pH was adjusted to 7.4 ± 0.2 (at 25°C) (Tepper et al., 1993).

Nitrate agar (NA)

The NA was prepared following the method described by Society of American Bacteriologist (SAB) (1944) by adding 5.0 g of peptone, 3.0 g of beef extract, 1.0 g of potassium nitrate and 12.0 g of agar to a liter of distilled water and the final pH was adjusted to 6.8 ± 0.2 (at 25°C).

Preparation of soil suspension

The soil suspension was prepared by dissolving 1 g of soil in 10 ml of sterile distilled water followed by gentle shaking. One gram of soil was dissolved in 10 ml of sterile distilled water to make soil suspension and shake gently. Serial dilution was carried out to reduce microbial density and to get a single colony isolate. One millilitre of soil suspension from each of the six soil samples was taken using micropipette and added to sterilized test tube containing nine ml of distilled water and shaken well to give six serial dilutions.

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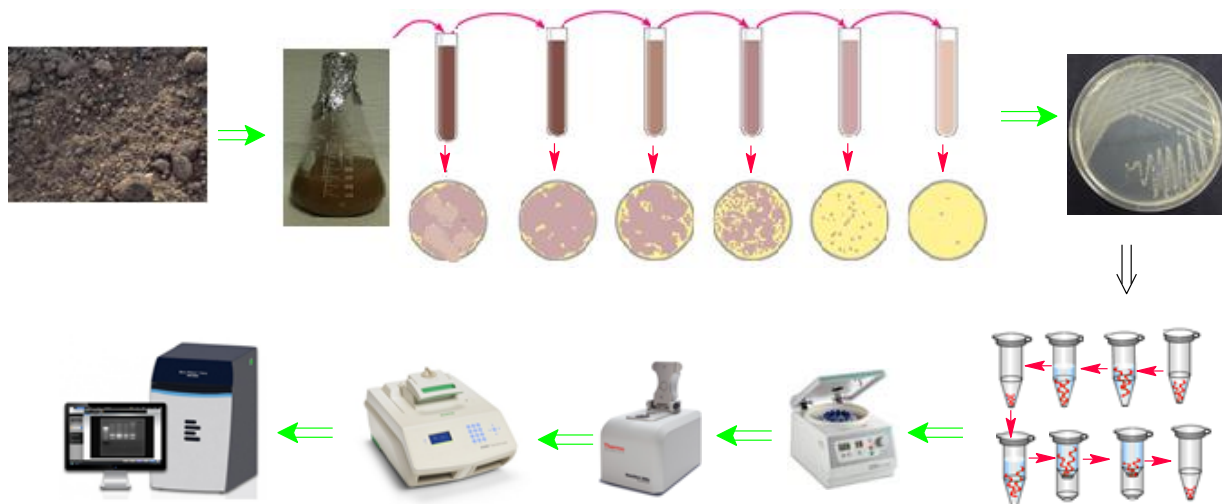


Figure 1. A simplified diagram for the method used.

Isolation of microorganisms from pesticide-polluted soil

One hundred microliters from each serial dilution were added to the surface of the nutrient agar (NA) medium on the center of each Petri dish and distributed carefully to the whole plate. The cultures were incubated at 30°C for 24 h. Hundred microliters from each serial dilution was added to the nutrient agar (NA) surfaces in the center of each plate and distributed carefully to the whole plate. The inoculums were spread on the NA surfaces using flamed glass spreader and incubated for 24 h at 30°C (Figure 1).

Purification and identification of the bacterial isolates

The dominant morphologically different bacterial colony types were chosen from the plate count agar. Predominant bacteria from morphologically different colony types were selected from plate count agar. The purification of different isolates was carried out by repeated subculturing on the selective media of nitrate agar. This was followed by streaking the isolates into sterile nutrient agar and left to propagate at 37°C for 24 h. The propagated bacterial colonies were sub-cultured on slope nutrient agar medium and preserved in a refrigerator at 4°C for further tests.

Culture and morphological characteristics

Barrow and Felthman (2003) procedure was followed for identification and purification of the isolates. Morphological tests performed include; Grams staining, shape, endospore staining, motility, and growth in the presence of air. The set of bio-chemical tests used in the identification of microorganisms are catalyzed test, oxidize test, glucose (acid) and O/F test.

Molecular identification

Genomic DNA extraction

Purified isolated bacterial cells were picked from pure culture and re-suspended in 10 µl of nuclease-free water. Genomic DNAs were

extracted from isolates at Macrogen Ltd., Korea, using the following protocol; Cells were boiled in water bath at 95°C for 3 min followed by cooling down on ice for 1 min. The suspensions were then centrifuged at 1500 rpm for 5 min. The extracted genomic DNAs were used as a template for PCR analysis.

PCR amplification of 16S rDNA gene

The 16S rDNA gene was amplified from genomic DNA using Macrogen universal primer sets 27F 5'-AGAGTTTGATCMTGGCTCAG -3' and 1492R 5'-TACGGYTACCTTGTTACGACT-3'. Amplification was performed according to the standard protocol using a EF-Taq (SolGent, Korea) as follows: initial denaturation at 95°C for 2 min, 35 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 1 min and a final extension step of 10 min at 72°C. PCR products were separated by gel electrophoresis on 1.2% agarose gels, stained with ethidium bromide and visualized with a UV transilluminator.

Purification of PCR products

PCR products were cut from the gel, purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA) and quantified with Nano Drop spectrophotometer before sequencing.

Sequencing of 16S rDNA gene

Purified PCR products were sequenced using PRISM Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). The PCR reaction was performed with 20 ng genomic DNA as a template and both forward primer (27F) and reverse primer (1492R) in a 30 µl reaction mixture in separate reactions. The PCR reactions were performed under the same conditions described above. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95°C for 5 minutes, followed by 5 minutes on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). The homology of the 16S rDNA gene

sequences was checked with the 16S rDNA sequences of other organisms that have been submitted to a database of NCBI (<https://www.ncbi.nlm.nih.gov>). The determined sequences of 16S rDNA gene were analyzed in the EZBIOCLOUD database (<http://www.ezbiocloud.net/eztaxon>).

Phylogenetic analysis

Multiple sequence alignment was performed using CLUSTALW program (<http://www.genome.jp/tools-bin/clustalw>) and edited using BioEdit 7.2.5 software (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). The sequences used in this study were as follows: AM: 747812 (*Brevibacterium halotolerans* DSM 8802T), JH: 600280 (*Bacillus mojavensis* RO-H-1T), AMXN: 0100002 1(*Bacillus subtilis* subsp. *inaquosorum* KCTC 13429T), CP: 002905(*Bacillus subtilis* subsp. *spizizenii* NRRL B-23049T), AYTO: 01000043 (*Bacillus tequilensis* KCTC 13622T), JH: 600273 (*Bacillus vallismortis* DV1-F-3T), ABQL: 01000001 (*Bacillus subtilis* subsp. *subtilis* NCBI 3610T), JTKJ: 01000077 (*Bacillus methylotrophicus* KACC 13105T), FN: 597644 (*Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* DSM 7T), AJVF: 01000043 (*Bacillus siamensis* KCTC 13613T), CP: 000560 (*Bacillus amyloliquefaciens* subsp. *plantarum* FZB42T), AB: 021181 (*Bacillus atrophaeus* JCM 9070T), AYTN: 01000016 (*Bacillus sonorensis* NBRC 101234T), AE: 071333 (*Bacillus licheniformis* ATCC 14580T), AJ: 831843 (*Bacillus aerius* 24KT), ASJD: 01000027 (*Bacillus safensis* FO-36b^T), ABRX: 01000007 (*Bacillus pumilis* ATCC 7061^T), Aj: 831844 (*Bacillus aerophilus* 28K^T), ASJC:01000029 (*Bacillus altitudinis* 41KF2b^T), AJ: 831841 (*Bacillus stratosphericus* 41KF2a^T), AMSH: 01000114 (*Bacillus xiamenensis* HYC-10^T), CP: 000764 (*Bacillus cytotoxicus* NVH 391-98^T), ACMX: 01000133 (*Bacillus pseudomycooides* DSM 12442^T), ACNF: 01000156 (*Bacillus thuringiensis* ATCC 10792^T), CP:006863 (*Bacillus toyonensis* BCT-7112^T), AE: 016879(*Bacillus anthracis* Ames), AE:016877 (*Bacillus cereus* ATCC 14579^T), ACMU: 01000002 (*Bacillus mycooides* DSM 2048^T), BAUY: 01000093 (*Bacillus weihenstephanensis* NBRC 101238^T), and K: I271266 (*Lactobacillus brevis* ATCC 14869^T). The phylogenetic tree was constructed using a Neighbor-joining method utilizing a Mega software version 6.0 (Tamura et al., 2013).

RESULTS AND DISCUSSION

Previous work from this group (Elsaid et al., 2009; Elsaid et al., 2010a, b; Sharef et al., 2013; Abdurrahman et al., 2015; Ishag et al., 2016, 2017) evaluated the degradation efficiency of microorganisms especially bacterial strains isolated from pesticide-polluted storage soil in Sudan. The isolated strains have shown promising capability in degrading many of the frequently detected pesticide contaminants in storage soils and dump sites in the Sudan including; chlorpyrifos, malathion, dimethoate, pendimethalin, endosulfan alpha and endosulfan beta.

In this study, seven isolates were subject to preliminary biodegradation tests against the most frequently reported contaminants of hot spots in the Sudan. Three of them have shown high capability in the removal of pesticide contaminants from contaminated soils and therefore were

selected for further genetic identification.

The previously described works did not use molecular biotechnological tools and rather depend on biochemical identification, except Ishag et al. (2016, 2017) who utilized both biochemical and molecular biotechnological tools, however, their published work (Ishag et al., 2016, 2017) displayed the degradation pathways and the kinetics of the reaction only. Their molecular identification findings were considered in details in the current paper. This paper describes the molecular identification and characterization of these strains using 16S rDNA gene. The use of 16S rDNA gene for identifications of genus and species of bacteria has been reported by Barghouthi (2011), Kinuthia et al. (2010), Karisham and Hari (2014) and Maryam et al. (2014). This method (16s rRNA gene) can identify bacteria at the species level as well as assist in differentiating between closely related bacterial species. Moreover, the 16s rRNA is part of the translation process, in all types of bacteria and therefore it represent a perfect universal target. Besides it has a multi-copy gene, which increases the detection sensitivity. Furthermore, it consists of conserved and highly variable regions, which increases its detection specificity and also allows for the use of universal primer. In addition it evolves at relatively constant rates, which allows inferring phylogenetic relationships (Patel, 2001; Clarridge, 2004; Woo et al., 2008). The genomic DNA was extracted and analyzed on an agarose gel which indicated good quality of band for PCR analysis (Figure 2). Extracted DNA from the isolates was subject to PCR amplification of 16S rDNA gene using universal primers. These primers amplify a specific fragment of 16S rDNA gene in all isolates at the length of 1500 bp. Similar results of band amplification of 16S rDNA gene were obtained by Orengo et al. (2010) using those primers.

The amplified PCR products were purified and sequenced using the Sanger-dideoxy method. The results of sequencing indicated that the 16S rDNA gene consists of 1487, 1480 and 1479 nucleotides in isolates 1, 2 and 3 respectively. In order to identify genus and species, analysis of partial 16S rDNA sequences (Figures 3 to 5) was used for BLAST search against GeneBank database of *Bacillus* species in NCBI. The sequences were aligned by multiple sequence alignment using CLUSTALW program.

The Neighbor-joining method was used to construct the phylogenetic tree using Mega 6.0 software. The phylogenetic analysis found that the three isolates belong to the genus *Bacillus*. The 16S rDNA gene of isolate 1, isolate 2 and isolate 3 showed 100, 99.9 and 99% similarity with 16S rDNA gene of the *B. cereus* ATCC 14579, *B. subtilis* subsp. *inaquosorum* KCTC 13429 and *B. safensis* FO-36b, respectively (Table 1 and Figure 6).

This result indicated that the three isolates belong to species *cereus*, *subtilis* and *safensis*. The efficiency of the identified isolates in degrading frequently detected

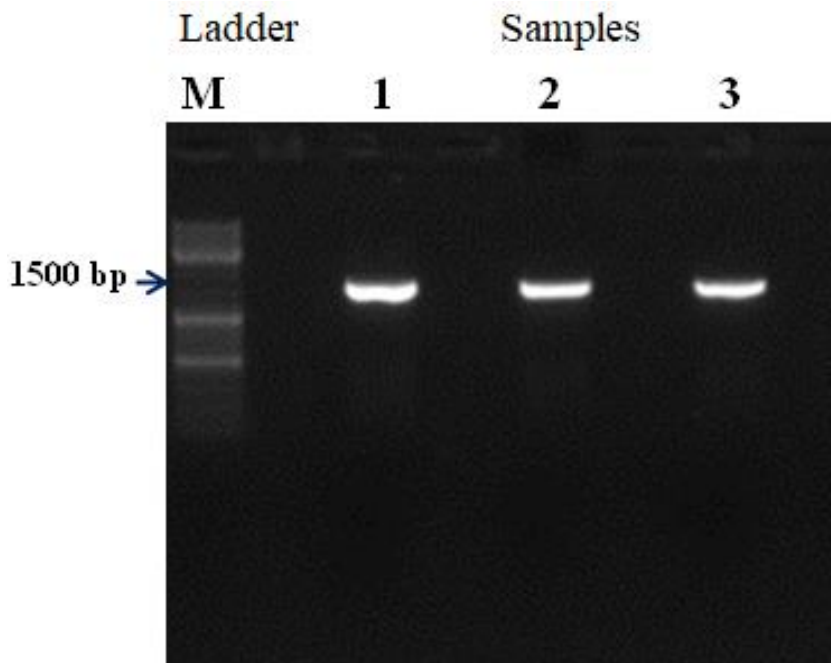


Figure 2. Genomic PCR analysis of Bacillus strains PCR amplification of 16S rDNA gene using universal primers. M, Marker; 1, Bacillus strain 1; 2, Bacillus strain 2; 3, Bacillus strain 3.

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1 GCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAG
76 TTAGCGGCGGACGGGTGAGTAACACGTGGGTAACTGCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAA
151 TACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCG
226 TCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA
301 CACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTCCGCAATGGACGAAAAGTCTG
376 ACGGAGCAACCGCGTGAAGTGAAGGCTTTCGGGTCGTAATACTCTGTTGTAGGGAAGAACAAGTGTCTAGT
451 TGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGGGTAATACG
526 TAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCC
601 CACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAAATCCATGTGTA
676 GCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTCTGGTCTGTAAGTACACTGAGGC
751 GCGAAAGCGTGGGAGCAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACCGATGAGTGTAAAGTGTTAGA
826 GGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACT
901 CAAAGGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTGAAAGCAACGCGAAGAACCCTTACCA
976 GGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTG
1051 TCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTG
1126 AGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTT
1201 ATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCAGGAGGTGGAGCTAATCTCATA
1276 AAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGC
1351 ATGCCGCGGTGAATACGTTCCCGGGCCTTGACACACCGCCCGTCACACCAGAGAGTTTGTAAACCCCGAAGTC
1426 GGTGGGTAACCTTTTTGGAGCCAGCCGCTAAGGTGGGACAGATGATTGGGGTGAAGTCGT
    
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Figure 3. The 16S rDNA gene sequences of Bacillus strain 1 (1487 bp).

pesticide contaminants (chlorpyrifos, malathion, dimethoate, pendimethalin, endosulfan alpha, endosulfan beta, temphos and fenthion) was described elsewhere (Ishag et al., 2016, 2017; Abdelbagi et al., 2018). These

findings agree with those of Kinuthia et al. (2010), Karisham and Hari (2014) and Maryam et al. (2014) who utilized 16S rDNA gene sequences to identify different species of Bacillus from pesticides polluted soil. The

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1 CTCAGACGACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGC
76 GCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCG
151 GATGCTTGTGTTGAACCGCATGGTTCAAACATAAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCA
226 TTAGCTAGTTGGTGGGTAACGGCTACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTG
301 GGAAGTACGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA
376 GCAACGCCGCGTGAGTGATGAAGGTTTTCCGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTGCAAT
451 AGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT
526 GGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCGG
601 CTC AACCGGGGAGGGTCAATTGAAAACCTGGGAACTTGAGTGCAGAAGAGGAGAGTGGAAATCCACGTGTAGCGGT
676 GAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAAGTACGCTGAGGAGCGAA
751 AGCGTGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCGTAAACGATGAGTGCTAAGTGTAGGGGGTT
826 TCCGCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGAGTACGGTTCGCAAGACTGAAACTCAAAG
901 GAATTGACGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTGAAAGCAACGCGAAGAACCCTTACCAGGTCT
976 TGACATCCTCTGACAACTCCTAGAGATAGGACGTCCCTTCGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTC
1051 AGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGATTGAGTTG
1126 GGCACCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGAC
1201 CTGGGCTACACACGTGCTACAATGGACAGAACAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATCT
1276 GTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCC
1351 GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTACACCACGAGAGTTTGAACACCCGAAGTCGGTGA
1426 GGTAACCTTTTAGGAGCCAGCCCGGAAGGTGGGACAGATGATTGGGGTGAAGTC

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Figure 4. The 16S rDNA gene sequences of *Bacillus* strain 2 (1480 bp).

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1 TCAGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGAAAGGGAGCTTGCTCCCGGATGTTAGC
76 GCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGAGCTAATACCG
151 GATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCA
226 TTAGCTAGTTGGTGGGTAATGGCTACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTG
301 GGAAGTACGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA
376 GCAACGCCGCGTGAGTGATGAAGGTTTTCCGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAGAGTAAC
451 TGCTCGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
526 GCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCGGC
601 TCAACCGGGGAGGGTCAATTGAAAACCTGGGAACTTGAGTGCAGAAGAGGAGAGTGGAAATCCACGTGTAGCGGTG
676 AAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAAGTACGCTGAGGAGCGGAA
751 CCGTGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCGTAAACGATGAGTGTGCTAAGTGTAGGGGGTTT
826 CGCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGAGTACGGTTCGCAAGACTGAAACTCAAAGG
901 AATTGACGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTGAAAGCAACGCGAAGAACCCTTACCAGGTCTT
976 GACATCCTCTGACAAACCTAGAGATAGGGCTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTC
1051 GCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTGAGTTGG
1126 GCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACC
1201 TGGGCTACACACGTGCTACAATGGACAGAACAAGGGCTGCAAGACCGCAAGGTTTAGCCAATCCCATAAATCTG
1276 TTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCC
1351 CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTACACCACGAGAGTTTGAACACCCGAAGTCGGTGA
1426 GTAACCTTTATGGAGCCAGCCCGGAAGGTGGGACAGATGATTGGGGTGAAGTC

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Figure 5. The 16S rDNA gene sequences of *Bacillus* strain 3 (1479 bp).

Table 1. Phylogenetic neighbors of bacteria on the basis of similarity to the partial 16S rDNA sequence.

Accession No.	Species (16S rRNA gene analysis)	Pairwise Similarity (%)	Diff/total nt	Identity (%)
AE016877	<i>Bacillus cereus</i> ATCC 14579T	100	0/1487	100
AMXN0100021	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i> KCTC 13429T	100	0/1479	99.90
ASJD0100027	<i>Bacillus safensis</i> FO-36bT	100	0/1479	99

Diff ≡ different; nt ≡ nucleotide.

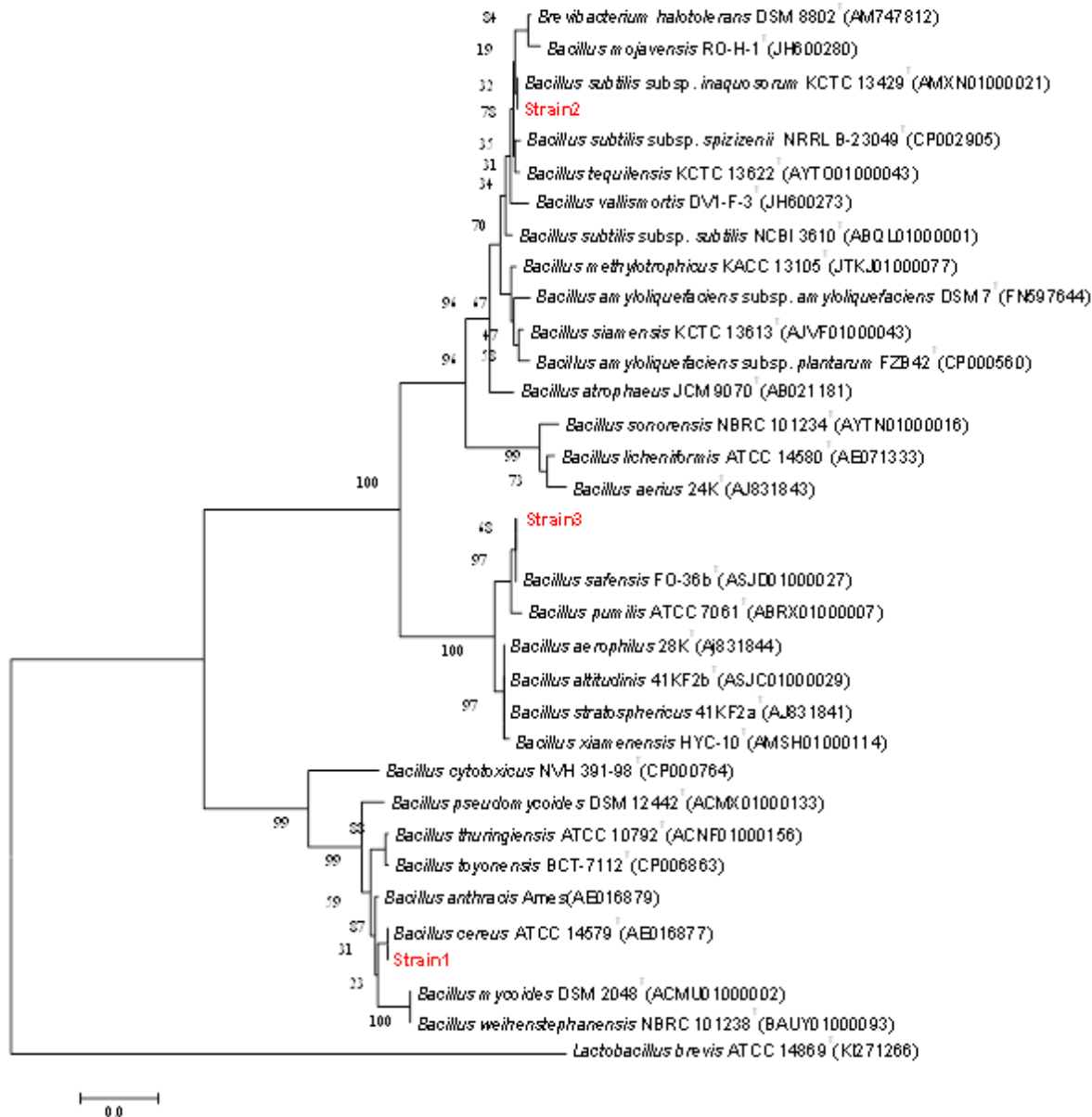


Figure 6. Phylogenetic relationships between the different strains of *Bacillus* isolated*. Numbers above and below branches indicate bootstrap values from Neighbor-Joining analysis. *Based on the analysis of partial 16S rDNA sequences.

identified isolates in these studies were also found capable of degrading pesticide contaminants. Further research should be done to clone genes responsible for degradation of pesticides to engineer indigenous microorganisms for bioremediation of pesticide in polluted soils and dumping sites.

Conclusion

Three indigenous isolates of *Bacillus* were isolated from

pesticide heavily contaminated soils in Sudan and were identified by 16S rDNA gene as *B. cereus*, *B. subtilis* and *B. safensis*. The identified isolates have shown promising potential in degrading many pesticide contaminants and can be further developed for future remediation of pesticides heavily polluted soils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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