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Biodegradation of Polyethylene glycol by *Bacillus subtilis* HTNAKIRS-1 strain: A novel strain for environmental remediation

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Abstract
Polyethylene glycol (PEG) is a polyether compound derived from petroleum that has high application in industrial manufacturing, pharmaceuticals, medicine, research, and cosmetics. As widespread as the application is, there would be a wide range of environmental pollution as well; its hydrophilic nature and strong affinity for water can promote the biodegradation of PEG. There are very few bacterial species reported for their involvement in PEG biodegradation. In this study we identified a novel *Bacillus subtilis* HTNAKIRS-1 strain, which can degrade PEG 8000, and this strain was studied for its biochemical characterisation along with molecular characterisation. The growth of the strain in 3% PEG8000 was studied, and their metabolites during the biodegradation were analysed by Gas Chromatography Mass Spectroscopy (GCMS). The strain showed promising results in PEG biodegradation, and 26 different metabolites were identified, i.e., aromatic carboxylic acids like phthalic acid, dibutyl phthalate, diethyl phthalate, aromatic hydrocarbons like naphthalene, glycerol, and its derivatives like 1-monopalmitin, 2-monopalmitin, and glycerol monostearate. These metabolite identifications paved the way to understand the involvement of different metabolic pathways interlinking in biodegrading PEG by the isolate.

Keywords: *Polyether, Polyethylene glycol, Bacillus subtilis, Biodegradation, GCMS*

Introduction

Polyethers are a complex of monomer molecules linked together with strong ether linkages, which are classified into aromatic and aliphatic polyesters. Aromatic polymers are petroleum derivatives, which are often used in plastic manufacturing. Aliphatic polyethers include polyethylene oxide and polypropylene oxide. Polytetrahydrofuran due to the presence of hydroxyl groups at chain ends is also called glycols, like polyether glycols (polyethylene glycols, polypropylene glycols). Polyethylene glycols (PEG) are synthetic polymers, which are hydrophilic in nature (Salahudeen *et al.*, 2025; Vazirzadeh *et al.*, 2025). Polyethylene glycols physically range from viscous liquids to flake solids; the type of PEG differs based on their molecular weight, which ranges from 200 to 40000 kDa. PEGs are the most manufactured products by industries due to their extensive usage in various fields like medical, lubricants, textiles, pharmaceuticals, etc (El-Bagoury *et al.*, 2026; Nath *et al.*, 2026). These polymer accumulations in the environment lead to acute pollution. Usage of PEG as raw material for many purposes leads to over accumulation. PEG contamination in waterbodies is the most observed issue where untreated wash off effluents from various industries can be one of the reasons. PEG may be non-toxic to human health, but few studies showed some acute effects on aquatic wildlife

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(Nascimento *et al.*, 2021). While many regulatory bodies assert that PEGs do not significantly bio accumulate or persist in the environment, the presence of contaminants underscores the need for careful monitoring and regulation of products containing PEG, but excess accumulation of these substances makes them carriers for some other hazardous chemicals. Polyethylene glycols are degradable compounds employing both physical and chemical approaches, which also hold some demerits compared to biodegradation (He *et al.*, 2025). Biodegradation of polyethylene glycols is a natural process where microorganisms like bacteria and fungi degrade the PEG by utilising it as a carbon source with the help of a few enzymes (Arrafi *et al.*, 2025; Geisler *et al.*, 2025). Microbes indulge different metabolic pathways in degrading these polyether compounds. Bacterial biodegradation includes both aerobic and anaerobic degradation. In anaerobes, degradation happens through the diol dehydratase pathway, which is analogous to PEG acetaldehyde lyase activity in bacteria, whereas in aerobic degradation, initially PEG is converted into PEG-aldehyde by the enzyme PEG-dehydrogenase (PEG-DH), later to PEG carboxylate by PEG-carboxylase, and later to glyoxylate. This glyoxylate enters the central metabolic pathway, TCA cycle, and glycerate pathway. PEG-dehydrogenase/PEG-DH and PEG-aldehyde dehydrogenase/PEGAL-DH,which are considered as pegA and pegC genes, are quite responsible genes for PEG degradation (Geisler *et al.*, 2025). It was also reported that *Sphingopyxis macrogoltabida* strain 103 has peg A, B, C, D, and E genes for polyethylene glycol (PEG) catabolism comprise a PEG-inducible operon, where the operon comprises promoters pegD which is permase, pegE is acyl-CoA synthetases and pegB promotor site containing the activator-binding molecules of an AraC/XylS-type regulator for transcription of the pegBCDAE operon, while the opposite direction of transcription occurs for the PegR gene, which codes for an AraC-type regulator in the operon's downstream region (Tani *et al.*, 2008). Few renowned PEG-degrading bacteria include *Pseudomonas aeruginosa, Pseudomonas stutzeri, and* Flavobacterium sp. *Rhodopseudomonas acidophila, Sphingomonas terrae, Sphingomonas* species, Pseudonocardia sp., Pantoea agglomerans, *Vibrio alginolyticus, Pseudoalteromonas caenipelagi, Microbulbifer pacifus, Pseudomonas marcincola, Methanospirillum hungatei, Acetobacterium woodii*, *Bacillus subtilis* (Azizi *et al.*, 2024; Kawai *et al.*, 2002). The present study aims to screen and identify PEG-degrading isolates, along with biochemical characterisation, growth studies in 3% PEG media, and analysing metabolites produced by the isolate during the biodegradation process through GCMS. The work also extends to detailing the metabolic pathways that the isolate could have exhibited in degrading PEG.

Materials and Methods

Collection and transport of soil sample from dumping area

Soil samples were collected from dumping yard, Vizag Steel Plant, J56G+46 Visakhapatnam, Andhra Pradesh, at plastic waste dry lands dumping yard area.

Isolation and screening of Polyethylene Glycol-degrading bacteria

From the collected soil samples, bacterial isolates were obtained by following the serial dilution method. The bacterial isolates were grown on Tryptone Soya Agar (TSA) and incubated at 37°C, and the axenic cultures were also maintained as per the standard protocols. The pure cultures were now grown on medium containing KH_2PO_4 (0.2 g/L), K_2HPO_4 (0.8 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), NH_4NO_3 (1.0 g/L), NaCl (0.005 g/L), $CaCl_2$ (0.15 g/L) solidified with 2% agar containing 3% polyethylene glycol 8000 (PEG 8000) as a carbon source (Nademo *et al.*, 2023; Rosari *et al.*, 2017). Plates are incubated at 37°C for 24 - 48 hrs. After 48 hrs of incubation, the bacterial colonies growth was first observed under phase contrast microscopy to study their morphological characteristics and Gram staining. Pure isolates were preserved by maintaining them on minimal basal media containing 30 g/L PEG 8000 at 4°C.

Biochemical characterisation and antibiogram

The isolate that is screened for PEG utilisation was tested for biochemical and morphological characteristics. The morphological characterisation can be examined by Gram staining and microscopy examination (Smith *et al.*, 2005). Biochemical tests such as the oxidase test, catalase test, indole test, methyl red test, Voges Proskaeurs test, citrate utilisation test, starch hydrolysis test, gelatin test, urease test, alkaline phosphatase test, and carbohydrate utilisation tests (sucrose, arabinose, trehalose, D-glucose, D-galactose, mannose, D-sorbitol, lactose) were examined by the standard protocols of the American Society of Microbiology (Dela *et al.*, 2012; Jo H *et al.*, 2023; Mac Williams 2009; McDevitt 2009; Purkaystha *et al.*, 2022; Reiner 2010; Shields *et al.*, 2010; Shields *et al.*, 2011). Mueller Hinton Agar Plates were used for antibiotic sensitivity test, disk method was used for studying antimicrobial

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action of the strain, standard antibiotics (Himedia) with following concentration were used, Chloramphenicol (C30), Kanamycin (K30), Amikacin (AK30), Cefazolin (CZ30)- 30mcg , Tetracycline (TE30)- 30mcg , Vancomycin (VA30)- 30mcg, Teicoplanin (TEI30)- 30mcg, Erythromycin (E15)- 15mcg, Gentamycin (GEN10)- 10mcg, Penicillin G (P10)- 10mcg, Streptomycin (S10)- 10mcg, Rifampicin (RIF5)- 5mcg, Moxifloxacin (MO5)- 5mcg, Trimethoprim (TR5)- 5mcg, Levofloxacin (LE5)- 5mcg, Methicillin (MET5)- 5mcg, Oxacillin (OX1)- 1mcg. Culture was revived in Tryptone Soya Broth and grown to 10^8 CFU/ml, and 100 µl of culture was spread on a sterile MH agar plate, and standard antibiotic discs were carefully placed on the surface of the agar using sterile forceps, and plates were incubated at 37°C for 24 hrs. Inhibition zones were measured with HiAntibiotic Zonescale-C.

Molecular characterisation by 16S rRNA sequencing and phylogenetic tree analysis

Genomic DNA was isolated from the bacterial cultures, and its quality was assessed on a 1.0% agarose gel, revealing a single band of high-molecular-weight DNA. Using 16SrRNA-F and 16SrRNA-R primers, a segment of the 16S rRNA gene was amplified. The PCR amplicon was then filtered to get rid of impurities. The ABI 3730xl Genetic Analyzer (Barcode Biosciences Pvt. Ltd., Bangalore, India) was used to run forward and reverse DNA sequencing reactions of PCR amplicons using 16SrRNA-Forward and 16SrRNA-Reverse primers and the BDT v3.1 Cycle sequencing kit. Using aligner software, the forward and reverse sequence data were combined to create the consensus sequence of the 16S rRNA gene. A BLAST search was conducted using the 16S rRNA gene sequence against the NCBI GenBank's 'nr' database. The top 10 sequences were chosen based on the highest identity score, and the Clustal W multiple alignment software was used to align them. MEGA 10 was used to create the phylogenetic tree and distance matrix.

Phylogenetic analysis

The Tamura-Nei model and the Maximum Likelihood approach were used to determine the evolutionary history (Kimura 1980). We display the tree with the highest log likelihood (-2136.95). A matrix of pairwise distances computed using the Tamura-Nei model was subjected to the Neighbour-Join and BioNJ algorithms, which automatically produced initial trees for the heuristic search. The topology with the highest possible log likelihood value was then chosen. Eleven nucleotide sequences were included in this investigation. The following codon locations were examined: noncoding, first, second, and third. A total of 1504 positions were included in the final dataset. MEGA11 was used to undertake evolutionary analysis (Kumar *et al.*, 2018).

Growth in Polyethylene Glycol media

Growth analysis is carried in Erlenmeyer flasks containing 100 ml of sterile liquid medium i.e., minimal medium consists of KH_2PO_4 (0.2 g/L), K_2HPO_4 (0.8 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), NH_4NO_3 (1.0 g/L), NaCl (0.005 g/L), $CaCl_2$ (0.15g/L) containing PEG 8000 as carbon source with concentrations of 30 g.L and pH 7.0±0.2 The culture inoculum of 10^8 CFU/mL was added to each flask. All the reactions were incubated at 37 °C in a rotatory shaker at 300 rpm. The bacterial growth was measured by optical density (OD) at a wavelength of 600 nm using a spectrophotometer, respectively, and non-inoculated sterile media was used as a blank against the test solutions.

Metabolite detection by GC-MS

PEG-digested culture after 72 hrs was centrifuged at 5000 x g for 30 minutes to remove the cells, and the supernatant was filtered by a 0.2-micron filter, and 500 µl of sample was evaporated using a rotor evaporator (Heidolph Rotary evaporator) at 50°C and 100 rpm. Later, the dried sample was dissolved in 200 µl of acetonitrile and sonicated for 15 mins. After sonication, the mixture underwent a silylation reaction where 200 µl of N,O-bis(trimethylsilyl)trifluroacetamide was added to the mixture and incubated at 60°C for 30 mins. Later, the sample was analysed using GCMS (Agilent Technologies) equipped with the 5977AMSD and 7890B GC system. The injection port temperature was maintained at 280°C for metabolite analysis. The oven temperature is initially maintained at 50°C for two minutes, then raised to 280°C at a rate of 10°C per minute, and the system is maintained at 280°C for ten minutes for complete analysis. Metabolism pathway and mechanism was analysed by comparing the metabolites through KEGG database (https://www.genome. jp/kegg/pathway.html).

Bioinformatic analysis

The whole-genome analysis was performed for the sample where Bioproject accession number: PRJNA1345961 (https://www.ncbi.nlm.nih.gov/bioproject/1345961), BioSample Accession number SAMN52822983 (https://www.ncbi.nlm.nih.gov/biosample/SAMN52822983). The predicted genes were annotated in Kyoto Encyclopedia of Genes and Genome (https://www.genome.jp/kegg/), Gene Ontology (https://geneontolo

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gy.org/), Cluster of Orthologous groups of proteins (http://eggnog5.embl.de/#/app/home).

Results

Isolation and screening of bacteria

Isolation of bacteria was carried out by growing on TSA plates where six pure cultures were isolated, whereas by primary screening using 30 g/L polyethylene glycol agar plates, only one isolate showed the ability todegrade/utilise polyethylene glycol as a sole carbon source. The isolate was further studied by growing them in elevated concentrations of polyethylene glycol agar plates at 3%, 6% which showed effective growth at pH 7. This shows that the isolate can degrade polyether components. The isolate was observed as gram-positive rods exhibiting filamentous, wrinkled, opaque white colony morphology on TSA plates and blood agar plates, with round transparent opacity on 3% PEG agar plates (Fig. 1).

Biochemical Analysis and Antibiogram

This culture was designated as the HTNAKIRS-1 strain. Biochemical analysis for the HTNAKIRS-1 strain showed positive results for tests, i.e., oxidase, catalase, citrate utilisation, starch hydrolysis, nitrate reduction, and carbohydrate utilisation of glucose, sucrose, arabinose, trehalose, and lactose, and negative results for indole, methyl red test, voges proskaeurs , gelatin, urease, alkaline phosphatase, and carbohydrate utilisation of D-galactose, mannose, and D-sorbitol. The isolate showed susceptible ranges for the following antibiotics like C30, K30, AK30, CZ30, TE30, VA30, TEI30, E15, GEN10, P10, MO5, TR5, LE5, MET5, and OX1, (Table 1) where the strain showed resistance towards streptomycin (S10) and rifampicin (RIF5) antibiotics (Fig. 2).

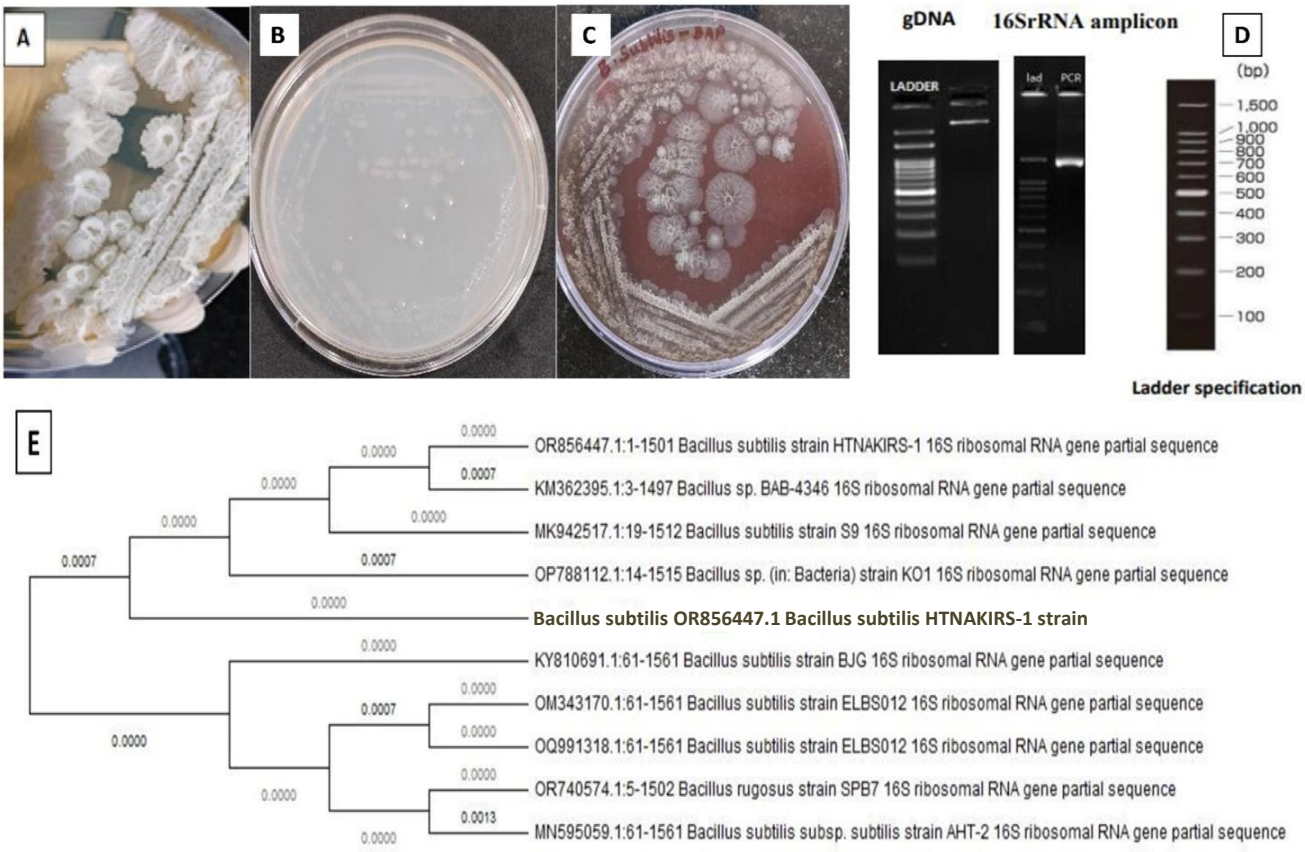

Figure1. *Pure isolate colony morphology of Bacillus subtilis HTNAKIRS-1 strain on TSA agar plate and 3% PEG Agar plate (a) growth of bacteria on Tryptone Soya Agar plate exhibiting filamentous, wrinkled, opaque white colony morphology (b) growth of bacteria on 3% PEG Agar plates exhibited round transparent opacity colony morphology (c) Isolate growth on blood agar plate exhibiting filamentous, wrinkled, opaque white colony morphology (d) Isolation of genomic DNA and distinct PCR amplicon band of 1500 bp along with ladder specification (e) Maximum likelihood phylogenetic tree of Bacillus subtilis strain HTNAKIRS-1 based on 16S rRNA sequencing, NCBI accession numbers were provided besides the species name.*

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Antibiotic	Range	Bacillus subtilis Zone of Inhibition(mm)
Chloramphenicol(C30)	Susceptible	27
Kanamycin(K30)	Susceptible	18
Amikacin(AK30)	Susceptible	16
Cefazolin(CZ30)	Susceptible	35
Tetracycline(TE30)	Susceptible	27
Vancomycin(VA30)	Susceptible	20
Teicoplanin(TEI30)	Susceptible	19
Erythromycin(E15)	Susceptible	26
Gentamycin(GEN 10)	Susceptible	16
Penicillin G(P10)	Susceptible	24
Streptomycin(S10)	Resistance	No zone
Rifampicin(RIF5)	Resistance	No zone
Moxifloxacin(MO5)	Susceptible	28
Trimethoprim(TR5)	Susceptible	19
Levofloxacin(LE5)	Susceptible	27
Methicillin(MET5)	Susceptible	23
Oxacillin(OX1)	Susceptible	21

Table 1
Zone of Inhibitions and its ranges for *Bacillus subtilis* HTNAKIRS-1 strain using standard antibiotics.

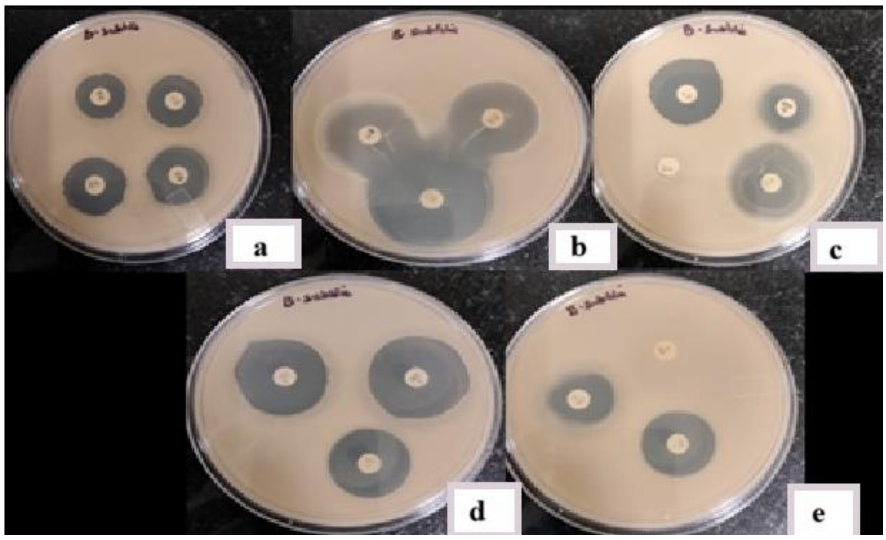

Figure 2
Inhibition zones by Standard Antibiotic against Bacillus subtilis HTNAKIRS-1 strain on Mueller Hinton Agar Plates ***a)*** *Plate consists antibiotic disc AK30, K30, TEI30, VA30* ***b)*** *Plate consists antibiotic disc C30, CZ30, TE30* ***c)*** *Plate consists antibiotic disc P10, S10 GEN10, E15* ***d)*** *Plate consists antibiotic disc LE5, MET5, MO5 e) Plate consists antibiotic disc TR5, OX1, RIF5.*

Molecular characterisation

Sanger sequencing method was used to resolve the 16S rRNA gene sequence for the HTNAKIRS-1 strain on an agarose gel, revealing a clear PCR amplicon band of 1500 bp (Fig.1). Using BLAST similarity searches, the acquired nucleotides were compared with reference sequences, and the database of NCBI GenBank was used to identify closely related sequences. The phylogenetic tree analysis showed that the two strains, the HTNAKIRS-1 strain, share 99.9% similarity with *Bacillus subtilis* (Fig. 1). The gene bank accession number for 16S rRNA of *the Bacillus subtilis* HTNAKIRS-1 strain is OR856447.1 (https://www.ncbi.nlm.nih.gov/nuccore/OR856447.1).

Degradation of Polyethylene Glycol

Bacillus subtilis HTNAKIRS-1 strain showed an increase in cell mass over time in minimal media containing 3% PEG 8000 as a carbon source. The growth was measured through absorbance (OD 600 nm) of culture, where at 72 hrs culture age 0.63 OD with cell count $2.3x10^7$ CFU/ml was noted, and the culture showed an extensive lag phase from 24 hr of inoculation (Fig. 3). The growth was only recorded till 72 hr time, as detection of metabolites is aimed for the mid-log phase of the bacterial growth.

Metabolites detection by GCMS

Metabolites of polyethylene glycol degradation by *Ba-*

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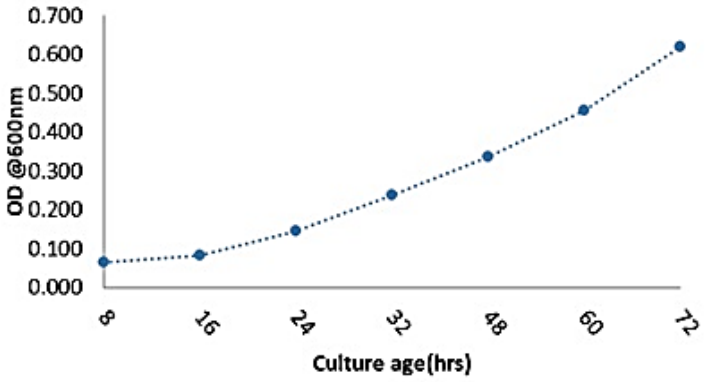

Culture Age	OD - BS
8	0.063
16	0.083
24	0.146
32	0.236
48	0.336
60	0.456
72	0.619

Figure 3
Growth of Bacillus subtilis HTNAKIRS-1 strain in 3%PEG 8000 and its growth measurement by absorbance (OD) at 600nm.

Bacillus subtilis HTNAKIRS-1 strain were analysed by GCMS. 26 efficient metabolites were identified, which are tabulated (Table 2). Quantification of metabolite concentrations was achieved by comparing peak areas and retention times from sample data with corresponding data from standard compounds found in the literature. Few main metabolites with high peak area like peak area of 11.75 % with 24.505 retention time and peak at 147 m/z was identified to be 1-Monopalmitin which is functionally related to hexadecenoic acid/Palmitic acid. Peak area of 7.03 and retention time of 19.095 min with a 149.0 charge-to-ion mass of m/z, which were identified as 1,2-benzene dicarboxylic acid/phthalic acid and dibutyl phthalate. 6.37 peak area (%) and retention time 24.285 with 28 m/z was identified as silane, peak area of 3.12 % with 24.253 retention time and peak at 129 m/z was identified to be 2-Palmitoyl glycerol / 2-Monopalmitin, 4.74 peak area (%) with retention time 26.022 with 147 m/z was identified as Glycerol monostearate (Fig. 4).

Table 2 *Metabolites identified after polyethylene glycol degradation by Bacillus subtilis HTNAKIRS-1 strain using GCMS*

Compound name	Retention time (min)	Molecular Structure	Peak Area (%)
L-Asparagine	5.819	$C_4H_8N_2O_3$	1.06
Urazole	6.182	$C_2H_3N_3O_2$	1.58
Azetidene	6.292	C_3H_7N	0.64
Ethylene glycol	6.525	$HOCH_2CH_2OH$	0.75
Decane	7.581	$CH_3(CH_2)_8CH_3$	1.08
2,2-Diphenylpropionitrile	9.836	$C_{15}H_{13}N$	0.66
Heptane	10.750	$CH_3(CH_2)_5CH_3$	0.83
1 Monopalmitin	24.505	$C_{19}H_{38}O_4$	11.75
Eiscosane	16.860	$C_{20}H_{42}$	0.37
1H-4-Azacycloprop[cd]indene	11.106	$C_9H_{15}N$	0.65
Diethylphthalate	15.208	$C_6H_4(COOC_2H_5)_2$	0.32
Heptadecane	16.387	$C_{17}H_{36}$	0.55
1,2,Benzene di carboxylic acid / Phthalic acid	18.156	$C_6H_4(COOH)_2$	0.63
Dibutyl phthalate	19.095	$C_6H_4(COOC_4H_9)_2$	7.03
Octadecanal	19.601	$C_{18}H_{36}O$	1.04
Octadecanenitrile	20.430	$C_{18}H_{35}N$	0.55
Bisphenol	21.518	$(CH_3)_2C(C_6H_4OH)_2$	0.54
9-Octadecenamide	22.899	$C_{18}H_{35}NO$	0.40
2-Palmitoyl glycerol / 2-Monopalmitin	24.253	$C_{19}H_{38}O_4$	3.12
Quinoline	23.715	C_9H_7N	0.42
Trans-3-ethoxy-b-methyl-b-nitrostyrene	25.834	$C_{11}H_{13}NO_3$	1.47
Naphthalene	23.585	$C_{10}H_8$	1.12
Cyclopentene-1-carboxylic acid	25.724	$C_6H_8O_2$	0.65
1H Indole	25.127	C_8H_7N	0.78
Ethylarcidine	23.760	$C_{15}H_{13}N$	0.25
Glycerol monostearate	26.022	$C_{21}H_{42}O_4$	4.74
1H-Benzo 4,5 furo3,2 indole	26.806	$C_{14}H_9NO$	0.49

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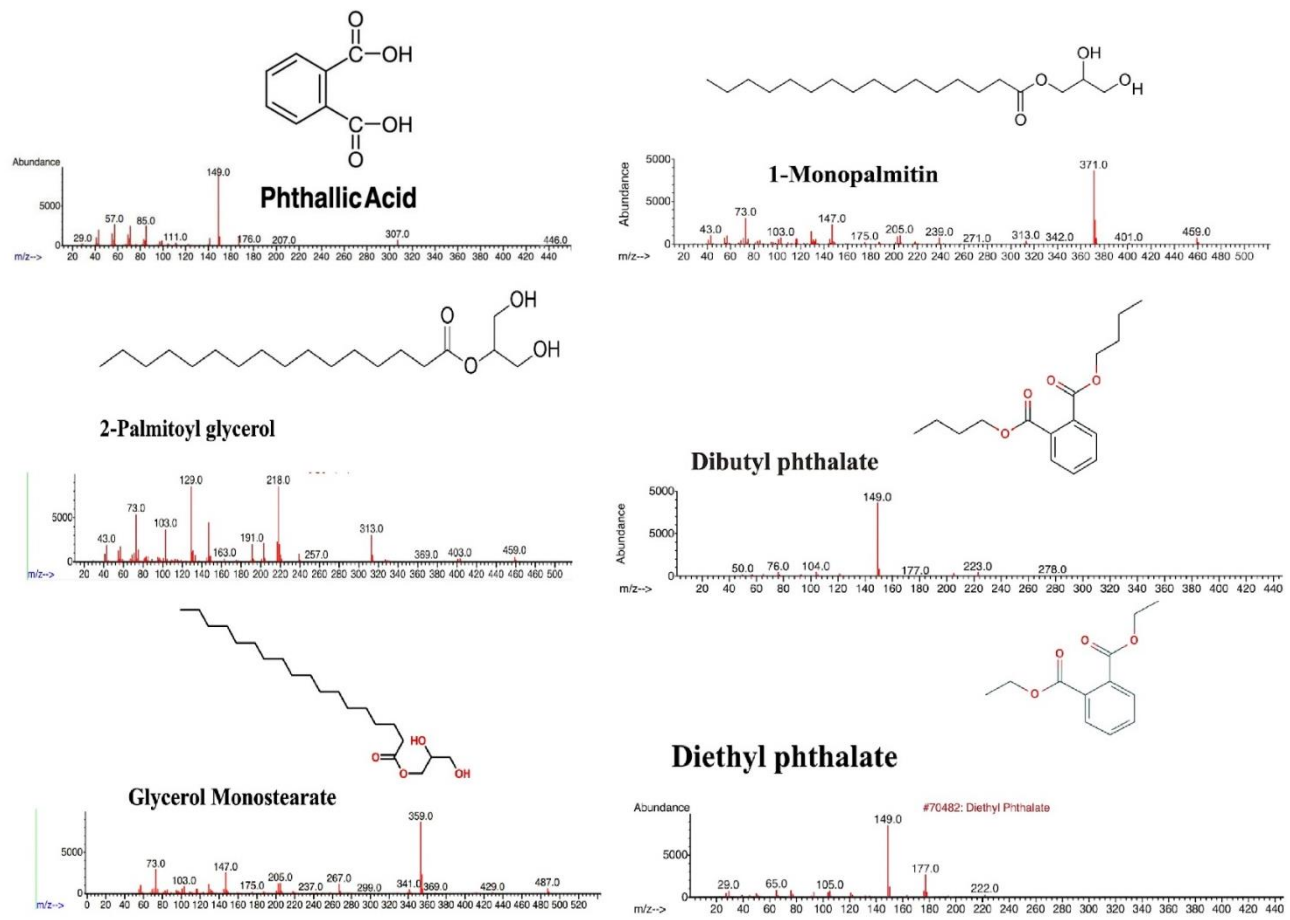

Figure 4 *Metabolites GCMS spectra and their specific peak ranges for compounds with maximum peak areas.*

Gene Annotation through whole genome sequencing

The gene bank accession number for *Bacillus subtilis* HTNAKIRS-1 strain is predicted that the strain has 283 genes involved in carbohydrate metabolism Glycolysis/Gluconeogenesis (28 genes), TCA cycle (18 genes), Pentose phosphate pathway (24 genes), Pentose and glucuronate interconversions (22 genes), Fructose and mannose metabolism (14 genes), Galactose metabolism (14 genes), Ascorbate and aldarate metabolism (7 genes), Starch and sucrose metabolism (31 genes), Pyruvate metabolism (28 genes), Glyoxylate and dicarboxylate metabolism (23), Propanoate metabolism (25 genes), Butanoate metabolism (22 genes), C5-Branched dibasic acid metabolism (8 genes), Inositol phosphate metabolism (10 genes). It has 15 genes involved in Fatty acid metabolism, 48 genes involved in lipid metabolism (Fatty acid biosynthesis, Fatty acid degradation, Mycolic acid biosynthesis, Glycerolipid metabolism, Glycerophospholipid metabolism, Sphingolipid metabolism, alpha-Linolenic acid metabolism). 35 genes were involved in Xenobiotics biodegradation and metabolism (Benzoate degradation, Aminobenzoate degradation, Chloroalkane and chloroalkene degradation, Chlorocyclohexane and chlorobenzene degradation, Xylene degradation, Ethylbenzene degradation, Styrene degradation.

Discussion

This study reveals that *the Bacillus subtilis* HTNAKIRS-1 strain has a unique characteristic that can degrade polyethylene glycols through a unique metabolism. Biochemical characterisation displayed that the isolate chose to ferment glucose, sucrose, arabinose, trehalose, and lactose. In general, *Bacillus subtilis* has a phosphortransferase system (PTS) that comprises specific regulatory proteins that help in glucose uptake and utilisation. *CcpA* (catabolite control protein A) is a regulator that responds to the presence of glucose and manages the expression of genes related to glucose metabolism (Yuan *et al.*, 2023; Atluri *et al.*, 2006; Mota *et al.*, 2001). It was also known that *Bacillus subtilis* also comprises *the*

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SacP locus for the utilisation of sucrose, where the *Ara* operon and the *araE*/*araR* divergent unit facilitate the utilisation of L-arabinose. The Ara operon consists of genes that encode the ABC transport system AraFGH and the arabinose–proton symporter AraE (Feng *et al*., 2017). The inherent metabolic abilities of microorganisms provide a highly effective, efficient way to remediate contaminated environments. Few bacteria have unique abilities in utilising polymer molecules as a carbon source and causing degradation of the compound, this natural occurrence helping the environment in eradicating manmade pollution. Usually, petroleum-based polyesters are non-degradable substances that can accumulate and slowly leach into soil and water bodies (Rajamani *et al*., 2023). So many soil biomes are involved in the degradation of these polymer molecules. The current study has revealed that the Bacillus subtilis HTNAKIRS strain exhibits an exceptional ability to degrade polyethylene glycols. The same species was reported previously in degrading polyethylene (PE) and polyethylene terephthalate (PET), which are accumulating in Jakarta Bay, where 2% PEG-Zobell Marine Agar was used for screening the isolates, which were found to be *Vibrioalginolyticus*, *Pseudoalteromonas caenipelagi*, *Microbulbifer pacificus, Pseudomonas marincola*, and *Bacillus subtilis,* where *B. subtilis* showed a polypropylene weight loss of 6.4% over a period of 40 days (Dela *et al*., 2012). Usually, Bacillus species are found to have the most bioremediation activities, as they have diverse enzymatic mechanisms (Kaur *et al*., 2024; Lee *et al*., 2024). Similarly, *Streptomyces coeruleorubidus* also showed utilizing PEG as a sole carbon source and showed significant loss in PEG concentration. Bacillus species usually undergo spore formation under adverse conditions of nutrient availability. This characteristic feature helps to tolerate and adjust in polymer contaminated environments (Belabbas *et al*., 2025). PEG degradation of *Bacillus subtilis* HTNAKIRS-1 strain revealed a few interesting metabolites by GCMS analysis, which makes predict the metabolic pathway using KEGG database software which is illustrated in Fig. 5. Polyethylene glycol is broken down into polyethylene aldehyde by the PEG-dehydrogenase enzyme, where PEG-aldehyde is converted to PEG-carboxylate in the presence of PEG-aldehyde dehydrogenase, followed by the formation of glyoxylate in the presence of PEG-carboxylate dehydrogenase. Glyoxylate condenses with acetyl CoA in the presence of the malate synthase enzyme, leading to malate (Pishva *et al*., 2025; Sarkar *et al*., 2011). Later, malate dehydrogenase oxidises malate to oxaloacetate by reduction of NAD+ to NADPH, while oxaloacetate enters the TCA cycle. Oxaloacetate in the presence of transaminase forms aspartate, followed by the formation of L-asparagine with the help of asparagine synthase. Aspartate may also enter the urea cycle, leading to the formation of urazole. From the TCA cycle, acetyl CoA enters the fatty acid biosynthesis pathway, forming Butyryl Acyl Carrier Protein. Butyryl ACP in the presence of thioesterase forms hexadecanoic acid or palmitic acid. Palmitic acid is a part of the formation of 2-palmitoyl glycerol; it also combines with CoA and ATP to form Palmitoyl CoA, palmitoyl in combination with malonyl CoA, leads to the formation of octadecanoic acid/stearic acid, from which octadecanal was generated further. Phosphophenol pyruvate carboxykinase converts oxaloacetate to Phosphophenolpyruvate which leads to entry in Shikimate pathway forming chorismate, later Chorismate enters Indole pyruvate pathway forming Indole. Palmitoyl CoA in the presence of acyl CoA dehydrogenase forms trans-enonyl CoA, and in the presence of the hydralase enzyme, trans-enonyl CoA forms L-β-hydroxyacyl CoA through dehydrogenase results β-ketoacyl CoA. β-ketoacylCoA in the presence of acyl CoA acetyl transferase/thiolase leads to the formation of acyl CoA. Acyl CoA again converts to triglycerides, diacylglycerol, and monoacylglycerol, leading to the formation of glycerol. Glycerol again with CO2 reaction in combination with stearic acid forms Glycerol monostearate, glycerol also forms glycol ethers like ethylene glycols. Fatty acid biosynthesis continues to the fatty acid elongation pathway, leading to the formation of hydrocarbons like decane, eicosane, and naphthalene. Hexadecanoic acid undergoes rapid intramolecular condensation leading to cyclisation and aromatisation, forming naphthalene, whereas naphthaquinone is formed by dehydrogenation of naphthalene. Ring opening and decarboxylation of naphthoquinone lead to benzaldehyde, while oxidation forms phthalic acid, which forms dibutyl phthalate and diethyl phthalate. Benzaldehyde results in acetophenone or benzoic acid, which leads to the formation of phenols and bisphenols. Naphthalene can also undergo degradation pathway leading to formation of catechol and enters TCA cycle. The bioinformatic analysis also revealed the specific genes supporting the PEG degradation pathway of *Bacillus subtilis* HTNAKIRS-1 strain.

Conclusions

This study investigated the growth of *Bacillus subtilis* HTNAKIRS-1 strain on PEG8000 as a sole carbon

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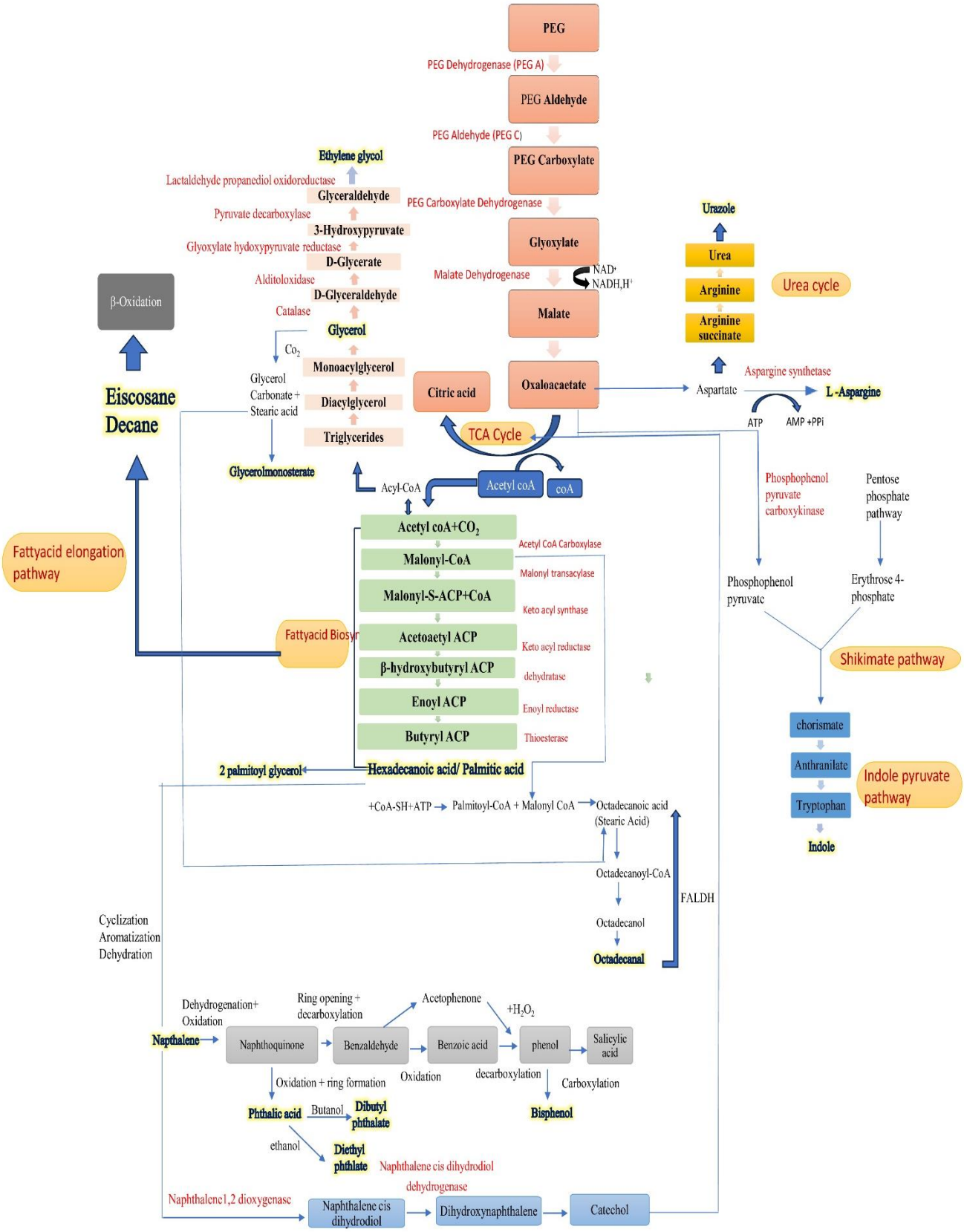

Figure 5. *Prediction of metabolic pathways using Kegg database software for metabolites produced by isolate Bacillus subtilis HTNAKIRS-1 strain during degradation of PEG 8000.*

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source. This strain showed the capability to degrade the polyethylene glycol molecules by undergoing networks with diverse metabolic pathways. Very few studies reported the polyesters biodegradation of Bacillus species, while this study provides a detailed overview of PEG biodegradation and its metabolites, along with an illustration of the metabolic pathway that the strain could have exhibited in utilising the polyethylene glycol molecules. The study also raises a further perspective, like the enhancement of biodegradation capability by different approaches like biostimulator, bioaugmentation, and genetic engineering studies to improve the strain's ability in biodegradation, which can be an effective eco-friendly approach in reducing polyether pollution in the ecosystem.

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Conflict of interest. All authors declare no conflicts of interest.

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