Isolation of quinalphos degrading microbes from industrial hotspots of Aurangabad

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***Abstract*—[Pesticides are widely used in agriculture to enhance crop yields, but their environmental impact necessitates effective bioremediation strategies. Quinalphos, an organophosphate pesticide, poses challenges due to its persistence in soil. Understanding the microbial communities capable of quinalphos degradation is crucial for developing sustainable remediation methods. This study aimed to characterize bacterial isolates enriched from quinalphos-treated soil to assess their potential for pesticide degradation. The integration of traditional biochemical methods and 16S sequencing provided a comprehensive approach to bacterial identification and metabolic profiling. Soil samples treated with a 2mmol/L concentration of quinalphos were enriched for bacterial isolates, and traditional biochemical tests were conducted to assess cultural and metabolic characteristics. Four putative isolates were identified, including *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. Differences in citrate utilization, lactose utilization, and urease production were observed among the isolates. This study highlights the importance of integrating traditional and molecular techniques for the identification and characterization of pesticide-degrading microbial communities. Isolation of high concentration quinalphos degrading microbes stands as primary step in bioremediation step for the development of efficient bioremediation strategies in mitigating pesticide pollution in soil.**

***Index Terms*—Quinalphos, remediation, coremediation, pesticide, soil.**

I. INTRODUCTION

Quinalphos play a crucial role in modern agriculture by protecting crops from pests and increasing yields. However, their widespread use has raised concerns about environmental pollution and human health risks [1]. Quinalphos, a commonly used organophosphate pesticide, poses significant challenges due to its persistence in the environment and potential toxicity to non-target organisms [2]. Bioremediation, employing microorganisms to degrade pesticides, offers a promising approach to mitigate the environmental impact of quinalphos contamination [3].

Identification of microbes that degrade quinalphos is one of the primary steps in microbial research. Traditional method involves biochemical tests. 16S sequencing offers several advantages over traditional methods. It allows for the comprehensive and unbiased analysis of bacterial communities, providing insights into microbial diversity that may be missed by culture-based approaches [4]. Additionally, 16S sequencing is faster and more cost-effective, enabling the simultaneous analysis of multiple samples and the rapid identification of bacterial taxa [5]. Moreover, 16S sequencing can detect and identify unculturable or difficult-to-culture bacteria, expanding our understanding of microbial ecosystems and their roles in various environments. Despite these advantages, 16S sequencing also has some limitations, including the inability to provide information on bacterial viability or functionality and the reliance on reference databases for taxonomic assignment, which may introduce biases or inaccuracies [6]. Nevertheless, the widespread adoption of 16S sequencing in research and clinical settings underscores its importance and efficacy as a versatile tool for bacterial identification and community analysis.

Dhanjal, et al. [7] found the degradation of quinalphos in aqueous streams and observed 80-87 % degradation using Bacilli and Pseudomonas. It states persistence of quinalphos and dihydroxyl quinalphos oxon after application, but exponential microbial degradation in 17 days. There is an exclusive use of quinalphos in pest control, albeit, research into its degradation has been relatively limited. Previous studies have touched upon aspects of its breakdown, such as a report on the metabolism of quinalphos by soil algae, although

specific metabolites remained unidentified [8]. Additionally, a Pseudomonas strain demonstrated the ability to degrade higher concentrations of quinalphos in the presence of glucose; however, the specific pathway of degradation was not explored [9]. Talwar, et al. [3] provides the only evidence of degradative pathway of quinalphos by Ochrobactrum sp. Bacterial organophosphate hydrolase enzyme are shown to degrade many organophosphate compounds [10]. The hydrolysis-mediated microbial degradation of organophosphate compounds is widely recognized as the crucial step in their detoxification process [11].

Understanding the microbial communities capable of quinalphos degradation is essential for developing effective bioremediation strategies [12]. In this context, our study focuses on characterizing bacterial isolates enriched from quinalphos-treated soil and elucidating their potential for pesticide degradation. By combining cultural, biochemical, and molecular techniques, we aim to identify and assess the metabolic capabilities of microbial isolates and their relevance in pesticide bioremediation efforts. This research contributes to the growing body of knowledge on microbial-mediated pesticide degradation and underscores the importance of microbial diversity in environmental remediation strategies.

II. MATERIALS AND METHODS

A. Sample collections

The rhizospheric and non rhizospheric soil samples were collected from various locations in the industrial area of Aurangabad. The samples were collected in sterile polythene bags and immediately transferred to the laboratory. The samples were then used for isolation of quinalphos degrading bacterial isolation.

B. Enrichment of bacteria with Quinalphos degradation trait

In a 500 ml Erlenmeyer flask, 108 CFU concentration of the isolated heavy metal tolerant bacteria were added to 100 ml of mineral salts medium. The mixture was then incubated for 7 days at a temperature range of 25-30°C on a shaker set at 160 rpm. The insecticides Quinalphos (Sigma, India) was introduced into the mineral salts medium at concentrations of 2 mmol/L as sole carbon source and checked for bacterial growth turbidometrically at 600nm [3]. The media was centrifuged and the supernatant λmax was checked. Pre and post fermentation at 240nm to confirm Quinalphos degradation. The bacterial load of quinalphos degrading bacteria was calculated.

C. Biochemical characterization of soil bacteria

The biochemical characterization experiments for the potential biosurfactant-producing bacterial isolates were conducted following the protocol of Ashikuzzaman, et al. [13]. Bacterial identification was achieved through IMViC tests and enzyme production assays, including catalase, oxidase and urease activity assessments. To distinguish between Gram-positive and Gram-negative microorganisms, Gram staining was used. Specific bacterial differentiation was achieved using carbohydrate utilization assays using sorbitol, lactose and mannitol. Furthermore, the bacterial motility and spore formation was also observed. Detailed descriptions of these tests are provided below.

D. Identification of heavy metals tolerant bacteria using 16S rRNA

The putative bacteria were cultured in 100 mL volumes until reaching an absorbance of 0.7. The culture was then transferred to 1 mL vials and centrifuged at 10,000 rpm for 15 minutes to collect all bacterial cells. This bacterial pellet was used for DNA extraction following the standard method outlined by Wilson [14]. The extracted DNA was dissolved in deionized double-distilled water for subsequent analysis.

For amplifying the 16S ribosomal DNA gene, specific universal primers 27F (AGA GTT TGA TCC TGG CTC AG) and 1492R (GGT TAC CTT GTT ACG ACT T) from Sigma (India) were used [15]. 1X reaction buffer (10 mM Tris [pH 8.3], 50 mM KCl, and 1.5 mM MgCl2), 20 µM dNTPs, 0.05 U Taq DNA polymerase enzyme (Sigma, USA), 0.5 mM of each primer, and 30 ng of template genomic DNA were all included in the 50 µL PCR reaction mixture. The thermal cycling settings were a five-minute initial denaturation at 94°C, thirty-second denaturation cycles at 95°C, one-minute annealing at 54°C, two-minute extensions at 72°C, and a final five-minute extension at 72°C. Resolving the PCR amplicons on a 1 percent (w/v) agarose gel to approximate the band size using a gel documentation device allowed for the confirmation of the amplicon quality.

The 16S rDNA sequences homology were compared to the NCBI GenBank database using nBLAST. The top ten sequences with the highest identity scores were selected and aligned using multiple alignment software programs. The phylogenetic analysis was done using MEGAX to confirm the bacterial identity [16].

III. RESULTS

A. Enrichment and isolation of bacterial isolates

Based on the enrichment, 4 microbes were found to be putative for quinalphos degradation. All isolates were negative for indole production. BS1, BS2, and BS3 were positive for MR and negative for VP, consistent with the pattern observed in Bacillus species. In contrast, BS4 was negative for both MR and VP, indicating a different metabolic profile. BS1, BS2, and BS3 were negative for citrate utilization, while BS4 was positive, suggesting differences in citrate utilization among the isolates. Sorbitol, Lactose, and Mannitol: All isolates were positive for sorbitol utilization, while only BS1, BS2, and BS3 utilized lactose. Mannitol utilization varied, with BS1 and BS3 being positive and BS2 and BS4 being negative. All isolates were positive for both oxidase and catalase production, indicative of aerobic metabolism. BS1, BS2, and BS4 were negative for urease, while BS3 was positive, suggesting differences in urease production among the isolates. Based on the combined cultural and biochemical characteristics, the probable isolates were identified as BS1: *Bacillus cereus*, BS2: *Bacillus megaterium*, BS3: *Bacillus subtilis* and BS4: *Pseudomonas aeruginosa*. The results suggest that exposure to quinalphos did not significantly alter the cultural or biochemical characteristics of the bacterial isolates. The presence of motility, spore formation, and positive reactions in the IMViC test, carbohydrate utilization test, and enzyme test indicate that the isolates retained their metabolic activity despite quinalphos treatment. The differences observed in citrate utilization, mannitol utilization, and urease production among the isolates highlight the diversity in metabolic capabilities within the bacterial community. Additionally, the presence of *Pseudomonas aeruginosa* (BS4) suggests the presence of non-Bacillus species in the sample, which may have different responses to quinalphos exposure.

Table 1 Biochemical characterization of putative quinalphos degrading bacteria from industrial region of Aurangabad, Maharashtra.

Sample code	Cultural characters			Biochemical Characterization										Probable Isolates
				IMViC test				Carbohydrate utilization test			Enzyme test			
	Motility	Spores	Gram staining	Indole	MR	VP	Citrate	Sorbitol	Lactose	Mannitol	Oxidase	Catalase	Urease	
BS1	+	+	+	-	-	+	+	-	-	-	-	+	+	*B. cereus*
BS2	+	+	+	-	-	+	+	+	-	+	-	+	-	*B. megaterium*
BS3	+	+	+	-	-	+	+	+	+	+	-	+	-	*Bacillus subtilis*
BS4	+	-	-	-	-	-	+	-	-	+	+	+	-	*Pseudomonas aeruginosa*

B. Identification of heavy metals tolerant bacteria using 16S rRNA Sequence

Bacillus cereus

The nBLAST analysis conducted on partial sequences of the 16S ribosomal RNA gene revealed intriguing insights into the genetic relatedness among various Bacillus species. Among the strains examined, multiple alignments showcased a close association with *Bacillus cereus*, with several strains displaying high identity percentages and substantial query cover against this species. Specifically, *Bacillus cereus* strains KTSMBNL 81, B11 838, D14, HKG201, NIT, and YH16106 all exhibited significant matches with 100% query cover and identity percentages ranging from 77.67% to 100%. This consistency in alignment shows the genetic proximity among these *Bacillus cereus* strains, signifying their close evolutionary relationships based on this gene segment (Figure 1).

Moreover, the analysis also unveiled alignments with other Bacillus species, such as Bacillus thuringiensis, Bacillus anthracis, Bacillus tropicus, and a species provisionally denoted as Bacillus so (in: firmicutes). These alignments, while not as predominant as those with *Bacillus cereus*, still revealed considerable similarity in the 16S ribosomal RNA gene sequences, hinting at potential genetic connections and evolutionary affinities among these Bacillus species.

The phylogenetic analysis shows genetic proximity among various strains of *Bacillus cereus*, indicating their likely shared ancestry and evolutionary divergence from a common ancestor. Furthermore, the presence of alignments with other Bacillus species highlights potential genetic similarities and evolutionary relationships, shedding light on the evolutionary history and relatedness within the Bacillus genus based on the analysed gene sequences. These results highlight the significance of genetic studies in understanding microbial diversity and evolutionary relationships among closely related bacterial species (Figure 2).

Sequences producing significant alignments Download Select columns Show 10

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus cereus strain KTSMBNL 81 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1568	1568	100%	0.0	100.00%	849	KT222889.1
Bacillus cereus strain B11_B38 16S ribosomal RNA gene, partial sequence	Bacillus cereus	758	758	98%	0.0	83.29%	1358	MF185161.1
Bacillus thuringiensis strain BTJ-S-1 16S ribosomal RNA gene, partial sequence	Bacillus thuringiensis	723	723	94%	0.0	83.09%	999	KF439054.1
Bacillus anthracis strain JS-18 16S ribosomal RNA gene, partial sequence	Bacillus anthracis	643	643	99%	2e-179	80.74%	1440	GQ280088.1
Bacillus cereus strain D14 16S ribosomal RNA gene, partial sequence	Bacillus cereus	593	593	99%	2e-164	79.84%	1420	KU922225.1
Bacillus tropicus strain cwf7 16S ribosomal RNA gene, partial sequence	Bacillus tropicus	593	593	98%	2e-164	79.79%	1376	OQ674805.1
Bacillus sp. (in: Bacteria) strain BH11 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	586	586	97%	3e-162	79.72%	1484	MZ004949.1
Bacillus cereus strain HKG201 16S ribosomal RNA gene, partial sequence	Bacillus cereus	584	584	100%	1e-161	79.56%	979	KF947110.1
Bacillus cereus strain NIT 7 16S ribosomal RNA gene, partial sequence	Bacillus cereus	573	573	100%	2e-158	79.36%	1443	KM885306.1
Bacillus cereus strain YH16106 16S ribosomal RNA gene, partial sequence	Bacillus cereus	571	571	99%	9e-158	77.67%	1438	KY767494.1

Figure 1 Homology analysis of *Bacillus cereus* using nBLAST of 16S rRNA sequence

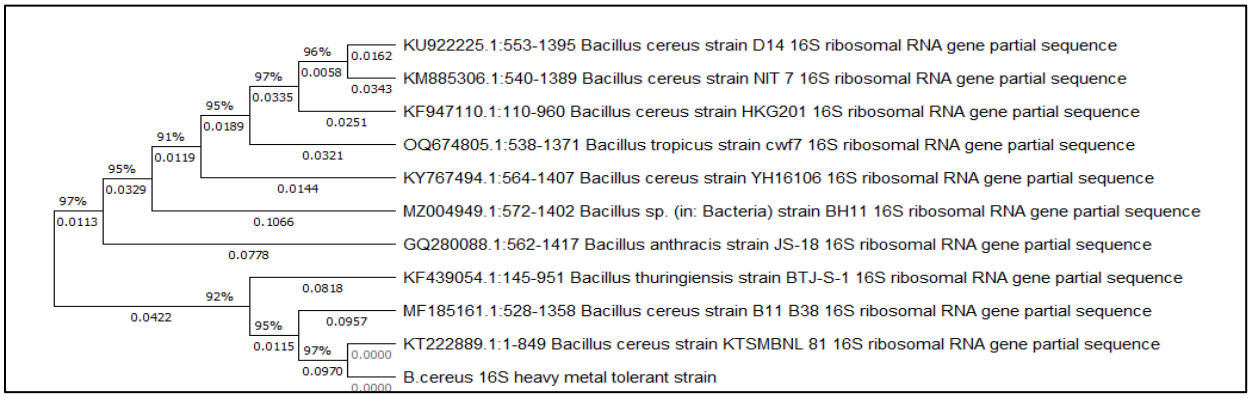

Figure 2 Phylogenetic analysis of *Bacillus cereus* strain using MEGA X

Bacillus megaterium

The BLAST analysis conducted on partial sequences of the 16S ribosomal RNA gene highlighted intriguing

findings pertaining to genetic relationships among various Bacillus and Priestia species. The alignments revealed notable similarities among several strains attributed to Priestia megaterium, showcasing high percentages of query cover and identity across multiple sequences. Notably, *Bacillus megaterium* strains YB3, DS8, ROA047, ROA024, and others exhibited consistent alignments with high percentages of query cover (ranging from 99% to 100%) and identity (ranging from 99.52% to 100%), indicative of substantial genetic relatedness within this species (Figure 3).

Moreover, alignments involving Bacillus sp. (in firmicutes) strain IC-1C and Bacillus aryabhattai strain PR-D07 displayed genetic similarities with Priestia species, hinting at potential evolutionary connections between different Bacillus species and the Priestia genus. The alignments, coupled with the high sequence similarities and consistent identity percentages, suggest a genetic affinity among these species, possibly indicating shared ancestry or evolutionary divergence from common genetic origins.

The BLAST phylogenetic analysis shows the genetic proximity and potential evolutionary relationships among various strains attributed to Priestia megaterium, indicating a close genetic association within this species group. Additionally, the alignments with other Bacillus species suggest broader genetic connections within the Bacillus genus, hinting at intricate evolutionary histories and potential shared genetic lineages among these bacterial species. The taxonomy browser in GenBank mentions both the isolates to be synonyms for each other. These findings emphasize the importance of genetic studies in unravelling the complex evolutionary relationships and genetic diversities among closely related bacterial taxa (Figure 4).

Sequences producing significant alignments Download Select columns Show 10

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus megaterium strain YB3 16S ribosomal RNA gene, partial sequence	Priestia megaterium	1157	1157	100%	0.0	100.00%	626	KT899485.1
Bacillus megaterium strain DS8 16S ribosomal RNA gene, partial sequence	Priestia megaterium	1142	1142	99%	0.0	99.68%	1474	EU835733.1
Bacillus sp. (in: Bacteria) strain IC-1C2 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	1136	1136	99%	0.0	99.52%	1494	MT649293.1
Bacillus megaterium strain ROA047 16S ribosomal RNA gene, partial sequence	Priestia megaterium	1136	1136	99%	0.0	99.52%	1431	MT525296.1
Bacillus megaterium strain ROA024 16S ribosomal RNA gene, partial sequence	Priestia megaterium	1136	1136	99%	0.0	99.52%	1483	MT510154.1
Priestia megaterium strain FDU301 chromosome, complete genome	Priestia megaterium	1136	15787	99%	0.0	99.52%	5272433	CP045272.1
Bacillus aryabhattai strain PR-D07 16S ribosomal RNA gene, partial sequence	Priestia aryabhattai	1136	1136	99%	0.0	99.52%	919	MT453908.1
Bacillus megaterium strain NEPZ-22 16S ribosomal RNA gene, partial sequence	Priestia megaterium	1136	1136	99%	0.0	99.52%	1480	MT184834.1
Priestia megaterium strain S188 chromosome, complete genome	Priestia megaterium	1136	13547	99%	0.0	99.52%	5278689	CP049296.1
Bacillus megaterium strain (A)LB_2-2 16S ribosomal RNA gene, partial sequence	Priestia megaterium	1136	1136	99%	0.0	99.52%	971	MT110975.1

Figure 3 Homology analysis of *Bacillus megaterium* using nBLAST of 16S rRNA sequence

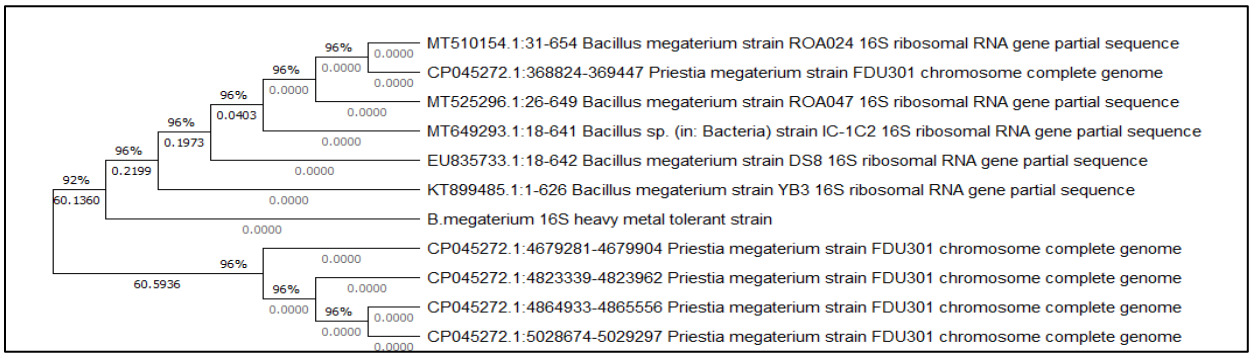

Figure 4 Phylogenetic analysis of *Bacillus megaterium* strain using MEGA X

Bacillus subtilis

The conducted nBLAST analysis revealed intriguing similarities and variations in the 16S ribosomal RNA gene sequences among different bacterial strains, primarily focusing on *Bacillus subtilis* and related species within the Firmicutes phylum.

Among the *Bacillus subtilis* strains analysed, a high degree of sequence similarity was observed across various isolates, as evidenced by the near-identical scores, query covers, and E-values. Notably, strains such as hswx89, Md1-42, A65 1, and CR26 showcased nearly identical total scores, reaching 2628-2676, indicating a very close relationship among these strains based on their 16S ribosomal RNA gene sequences. This consistency features the genetic similarity and probable evolutionary closeness between these *Bacillus subtilis* variants.

Contrastingly, while maintaining a significant degree of similarity, certain variations and distinctions were evident when examining other Bacillus species within the Firmicutes phylum. For instance, Bacillus so strains SNH M44, WM13-24, and SNH-K31 exhibited slightly lower total scores compared to *Bacillus subtilis* strains, with scores ranging from 2625 to 2632. Despite this, the query covers remained high (98-99%), signifying substantial overlap with the query sequence, suggesting a close relationship despite slight sequence differences.

Moreover, the analysis unveiled Geobacillus stearothermophilus as an outlier among the Bacillus strains, showing a similar total score of 2625, a query cover of 99%, and a high degree of sequence identity (99.65%). However, its classification under a different genus accentuates its genetic divergence from Bacillus but hints at potential shared ancestry or common genetic motifs.

The phylogenetic analysis illustrates the genetic proximity among *Bacillus subtilis* strains, highlighting their genomic similarity and probable shared evolutionary history. Additionally, while other Bacillus species within the Firmicutes phylum displayed slight variations in their 16S ribosomal RNA gene sequences, the overarching pattern indicates a significant genetic resemblance. The presence of Geobacillus stearothermophilus further emphasizes the genetic diversity within this group, hinting at both shared and divergent evolutionary paths.

Sequences producing significant alignments Download Select columns Show 10

select all *10 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus subtilis strain hswx89 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2676	2676	100%	0.0	100.00%	1449	JQ237657.1
Bacillus sp. (in: Bacteria) strain SNH_M44 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	2632	2632	99%	0.0	99.72%	1442	MN577389.1
Bacillus subtilis strain Md1-42 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2632	2632	99%	0.0	99.72%	1454	MF581448.1
Bacillus subtilis strain A65.1 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2628	2628	99%	0.0	99.65%	1463	ON366398.1
Bacillus sp. (in: Bacteria) strain WM13-24 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	2627	2627	98%	0.0	99.72%	1449	MN582985.1
Bacillus sp. (in: Bacteria) strain WM13-24 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	2627	2627	98%	0.0	99.72%	1449	OL721875.1
Bacillus sp. (in: Bacteria) strain SNH-K31 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	2625	2625	99%	0.0	99.58%	1448	MN493892.1
Geobacillus stearothermophilus strain HPA19 16S ribosomal RNA gene, partial sequence	Geobacillus stearothermophilus	2625	2625	99%	0.0	99.65%	1453	MF371320.1
Bacillus sp. (in: Bacteria) strain WC5 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	2625	2625	98%	0.0	99.72%	1475	JN975953.1
Bacillus subtilis strain CR26 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2625	2625	99%	0.0	99.65%	1449	KR780430.1

Figure 5 Homology analysis of *Bacillus subtilis* using nBLAST of 16S rRNA sequence

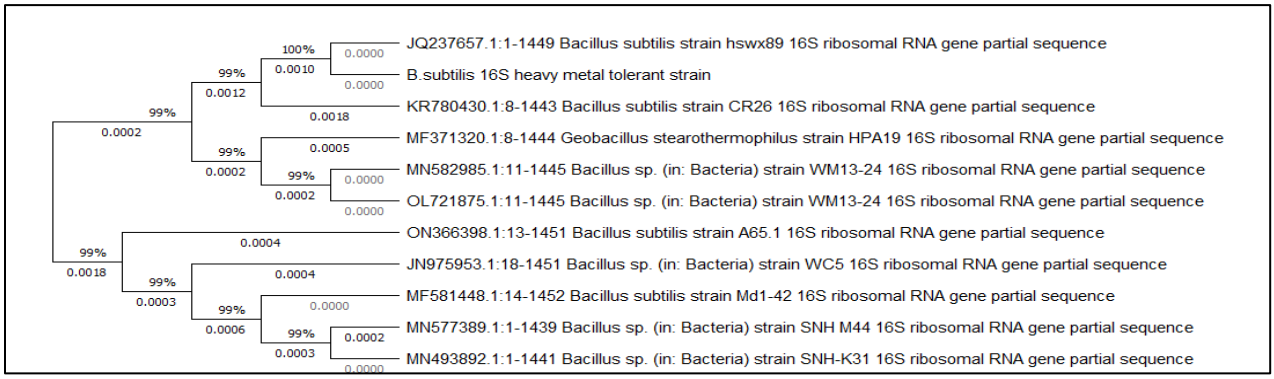

Figure 6 Phylogenetic analysis of *Bacillus subtilis* strain using MEGA X

Pseudomonas aeruginosa

The nBLAST analysis conducted on various strains of *Pseudomonas aeruginosa* presents a consistent pattern of high similarity in their 16S ribosomal RNA gene sequences while revealing subtle differences among individual strains.

Notably, all strains displayed remarkably high total scores ranging from 2710 to 2795, indicative of significant alignment and similarity to the query sequence. The query cover for each strain was consistently high, ranging from 98% to 100%, demonstrating extensive coverage of the queried sequence across these *Pseudomonas aeruginosa* variants.

Moreover, the E-values were consistently reported as 0.0, indicating a high significance of the matches and further reinforcing the close relationship between the queried sequences and those of the various *Pseudomonas aeruginosa* strains. Additionally, the percent identity values were consistently high, averaging around 99.27% to 100.00%, highlighting the sequence conservation within the 16S ribosomal RNA gene across these strains.

Despite the overall high degree of similarity, minor variations in the sequences were detected, as reflected in the slight differences in the total scores and percent identities. These variations, although minimal, suggest potential strain-specific genetic distinctions or evolutionary divergence within
Pseudomonas aeruginosa populations.

The phylogenetic analysis shows the genetic proximity and strong sequence conservation among different strains of *Pseudomonas aeruginosa*, typified by consistently high total scores, query covers, and percent identities. The subtle variations detected hint at the existence of strain-specific genetic traits or potential evolutionary divergence within this species.

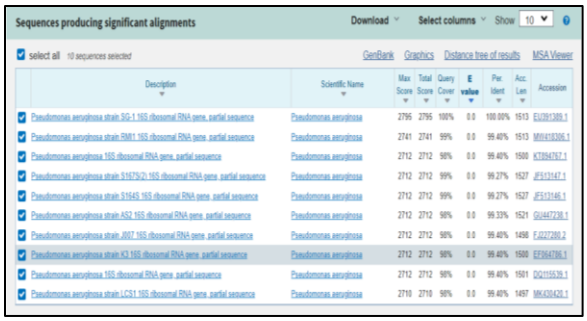

Figure 7 Homology analysis of *Pseudomonas aeruginosa* using nBLAST of 16S rRNA sequence.

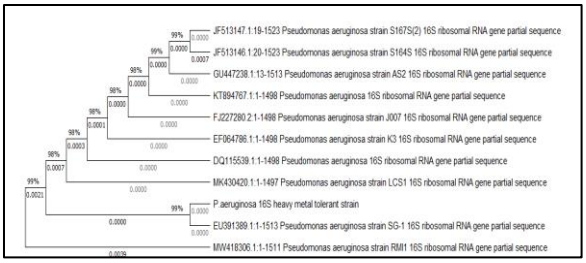

Figure 8 Phylogenetic analysis of *Pseudomonas aeruginosa* strain using MEGA X

IV. DISCUSSION

The confirmation of bacterial isolates using 16S sequencing provides valuable insights into their taxonomic identity and potential metabolic capabilities. Similar isolates with quinalphos-degrading traits have been reported in the literature, corroborating our findings. For instance, studies by Dhanjal, et al. [7] identified Pseudomonas and Bacilli strains capable of quinalphos degradation at 6.7 mmol/L concentration, supporting similar isolates in quinalphos degradation. Additionally, the presence of *Pseudomonas aeruginosa* (BS4) aligns with reports by Alengebawy, et al. [17], who demonstrated quinalphos degradation by Pseudomonas spp. This reinforces the relevance of our findings in the context of pesticide bioremediation research. Several other microbes like Ochrobactrum sp. are also found to be tolerant and degrading 2 mmol/L concentration of Quinalphos. The diverse groups of microbes with even greater potential to quinalphos degradation is necessary and evolving field for efficient biodegradation of quinalphos.

IV. CONCLUSION

The results highlight the effectiveness of both traditional biochemical methods and 16S sequencing for bacterial identification. Traditional biochemical methods, such as culture-based techniques and biochemical assays, provided valuable insights into the metabolic capabilities of the bacterial isolates, allowing for the identification of putative species based on their cultural and biochemical characteristics. 16S sequencing offered a rapid and comprehensive approach to bacterial identification, enabling the simultaneous analysis of multiple samples and the detection of diverse bacterial taxa. The combination of cultural and biochemical characterization with 16S

sequencing allowed for a more thorough and accurate assessment of the bacterial isolates, enhancing our understanding of their taxonomic identity and metabolic capabilities. Moving forward, the integration of both traditional and molecular techniques will continue to be valuable for bacterial identification and community analysis, facilitating research in diverse fields such as environmental microbiology, clinical microbiology, and biotechnology.

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