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Isolation and Molecular Identification of *Bacillus sonorensis* S12 16S rRNA gene from Poultry Feces samples of Latur Region Poultry Farm (MS) India

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Abstract

The accumulation of poultry waste, comprising various materials such as broiler and layer carcasses, feathers, bones, blood, hatchery debris, and deceased birds, poses significant environmental and health concerns. These waste materials can lead to microbial contamination, foul odours, and the proliferation of pests like flies and rodents, thereby contributing to environmental pollution. The Samples were collected according to standard microbiological procedures during study period September 2022 to June 2023. 2-5 gm of samples were collected in sterile screw cap tube using sterile spatula and immediately transported with specially prepared ice box to the laboratory for further analysis. Biochemical assays confirmed the bacterial specimens, and molecular characterization was conducted through polymerase chain reaction (PCR) and sequencing of the 16S rRNA gene of *B. sonorensis*. The newly sequenced 16S rRNA gene sequences demonstrated 100% homology to *B. sonorensis*, as analyzed using the NCBI-BLAST tool.Phylogenetic analysis and nucleotide base composition studies were performed using 60 sequences of the 16S rRNA gene from various *Bacillus* isolates, including *Bacillus sonorensis*. For this purpose, 16S rRNA gene sequences were retrieved from NCBI in FASTA format. The phylogenetic analysis, conducted using the Maximum Likelihood method, revealed the relationships and percent similarity of the Bacillus sonorensis 16S rRNA gene.

Keywords: Poultry Feces; 16S rRNA Gene; *B. sonorensis;* phylogenetic analysis; BLAST Tool

1 Introduction

The rapid growth of the poultry sector in India has indeed brought about numerous benefits, including increased production, economic growth, and job opportunities. However, alongside these advantages come significant challenges, particularly in managing the resulting poultry waste. The accumulation of poultry waste, comprising various materials such as broiler and layer carcasses, feathers, bones, blood, hatchery debris, and deceased birds, poses significant environmental and health concerns. These waste materials can lead to microbial contamination, foul odours, and the proliferation of pests like flies and rodents, thereby contributing to environmental pollution. While efforts are being made to find ways to reuse poultry waste, such as converting them into fertilizers or animal feed supplements, there is a pressing need to address the potential health risks associated with these waste materials. One concern is the possibility that these waste deposits could serve as reservoirs for the multiplication of pathogenic microorganisms [1]. In intensive poultry production, newly hatched chicks are unable to maintain contact with their mothers, which results in a slower colonization of beneficial microbial flora in their intestinal tracts. Consequently, this makes them more vulnerable to infections from pathogenic microorganisms such as *Salmonella typhimurium, Escherichia coli*, and *Clostridium perfringens* Mandana Salehizadeh [2].This also results in the generation of large quantities of poultry waste, typically consisting of broilers and layers, feathers, bones, blood, hatchery debris, and deceased birds. These wastes pose significant environmental pollution issues due to microbial contamination, offensive odours, and the attraction of flies and rodents. In developing countries like India, proper disposal units for this waste are lacking. However, efforts

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are underway to repurpose these materials into beneficial products such as fertilizers and animal feed supplements. Given that these wastes consist of tissues and blood, we hypothesize that they may serve as a reservoir for the multiplication of various pathogenic microorganisms capable of causing severe disease outbreaks [1] [3].This study aims to identify potential pathogens that can survive and thrive in poultry waste, which is a crucial step in understanding and mitigating the risks posed by these waste materials. By identifying and studying these pathogens and assess the potential hazards they pose to human and animal health and develop strategies to manage and minimize these risks. Overall, this research addresses an important gap in knowledge regarding the microbial ecology of poultry waste and contributes to efforts aimed at ensuring the sustainable and safe management of poultry production in India. Therefore, the present study was conducted to identify the potential pathogens that can survive in this poultry waste and pose a hazard.

2 Material and Methods

2.1 Study Area

The poultry waste samples were collected from the poultry shed in and around the Udgir, a suburban situated in the east side of Latur, Maharashtra State, India. Samples were collected from two poultry farm of different regions.

2.2 Sample Collection

The Samples were collected according to standard microbiological procedures [4, 5] during study period September 2022 to June 2023. 2-5 gm of samples were collected in sterile screw cap tube using sterile spatula and immediately transported with specially prepared ice box to the laboratory for further analysis. The media and reagents used for the study such as Nutrient Broth, Nutrient Agar. The collected samples were processed aseptically in the laboratory. 200 mg of each sample were inoculated into the 100 ml of freshly prepared nutrient broth and incubated at 24 hrs at 37^0C. Isolation of chicken feces samples was done on sterile nutrient agar. The plates were incubated for 1 week at 37^0C. The colony characters were observed and gram staining was performed.

2.3 Molecular Characterization of *Bacillus sp*ecies

Positive samples of *Bacillus sp.* identified through morphological and biochemical assays, were further analyzed using molecular characterization methods with a PCR-based assay. The details are provided below:

2.4 Genomic DNA Extraction from the selected cultures

DNA extraction from bacterial colonies was performed using a Sodium Dodecyl Sulfate (SDS)-based method [6, 7, 8].The bacterial cell suspension was treated with a lysis buffer containing SDS, Trisaminomethane Hydrochloric Acid (Tris HCl), and Ethylene-diaminetetra-acetic acid (EDTA). Cell debris and other impurities were removed through several sequential steps involving centrifugation. Genomic DNA was precipitated using chilled ethyl alcohol and collected as a pellet by centrifugation. The pellet was then dissolved in TE buffer and stored at 4°C until further use.

2.5 Amplification of 16S rRNA gene using PCR

For the PCR reaction, the total reaction volume was 50 µl, containing 5 µl of DNA template, 1U Ampl Taq DNA polymerase, 10 pmol of each primer (forward and reverse, purchased from Sigma-Aldrich, Hyderabad), 200 µmol of each Deoxyribonucleoside triphosphate, 1.5 mmol of MgCl2, 10 mmol of Tris-HCl (pH 8.8), 50 mmol of KCl, and 0.1% Triton X-100.

2.6 Local sequence Alignment

Basic local Alignment Search Tool (BLAST) was performed for the different isolates of *Bacillus sonorensis*16S rRNA gene sequence retrieved from NCBI to identify the homology or similarity its relatives in different isolated of *Bacillus sonorensis* using the online NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This software takes the data in FASTA format and produces the BLAST table.

2.7 Phylogenetic Analysis

Phylogenetic analysis of the 16S rRNA gene sequence of *Bacillus sonorensis* was conducted using Maximum Likelihood methods with MEGA7. The software generated phylogenetic trees illustrating the ancestral relationships among the sequences. Sequences within the same cluster were found to be closely related.

3 Result and Discussion

Bacillus sonorensis strain S12 16S rRNA gene, complete sequence was retrieved from the NCBI in FASTA format. The Sequence of the (PP654466) is as the following: PP654466.1 *Bacillus sonorensis* strain S12 16S rRNA gene, complete sequence.

TACCGGATGCTTGATTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGA
CCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGG
GTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGC
AATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGT
TAGGGAAGAACAAGTACCGTTCGAACAGGGCGGTGCCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCG
CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAAC
TTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCA
GTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAG
ATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCA
GCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCT
CTGACACCCCTAGAGATAGGGCTTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCT
CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTT
GGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCT
TATGACCTGGGCTACACACGTGCTACAATGGGCAGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAAT

3.1 Specific set of primers of *Bacillus sonorensis* strain S12 16S rRNA gene Sequence

Specific primer of *Bacillus sonorensis* strain S11 16S rRNA gene sequence was designed using Primer 3 (V040) tool, as illustrated in Figure 1. We obtained a set of primer for 16S rRNA sequence of covering 181 nucleotide sequence length. The length of sense primer is 315 nucleotide (identified with red colour) and antisense primer length is 495 nucleotide (identified with Blue colour). We also check self-complementary alignment of specific primers sequences and PCR Protocols are describing in Table 1.

```
No mispriming library specified
Using 1-based sequence positions
OLIGO            start  len      tm     gc%   any    3' seq
LEFT PRIMER        315   20   59.99   55.00  6.00  0.00 GCCTTGACGGTACCTAACCA
RIGHT PRIMER       495   20   60.08   50.00  3.00  0.00 CTCAAGTTCCCCAGTTTCCA
SEQUENCE SIZE: 1120
INCLUDED REGION SIZE: 1120

PRODUCT SIZE: 181, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 0.00

    1 TACCGGATGCTTGATTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTAGCTACCACT

   61 TACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGA

  121 TGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCC

  181 TACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCG

  241 CGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGT

  301 TCGAACAGGGCGGTGCCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAG
                    >>>>>>>>>>>>>>>>>>>>

  361 CAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCG

  421 CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAA
                                                              <<<<<

  481 ACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGT
      <<<<<<<<<<<<<<<

  541 AGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGG

  601 CGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG

  661 AGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCC

  721 GCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC

  781 GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCT

  841 CTGACACCCCTAGAGATAGGGCTTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTT

  901 GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATC

  961 TTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAG

 1021 GTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATG

 1081 GGCAGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAAT
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Figure 1 *Bacillus sonorensis*16S rRNA gene Primer3 Output

Table 1 Detail of Primers sequences used to amplify *Bacillus sonorensis* 16S rRNA gene

Gene	Primer type	Sequences	Primer Length Product Size	PCR
16S rRNA PCR Primer	Forward Primer	GCCTTGACGGTACCTAACCA	20	181
	Reverse Primer	CTCAAGTTCCCCAGTTTCCA	20	

3.2 Amplification of*Bacillus sonorensis strain* S11 16S rRNA gene

3.2.1 Sequence using PCR

Prepared PCR reaction mixtures were prepared with methods. Amplification of 16S rRNA gene was performed in PCR Thermo cyclers (Applied Biosystems Ver 96) for 30 cycles by using run methods: denaturation at 95°C for 50 seconds and extension at 72°C for 1.80 min. The cycles were antedate by a denaturation step at 95°C for 4 min, afterwards extension in step at 72°C for 3.50 min.

3.2.2 Local sequence alignment

We have sequenced one isolate *Bacillus sonorensis*16S rRNA and different isolates of *Bacillus sonorensis*16S rRNA gene sequences were retrieved from the NCBI (https://www.ncbi.nlm.nih.gov/nuccore) in FASTA format and performed local sequence alignment by using online NCBI BLAST tool download BLAST table in that description of the gene, accession numbers, percent of similarity, etc. (Table 2).

Table 2 BLAST table of 16S rRNA of *Bacillus sonorensis.*

Sr. No	Description	Max Score	Total Score	% Identify	Accession No.
1	Bacillus sonorensis strain 1779 16S ribosomal RNA gene, partial sequence	2069	2069	100	MT597627.1
2	Bacillus licheniformis strain EB69 16S ribosomal RNA gene, partial sequence	2069	2069	100	MT509772.1
3	Bacillus sonorensis strain S8-TSB-4 16S ribosomal RNA gene, partial sequence	2069	2069	100	MK026799.1
4	Bacillus sp. (in: Bacteria) strain GAM-2 16S ribosomal RNA gene, partial sequence	2069	2069	100	MK063725.1
5	Bacillus licheniformis strain B6 16S ribosomal RNA gene, partial sequence	2069	2069	100	MH384784.1
6	Bacillus sonorensis strain HR01 16S ribosomal RNA gene, partial sequence	2069	2069	100	MK283754.1
7	Bacillus sonorensis strain MPTD13 16S ribosomal RNA gene, partial sequence	2069	2069	100	MK392132.1
8	Bacillus velezensis strain HBUM07076 16S ribosomal RNA gene, partial sequence	2069	2069	100	MF662502.1
9	Bacillus sonorensis strain HBUM07075 16S ribosomal RNA gene, partial sequence	2069	2069	100	MF662501.1
10	Bacillus sp. (in: Bacteria) strain hsn03 16S ribosomal RNA gene, partial sequence	2069	2069	100	MF925340.1
11	Bacillus sp. (in: Bacteria) strain ZJ-8 16S ribosomal RNA gene, partial sequence	2069	2069	100	KY283149.1
12	Bacillus sp. (in: Bacteria) strain ZJ-2 16S ribosomal RNA gene, partial sequence	2069	2069	100	KY283143.1
13	Bacillus sonorensis strain 8B-B7 16S ribosomal RNA gene, partial sequence	2069	2069	100	MF062626.1
14	Bacillus sonorensis strain KNFB35 16S ribosomal RNA gene, partial sequence	2069	2069	100	PP708954.1
15	Bacillus sonorensis strain SRCM101395, complete genome	2069	16516	100	CP021920.1

16	Bacillus sonorensis strain CPO PLC1 16S ribosomal RNA gene, partial sequence	2069	2069	100	PP661233.1
17	Bacillus sonorensis strain S12 16S ribosomal RNA gene, partial sequence	2069	2069	100	PP654466.1
18	Bacillus sonorensis strain S3 16S ribosomal RNA gene, partial sequence	2069	2069	100	PP381848.1
19	Bacillus sonorensis strain V5768 16S ribosomal RNA gene, partial sequence	2069	2069	100	PP257132.1
20	Bacillus sp. (in: Bacteria) strain XSJ001YB 16S ribosomal RNA gene, partial sequence	2069	2069	100	KY077256.1
21	Bacillus sp. (in: Bacteria) strain mxJ22YB 16S ribosomal RNA gene, partial sequence	2069	2069	100	KY077250.1
22	Bacillus sonorensis strain PMC204 chromosome, complete genome	2069	16516	100	CP139190.1
23	Bacillus sp. (in: firmicutes) strain KICET-3 16S ribosomal RNA gene, partial sequence	2069	2069	100	OR807426.1
24	Bacillus sp. KICET-3 chromosome, complete genome	2069	16516	100	CP137345.1
25	Bacillus sp. LY-10 16S ribosomal RNA gene, partial sequence	2069	2069	100	KU363164.1
26	Bacillus sp. (in: firmicutes) strain RUJHM_91 16S ribosomal RNA gene, partial sequence	2069	2069	100	OR268217.1
27	Bacillus sp. Bac266k 16S ribosomal RNA gene, partial sequence	2069	2069	100	KP795921.1
28	Bacillus sp. Bac265k 16S ribosomal RNA gene, partial sequence	2069	2069	100	KP795920.1
29	Bacillus sonorensis strain LG-2 16S ribosomal RNA gene, partial sequence	2069	2069	100	KT725252.1
30	Bacillus sonorensis strain ALP-39 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ825022.1
31	Bacillus licheniformis strain MER_TA_88 16S ribosomal RNA gene, partial sequence	2069	2069	100	KT719497.1
32	Bacillus sonorensis strain HBUAS67378 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ804958.1
33	Bacillus sonorensis strain HBUAS67353 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ804933.1
34	Bacillus sonorensis strain HBUAS67347 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ804927.1
35	Bacillus sonorensis strain HBUAS67339 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ804919.1
36	Bacillus sonorensis strain HBUAS67309 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ804889.1
37	Bacillus sonorensis strain HBUAS67277 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ804857.1
38	Bacillus sonorensis strain T-2 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ750673.1

39	Bacillus sp. 09-E3 16S ribosomal RNA gene, partial sequence	2069	2069	100	KC920591.1
40	Bacillus sonorensis strain HBUAS67243 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ552705.1
41	Bacillus sonorensis strain HBUAS67198 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ552660.1
42	Bacillus sonorensis strain HBUAS67193 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ552655.1
43	Bacillus sonorensis strain HBUAS67189 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ552651.1
44	Bacillus sonorensis strain HBUAS67184 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ552646.1
45	Bacillus sonorensis strain HBUAS67182 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ552644.1
46	Bacillus sonorensis strain 5 16S ribosomal RNA gene, partial sequence	2069	2069	100	OQ195173.1
47	Bacillus sonorensis strain MSH6-1 16S ribosomal RNA gene, partial sequence	2069	2069	100	OM074006.1
48	Bacillus sonorensis strain HBUAS74061 16S ribosomal RNA gene, partial sequence	2069	2069	100	OP904276.1
49	Bacillus sonorensis strain HBUAS74058 16S ribosomal RNA gene, partial sequence	2069	2069	100	OP904273.1
50	Bacillus sonorensis strain HBUAS74049 16S ribosomal RNA gene, partial sequence	2069	2069	100	OP904264.1
51	Bacillus sp. (in: Bacteria) strain AAU1 16S ribosomal RNA gene, partial sequence	2065	2065	99.91	MF280233.1
52	Bacillus sonorensis partial 16S rRNA gene, strain 1-9AIA	2065	2065	99.91	FN397516.1
53	Bacillus sp. (in: firmicutes) strain Zab1 16S ribosomal RNA gene, partial sequence	2063	2063	99.91	PP683260.1
54	Bacillus sonorensis strain HBUAS71556 16S ribosomal RNA gene, partial sequence	2063	2063	99.91	PP425818.1
55	Bacillus sp. (in: firmicutes) strain RV157 16S ribosomal RNA gene, partial sequence	2063	2063	99.91	PP212042.1
56	Bacillus sonorensis strain JSNBEBT50_4C 16S ribosomal RNA gene, partial sequence	2063	2063	99.91	PP086720.1
57	Bacillus sonorensis strain HBUAS71466 16S ribosomal RNA gene, partial sequence	2063	2063	99.91	OR673057.1
58	Bacillus sonorensis strain HBUAS71454 16S ribosomal RNA gene, partial sequence	2063	2063	99.91	OR673045.1
59	Bacillus sonorensis strain US27 16S ribosomal RNA gene, partial sequence	2063	2063	99.91	OR251353.1
60	Bacillus sonorensis strain HBUAS67371 16S ribosomal RNA gene, partial sequence	2063	2063	100	OQ804951.1

This BLAST results clear that the newly sequenced (PP654466.1) *Bacillus sonorensis* 16S rRNA gene have showing 100% identity with different *Bacillus sonorensis*16S rRNA genes was submitted at NCBI Genebank and such as Bacillus sonorensis strain 1779 16S ribosomal RNA gene, partial sequence MT597627.1, Bacillus licheniformis strain EB69 16S

ribosomal RNA gene, partial sequence MT509772.1, Bacillus sonorensis strain S8-TSB-4 16S ribosomal RNA gene, partial sequence MK026799.1., Bacillus sp. (in: Bacteria) strain GAM-2 16S ribosomal RNA gene, partial sequence MK063725.1., Bacillus licheniformis strain B6 16S ribosomal RNA gene, partial sequence MH384784.1., Bacillus sonorensis strain HR01 16S ribosomal RNA gene, partial sequence MK283754.1., Bacillus sonorensis strain MPTD13 16S ribosomal RNA gene, partial sequence MK392132.1., Bacillus velezensis strain HBUM07076 16S ribosomal RNA gene, partial sequence MF662502.1., Bacillus sonorensis strain HBUM07075 16S ribosomal RNA gene, partial sequence MF662501.1., Bacillus sp. (in: Bacteria) strain hsn03 16S ribosomal RNA gene, partial sequence MF925340.1., Bacillus sp. (in: Bacteria) strain ZJ-8 16S ribosomal RNA gene, partial sequence KY283149.1., Bacillus sp. (in: Bacteria) strain ZJ-2 16S ribosomal RNA gene, partial sequence KY283143.1., Bacillus sonorensis strain 8B-B7 16S ribosomal RNA gene, partial sequence MF062626.1., Bacillus sonorensis strain KNFB35 16S ribosomal RNA gene, partial sequence PP708954.1., Bacillus sonorensis strain SRCM101395, complete genome CP021920.1., Bacillus sonorensis strain CPO PLC1 16S ribosomal RNA gene, partial sequence PP661233.1., However, other uncultured 16S rRNA gene sequence with accession number MF280233.1., FN397516.1., PP683260.1., PP425818.1., PP212042.1., PP086720.1., OR673057.1., OR673045.1., and OR251353.1 shown 99.92 homology with newly sequenced of 16S rRNA of *Bacillus sp.*

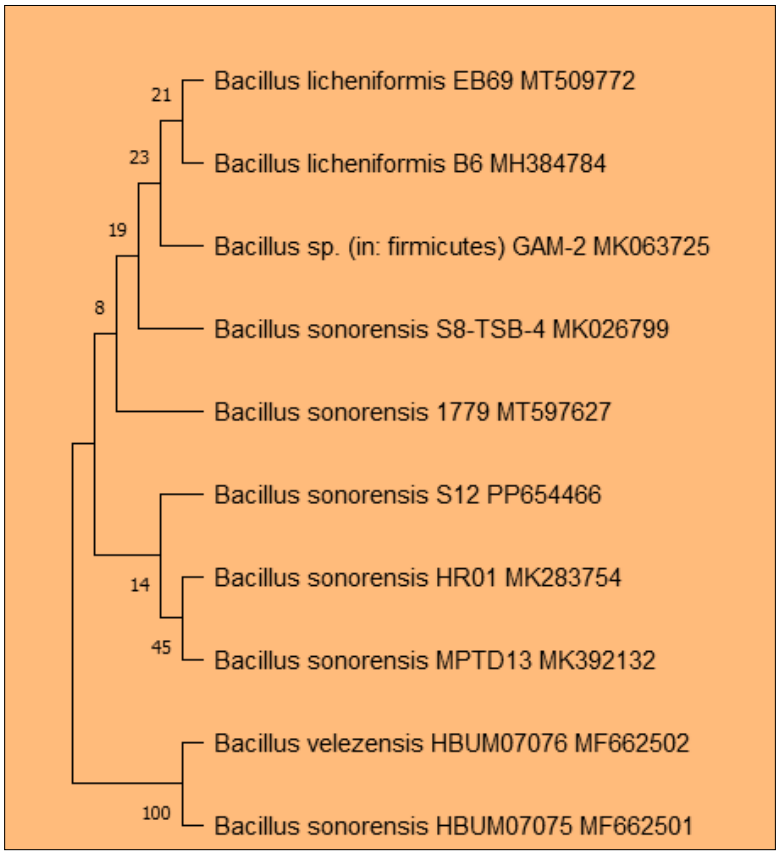

Figure 2 (a) Molecular Phylogenetic Analysis of 16S rRNA gene using Maximum Likelihood Method. The evolutionary history was inferred using maximum pairs

3.3 Maximum Parsimony Analysis of Taxa

The evolutionary history was inferred using the Maximum Parsimony method. The most parsimonious tree with length = 0 is shown. The consistency index is 0.603204 (0.600286), the retention index is 0.371346 (0.371346), and the composite index is 0.223997 (0.222914) for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (200 replicates) are shown next to the branches [3]. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (pg. 126 in ref. [1]) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). This analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 1120 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2]

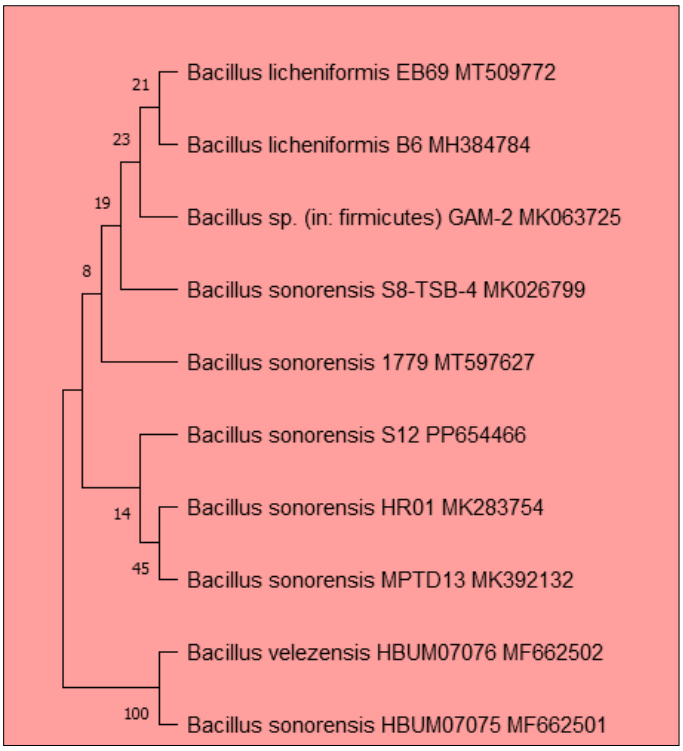

Figure 2 (b) Molecular Phylogenetic Analysis of 16S rRNA gene using Maximum Likelihood Method. The evolutionary history was inferred using maximum pairs

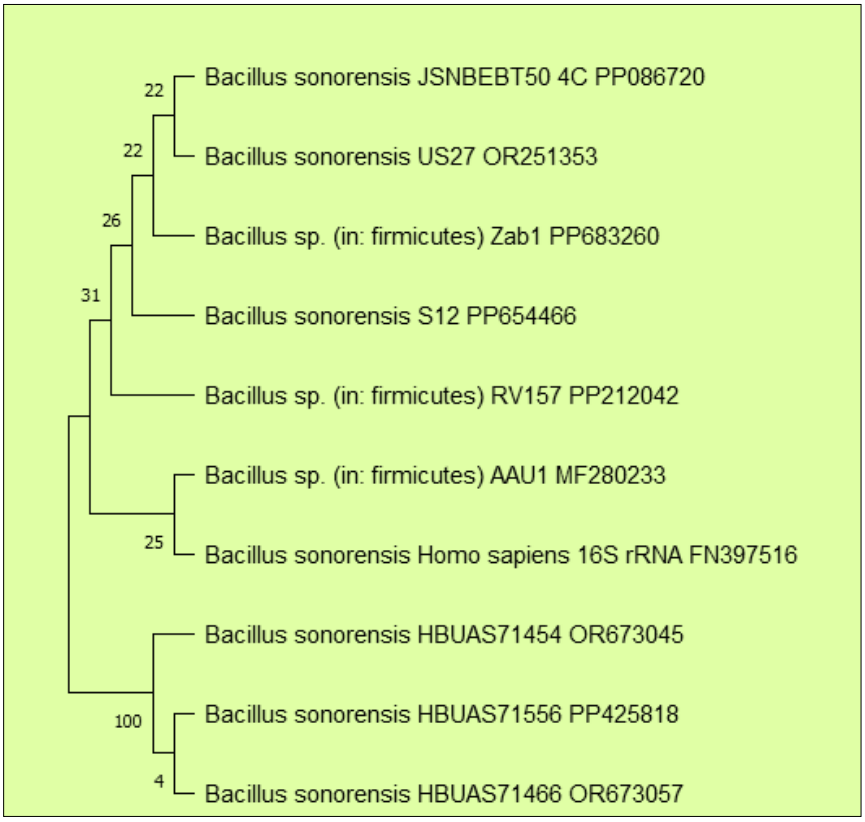

Figure 2 (c) Molecular Phylogenetic Analysis of 16S rRNA gene using Maximum Likelihood Method. The evolutionary history was inferred using maximum pairs

4 Phylogenetic analysis

Phylogenetic analysis included the newly sequenced *Bacillus sonorensis* 16S rRNA and the NCBI database were searched for 16S rRNA sequences of the different isolates. Alignments of *Bacillus sonorensis* 16S rRNA gene sequences were generated using the MEGA11 tool. Individual dendrograms were created using different methods, maximum likelihood methods. Phylogenetic groups and subgroups were defined according to the length and branching order of the integrate gene tree. The resulting groups were supported by high bootstrap values.

In Phylogenetic analysis, aligning nucleotide sequences is a crucial step, especially in studies involving genes from diverse taxa. Although it may seem evident that Phylogenetic analysis must start with proper data alignment, this process remains one of the most challenging and least understood aspects of molecular data analysis. Accurate alignment of genomic sequences is essential for constructing a phylogenetic tree. Additionally, phylogenetic analysis frequently involves examining molecular evolution for signs of directional selection [8, 9, 10]. The evolution of the 16S rRNA was examined in various isolates of *Bacillus sonorensis*, revealing adaptive changes in their sequences. The phylogenetic analysis of the 16S rRNA gene dataset from *Bacillus sonorensis*produced a tree that aligns with the contemporary systematic understanding of the relationships among different species within the Bacillus genus, primarily based on DNA sequence homology [Figure 2 a, b, c].

To identify the genus of bacterial isolates collected from poultry feces samples in Latur region; we amplified and sequenced the 16S rRNA gene of the bacterial group. The resulting sequences were then compared against NCBI's 16S rRNA GenBank using BLAST [8, 11].The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model [12].The phylogenetic analysis performed using 20 sequences of 16S rRNA gene from newly and retrieved 16S rRNA sequences, including *Bacillus sonorensis*.The consensus tree derived from the 10 most parsimonious trees is presented. Branches corresponding to partitions that appear in fewer than 50% of the trees are collapsed. The consistency index is 1.000000 (1.000000), the retention index is 1.000000 (1.000000), and the composite index is 1.000000 (1.000000) for both all sites and parsimony-informative sites (values in parentheses).The most parsimonious (MP) tree was generated using the Subtree-Pruning-Regrafting (SPR) algorithm [12].The MP tree was generated using the Subtree-Pruning-Regrafting (SPR) algorithm with a search level of 0, where the initial trees were constructed through the random addition of sequences (10 replicates). The tree is drawn to scale, with branch lengths calculated using the average pathway method, expressed in units of the number of changes across the entire sequence. The analysis included 39 nucleotide sequences; covering codon positions 1st, 2nd, 3rd, and noncoding regions. All positions with gaps and missing data were excluded. The final dataset comprised 578 positions. Evolutionary analyses were performed using MEGA7 [13].

Phylogenetic trees were constructed using the Maximum Likelihood method for the sequences of newly isolated bacteria from poultry feces. The Maximum Likelihood method is the most suitable model for understanding the evolutionary history of an organism. Bootstrap consensus trees, inferred from 1000 replicates, were used to represent the evolutionary history of the analyzed taxa. The Maximum Likelihood trees were generated using the Nearest Neighbor-Interchange heuristic algorithm. All positions with gaps and missing data were excluded from the dataset using the Complete Deletion option. Phylogenetic analyses were performed in MEGA11, resulting in three major clusters in Figure 2 (a, b, c), it is classified as Clade a (Yellow Colour), Clade b (Red Colour) and Clade c (Purple colour). As illustrated in Figure 2 (a, b, c), the newly sequenced 16S rRNA gene sequences were clustered with other Bacillus sonorensis isolates in Clade a, exhibiting 100% homology as determined by local alignment analysis. Clade b Uncultured bacterial culture shown homology with Calde A 100% But Clade c shown 99.92% homology with Clade a. Phylogenetic analysis of the 16S rRNA gene of *Bacillus sonorensis*, using the Maximum Likelihood method, revealed the relationships and percent similarity of the 16S rRNA gene among different bacterial isolates, including *Bacillus sonorensis*. Molecular techniques confirmed the predominant presence of *Bacillus sonorensis* in the collected poultry feces samples.

5 Conclusion

Phylogenetic analysis of Bacillus species, including new isolates from Poultry feces sampels , revealed that they belong to the same strain and are affiliated with Bacillus sonorensis . In recent years, Next Generation Sequencing technologies have significantly expanded genome databases, resulting in a remarkable increase in the availability of sequenced genomes, both drafts and complete. However, accurately assigning sequenced strains to their corresponding species using accepted taxonomic tools is essential before conducting comparative analyses with other genomes. The necessity for whole genome sequences of all type strains, which serve as the only species references publicly available in culture collections, is evident. In the present study, we identified and characterized *Bacillus sonorensis*from poultry feces samples using molecular biology techniques. New 16S rRNA sequences of Bacillus sonorensis isolates were aligned with

those of other *Bacillus* species, and a phylogenetic tree was constructed to determine the molecular evolution and population structure of Bacillus species using bioinformatics tools. The phylogenetic association of the different *Bacillus sonorensis* species were demonstrated through Maximum Likelihood-based phylogenetic analyses of the 16S rRNA sequences. Our study showed evidence of positive selection of the 16S rRNA gene during the divergence of different *Bacillus*species isolates throughout evolution. These evolutionary changes have led to necessary modifications in the genetic control of ontogeny, which may have caused adaptive changes in the 16S rRNA gene.

Compliance with ethical standards

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