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PRODUCTION OF BIO-DEGRADABLE PLASTICS USING DIFFERENT WASTE RESOURCES BY *STREPTOMYCES GEYSIRIENSIS* SP. VITJK02

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ABSTRACT

Plastic polymers are invariably used every day for multiple applications causing huge environmental pollution globally. Biopolymers are biodegradable and considered to be a better alternative to synthetic plastics. This study explores the microbial production of Poly 3-hydroxyalkanoates (PHAs/ Poly 3-hydroxybutyrates (PHBs) using low-cost waste substrate. The polymer of hydroxyalkanoate is produced as intracellular granules, accumulates inside and serves as a source of energy for microorganisms. PHA-producing bacteria were isolated and screened using the Nile red and Sudan black B staining technique. Agro-industrial wastes like Rice bran. Sesame seed oil cake, used cooking oil, and glucose were used as carbon sources. The PHA-producing strain was characterized and identified by 16S rRNA partial genome sequencing. The extracted PHA was analyzed for PHA and PHB monomeric compounds by GC-MS analysis. PHA-producing isolate was identified as *Streptomyces* species and named as *Streptomyces geysiriensis sp.* VITJK02 by molecular taxonomic characterization. Among the carbon sources used rice bran served as a better carbon source for PHA production, and the yield was calculated to be 37.55%. Octadecanoic acid, 3-hydroxy-methyl ester, 2-butenoic acid, 1-methyl ethyl ester, 9-hexadecenoic acid, butanoic acid, 2,7-dimethyl oct-7-en-5yn-4-yl ester, butanedioic acid, dimethyl ester, pentadecanoic acid, and valeric acid, 2-tetradecyl ester were a few of the major PHA monomeric compounds observed in our study. Since PHA/PHB bioplastics are a better alternative to petroleum hydrocarbon-based synthetic plastic polymers, cheap household wastes can be used to produce PHA to minimize the cost.

1. INTRODUCTION

One of the major causations of water pollution is the improper disposal of solid waste like garbage; the environment is currently suffering because of improper use and disposal of plastics. Plastic pollution has a huge impact on the environment, animals, plants, and humans directly and indirectly. We need to follow the three R's in plastics use: reduce, reuse, and recycle. Although plastics are frequently used in our daily lives, their use must be minimized as much as possible, and they need to be recycled properly to reduce plastic pollution. PHAs are a type of naturally occurring bio-polyesters produced by a variety of microorganisms. PHA synthase gene (phaC) is responsible for the synthesis of PHA by bacteria (Tan et al., 2019). PHA is commercially important due to its eco-friendly nature, biodegradability, biocompatibility, crystallinity, and chemical diversity, and it could be produced from cheap and renewable carbon resources (Shah et al., 2008). PHA molecule is made up of 600 to 35,000 (R)-hydroxy fatty acid monomer units (Khanna & Srivastava, 2005). Based on number of carbon atoms, PHA monomers can be classified as short (3-5 carbon atoms), medium (6-14 carbon atoms), and long (≥15 carbon atoms) chain length (Al-Hamdani, 2016). Some of the SCL-PHAs (Short chain length-PHAs) polymers are 3-hydroxypropionate (3HP), 3-hydroxybutyrate (3HB), 4-hydroxybutyrate (4HB), 3-hydroxyvalerate (3HV), 4-hydroxyvalerate (4HV) and 5-hydroxyvalerate (5HV). MCL-PHAs (Medium chain length-PHAs) polymers are 3-hydroxyheptanoate (3HHp), 3-hydroxyoctanoate (3HO), 3-hydroxyhexanoate (3HHx), 3-hydroxydodecanoate (3HDD), 3-hydroxynonanote (3HN), and 3-hydroxydecanoate (3HD) (Ojha & Das. 2020). LCL-PHAs (Long chain length-PHAs) polymers are 3-hydroxypentadecanoate (3-HHxD), 3-hydroxyoctadecanoate (3-HOD), 3-hydroxy-9,12-octadeca-dienaote, 3-hydroxy-9-octadecenoate, etc (Roja et al., 2019; Koller. 2018). Structurally, mechanically, and thermally, the MCL-PHA was different from SCL-PHA. Due to expensive fermentation procedures and a lack of potential MCL-PHA-pro-

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ducing bacteria, the research on MCL-PHA is limited (Sathiyanarayanan et al., 2017). This classification aids in understanding the differences in physical and mechanical properties of various types of PHA. PHBs possess thermoplastic characteristics and is biodegradable. PHAs can be completely broken down into CO2 and water by a variety of microorganisms.

PHA can be commonly modified using physical and chemical methods (Zinn & Hany, 2005). However, recombinant DNA technology facilitates the production of PHA containing desired functional groups (Thompson et al., 1997). PHB is the most well-known polyester in the PHA series, with a similar molecular structure and physical properties (Hahn et al., 1994). PHA is a microbial thermoplastic that degrades into a potentially useful polyester that can replace petroleum-derived thermoplastics (Zhang Z et al., 2023). Based on their UV tolerance, water solubility, and good barrier from oxygen penetration, they have been used in agricultural, domestic, industrial, and Bio-medical applications (Vu et al., 2020; Saxena & Tiwari, 2011). Some other applications of PHA-based bioplastics in the industry include packaging, disposable bottles, preparation of bioplastic films for the protection of crops, hydrating agents, additives for animal nutrients, and textile fibers. Biomedical use includes wound healing, artificial blood vessels, tissue repair, regenerative medicine, cartilage regeneration, heart valves, bone implants, scaffold making, and drug carriers. Environmental uses include PHA surface coating, antifouling agent, and nanocomposite uses including biomedicine, hydrogels/nanogels, cancer treatment, stem cell engineering, coating for biomedical agents, coating for the paper board, automotive tools, microchips, and nanochips, etc. (Pratap & Krishnan, 2023).

When compared to synthetic plastics, PHA has much higher manufacturing costs. PHA costs are influenced by both carbon sources and fermentation processes. The cost of producing PHA from fossil-based polymers is very high. For large-scale production of PHA sugar-rich substrates and pure or recombinant bacterial strains are needed (Pratap & Krishnan, 2023). The production cost of PHA can be decreased by using cheap carbon sources such as household oil waste, fruit and vegetable waste, spent coffee grounds (SCGs), chicken feather waste, organic sludge, dairy waste, etc. Many reports are available on the production of PHA/PHB using various carbon sources and actinobacterium isolates. Some of these include Arthobacter sp. PCH145, which produces 15% of PHA, Paenarthrobacter sp. PCH191, which produces 26.6%, and Microbacterium sp. PCH192, which produces 67.2% of PHA (Kumar et al., 2018). Rhodococcus sp. strain BSRT1-1 yields 46.8% PHB content when fructose is utilized as a carbon source (Trakunjae et al., 2021). Rubrobacter xylanophilus produced 38.02%, 3%, 4.21%, and 51.53% of PHB content using glucose, arabinose, fructose, and glycerol as carbon sources, respectively. Rubrobacter spartanus utilizes glucose and glycerol as substrates, producing 38.59% and 49.65%, respectively (Kourilova et al., 2021). Propionibacterium sp. produced 13% and 5% PHA content utilizing 1% palm oil and 2% waste glycerol as carbon sources, respectively (Jamil, 2022). Streptomyces parvulus created PHB by using whey as a low-cost carbon source (Rasheed et al., 2013). Streptomyces sp. MAPPL011 produced 6.9%, 28.55%, 7.5%, and 7.94% of PHB content when agricultural waste like rice bran, oil cake, paddy straw, and cane molasses were chosen as carbon sources (Krishnan, Chinnadurai & Perumal, 2017). This study aims to isolate the PHA-producing microorganism from agricultural soil samples and produce PHA using cheap agriculture and household wastes including rice bran, used cooking oil, and sesame seed oil cake were provided as a carbon source for the bacteria for PHA production to minimize the production cost. To the best of our knowledge, this is the first report on the production of PHA using Streptomyces geysiriensis sp. VITJK02. The yield of PHA produced by Streptomyces sp. MAPPL011 was comparatively higher than earlier reports. The maximum growth was observed at 120h, and accumulation of PHA was observed on the same day by Streptomyces geysiriensis sp. VITJK02. When the rice bran was used as a cheap carbon source, the yield of PHA by Streptomyces geysiriensis sp. VITJK02 strain (37.55%) was more than Streptomyces sp. MAPPL011 (6.9%). Another innovative aspect of the study is to maximize the applications like bioplastics are used not only as an alternative to hydrocarbon-based plastic polymers but also in other biomedical applications.

1.1 Nomenclature

- PHAs (Polyhydroxyalkanoates)
- PHBs (Polyhydroxybutyrates)
- SCL-PHAs (Short chain length-PHAs)
- MCL-PHAs (Medium chain length-PHAs)
- LCL-PHAs (Long chain length-PHAs)
- CO_2 (Carbon dioxide)
- GC-MS (Gass chromatography- Mass spectrometry)
- phaC (Polyhydroxyalkonates synthase)
- r-DNA (Recombinant DNA)
- SCGs (Spent coffee grounds)
- MSM (Minimal salt medium)
- UV (Ultra violet)
- PCR (Polymerase chain reaction)
- MEGA (Molecular Evolutionary Genetics Analysis)
- BLAST (Basic Local Alignment Search Tool)
- NCBI (National Center for Biotechnology Information)
- rRNA (Ribosomal ribonucleic acid)
- SEM (Scanning electron microscopy)
- RT (Retention time)

2. MATERIALS AND METHODS

2.1 Collection of agricultural soil samples

Agricultural soil samples were collected from Chandrappa Naidu Kandriga Village, Taduku, Puttur Mandal, Tirupati district, Andhra Pradesh, India. The soil samples were collected in a sterilized sample collecting container after removing the surface soil. All samples were labeled, transported to the laboratory, and refrigerated at 4°C for further processing.

2.2 Isolation of PHA-producing organisms

The soil sample was serially diluted and plated on nutrient agar medium [0.5% of peptone- nitrogen source),

0.3% of beef extract (water-soluble consists of vitamins, carbohydrates, nitrogen, and salt), and 1.5% of agar] for the isolation of PHA producing organism. All the plates were incubated at 37°C for two days, pure colonies were isolated by the streak plate method on minimal salt medium [30 g/L of Na2HPO4.7H2O, 15 g/L of KH2PO4, 2.5 g/L of NaCl, 5 g/L of NH4Cl, 2 g/L of MgSO4, 0.1 g/L of CaCl2, 10 g/L of glucose and 20 g/L agar] (Sriyapai et al., 2022). The glycerol stock cultures were kept at -20°C on 20% glycerol stock.

2.3 Screening and Detection of PHA-producing Microorganisms

2.3.1 Nile red and Sudan black B staining for PHA-producing isolate

Nile red (0.5µg mL-1) was added to a sterilized MSM medium containing 1% glucose. The plates were incubated at 37°C for 48 h after adding microbial colonies (Tan et al., 2014). After incubation, the plates were exposed to UV light and observed for orange color fluorescence. Microbial colonies were cultured on MSM medium with 1% glucose and all the plates were incubated at 37°C for 72 h. To stain microbial colonies, the culture plates were flooded with 0.02% alcoholic Sudan black B incubated for 30 min (Al-Hamdani, 2016). The excess stain was removed by rinsing with 100% ethanol and observed for bluish-black color.

2.4 Identification of PHA-producing isolate

2.4.1 Phenotypic characterization of PHA-producing isolate

Morphological, biochemical, and physiological characterization of the isolate was carried out according to Bergey's manual (Bergey, 1994). The colony morphology like shape, margin, opacity, elevation, and texture was studied by microscopic examination.

2.4.2 Phylogenetic characterization of PHA-producing isolate

The genomic DNA was extracted from a PHA-producing isolate VITJK02 using the PCR template preparation kit (Roche) (Kieser et al., 2000). The extracted DNA was amplified by using 27F and 1492R and 518F and 800R universal primers and the PCR reaction mixture consist of double distilled water (18.3µl), tag DNA polymerase buffer (10x) (2.5µl), dNTP mix (2mM) (2.5µl), forward primer (10µM) 27F, 518F (0.5µl), reverse primer (10µM) 800R and 1492R (0.5µl), template DNA (0.5µl), taq DNA polymerase enzyme (5U/µl) (0.2µl) with amplification conditions of initial denaturation at 94°C for 5min, denaturation at 94°C for 45sec, annealing at 55°C for 45sec, extension at 72°C for 1min for 34 cycles and the final extension reaches to 72°C for 5min. After the program, PCR products were subjected to 1% agarose gel electrophoresis, and the amplified bands observed were compared with 1kb ladder. The 16S rDNA sequence obtained was subjected to BLAST search using NCBI GenBank database to find out the sequence similarity. The MEGA 5.1 software program (Tamura et., 2011) was used to construct a phylogenetic tree.

The 16S rRNA gene was 1500bp long and has conserved regions as well as hypervariable regions with specific codes useful for actinobacterial identification.

2.4.3 Scanning electron microscopic (SEM) analysis

The PHA-producing isolate was cultured in an MSM agar plate for SEM analysis (Carl Zeiss, Model No: EVO /18). The agar blocks were cut with a sterile blade and the culture was streaked around the edges, covered with a coverslip, and incubated for 7 days at 37°C. After incubation, 2.5% glutaraldehyde was added as a fixative. After overnight incubation, slide was washed with different ethanol gradients (30%, 60%, 70%, and 90%) to remove the loosely attached cells and excess of glutaraldehyde. It was allowed to dry and observed under SEM.

2.5 Growth kinetics

The PHA-producing isolate was inoculated into a 100ml MSM medium and incubated at 37°C at 200 rpm for various time intervals. The cell dry weight was determined after 24h, 48h, 72h, 96h, 120h, 144h, and 168h of incubation. To determine cell dry weight, microorganism-containing media was pelleted by centrifugation at 9000rpm for 10 minutes at 4°C. The supernatant was discarded, and a pellet containing cells was collected and dried overnight in a hot air oven at 100°C. The dry weight of the cells was calculated as g/L of the medium. All experiments were done in triplicates.

2.6 Extraction of PHA

PHA-producing isolate was grown on 250ml of MSM broth with 1% substrate in 500ml Erlenmeyer flasks. Different substrates like glucose, used cooking oil, sesame seeds oil cake, and rice bran were used separately for PHA production. It was incubated at 37°C for 7 days in a rotary orbital shaker at 200rpm. After incubation, the cells were harvested by centrifugation at 9000 rpm for 15min (Sriyapai et al., 2022). The pellet was collected, dried and the cell dry weight was determined in g/L. Sodium hypochlorite (10 ml) was added to the pelleted dry biomass and incubated for 2 hr at 50°C to lyse the cells before centrifugation at 9000 rpm for 10 min (Adnan et al., 2023). After centrifugation, a mixture of ethanol and acetone (1:2 ratio) was used to wash the pellet. The polymer-containing pellet was dissolved in chloroform and filtered through Whatman No.1 filter paper (Narayanan et al., 2022). The filtrate containing PHA was dried in a hot air oven at 60°C, and collected as a white precipitate. Experiments were performed in triplicates. The concentration of PHA (g/L) and percentage of PHA content was calculated as:

$$Percentage\ of\ PHA = PHA\ weight/cell\ dry\ weight \times 100$$

The extracted PHA was subjected to GC-MS (GC-7890B; MS-5977A) analysis to identify the PHA and PHB monomeric compounds. The split injection mode was used in the GCMS analysis, and its ratio is 50:1, liner volume: 0.99 ml, injection volume: 1.0 µl, injector temperature 260°C, column: DB-5-30m x 0.25 mm x 0.25 µ or equivalent, carrier gas: helium, column flow 1.0 ml/min, initial temperature 45°C, Interface temperature: 280°C, Mass range: 45-600 amu, Temperature rise/min:10-20°C.

3. RESULTS

3.1 Isolation and screening of PHA-producing bacteria

Twelve bacterial strains (VITJK01 to VITJK12) were isolated from collected soil samples. All the isolates were screened for PHA-producing potential. Unstained isolates are shown in Figure.1 A. Four isolates emitted orange colour fluorescence light under UV light after Nile red staining (Figure 1 B), and bluish-black colonies after Sudan Black B staining (Figure 1C).

3.2 Growth curve analysis

In order to identify the stationary phase for PHA extraction, a growth curve of the potential isolate was plotted based on the dry weight of the isolate. To plot the growth curve, the broth was centrifuged and the pellet obtained was dried, and the dry weight was recorded every 24 hours for 7 days. The stationary phase of the isolate was identified as 120 h and the pHA extraction was done on the same day. The growth curve was also used to study the growth rate of the isolate (Figure 2).

3.3 Characterization of PHA-producing microorganisms

Based on physiological characterization, the isolate was irregular in shape, smooth surface, moist texture, raised elevation, undulated margin, and white in color on day 2 (Figure 3 A) and chocolate brown color on day 7 (Figure 3 B). The isolate was examined under SEM to study the structure and surface morphology and it was observed as a rod-shaped chain-like structure. The cell was approximately 0.5-1.0m in size (Figure 3 C).

3.4 16S rRNA partial genome sequencing

A BLAST search of the VITJK02 sequence showed a sequence similarity of 99.92% with Streptomyces geysiriensis (Accession number NR_112459.1). The phylogenetic tree of VITJK02 was constructed using MEGA Software by the neighbor-joining method (Figure 4). Based on phylogeny and molecular taxonomy the PHA-producing isolate was identified as Streptomyces species and designated as Streptomyces geysiriensis sp. VITJK02. The 16S rRNA sequence of the PHA-producing isolate (VITJK02)

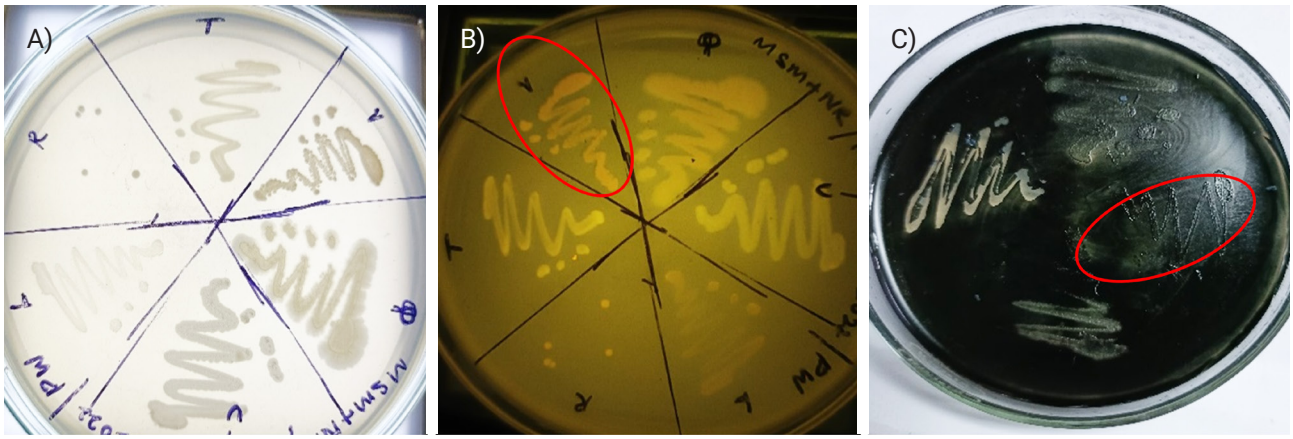

FIGURE 1: Nile red and Sudan black B staining for PHA-producing isolate VITJK02. A) Under normal light. B) Orange color fluorescence under UV light after Nile red staining. C) Bluish-black color after Sudan Black B staining.

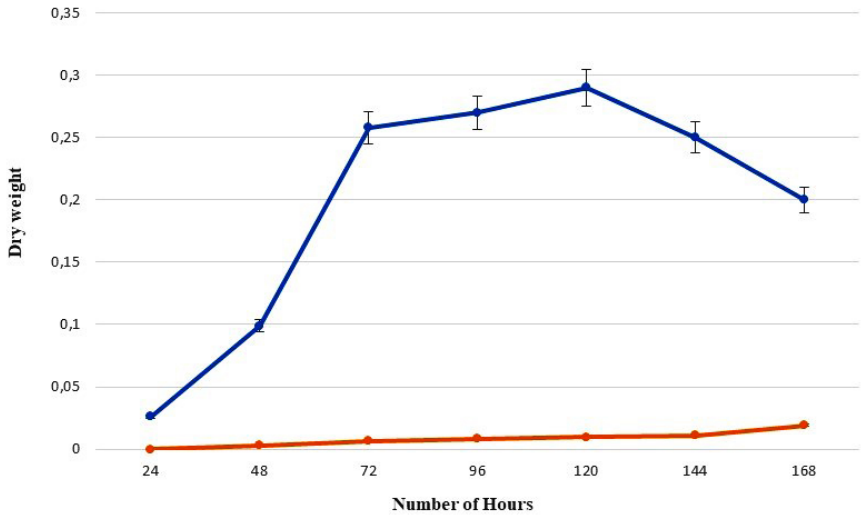

FIGURE 2: Growth curve analysis of a potential isolate *Streptomyces geysiriensis* sp. VITJK02.

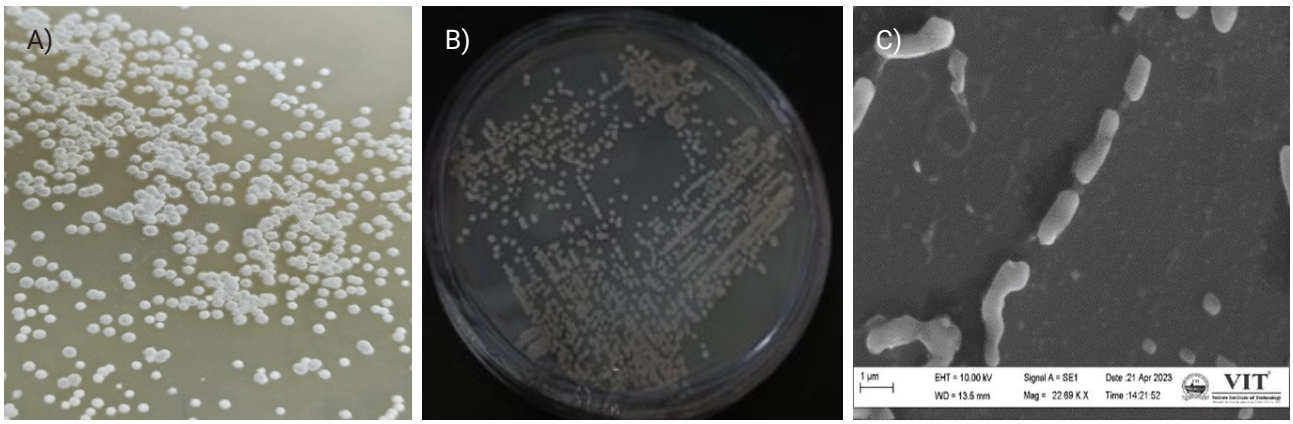

FIGURE 3: Morphological characterization of PHA-producing isolate *Streptomyces geysiriensis* sp. VITJK02. A) Growth at 48 hours, B) Growth at 7 days (producing brown color pigment), C) SEM image.

was submitted to GenBank under the Accession number OQ373315.

3.5 PHA Production and extraction

The PHA-producing isolate was cultured in 500 ml Erlenmeyer flasks each containing 250 ml MSM broth with 1% sesame seed oil cake, 1% of rice bran, 1% used cooking oil and 1% glucose (control) as a carbon source, and incubated for 7 days in an orbital shaker at 200 rpm at 37°C. For 1% rice bran the PHA yield was calculated to be 1.6 g/L from 3.5 g/L of cell dry weight. For 1% sesame seed oil cake it was 1.4 g/L from 4.2 g/L of cell dry weight. For 1% glucose it was 1.2 g/L from 3.8 g/L of cell dry weight. The isolate VITJK02 with rice bran as a carbon source yielded the highest PHA (34%), sesame seed oil cake (33%), glucose showed (31.5%) and used cooking oil showed less PHA yield (Figure 5). The extracted PHA yield was analyzed using GC-MS for PHA and PHB monomeric compounds.

3.6 GCMS analysis

The monomeric chemical composition of the polyhydroxyalkanoate (PHA) produced was characterized by gas chromatography-mass spectroscopic analysis. Multiple peaks were observed in the test system when compared to the control system. The majority of the PHA monomer-

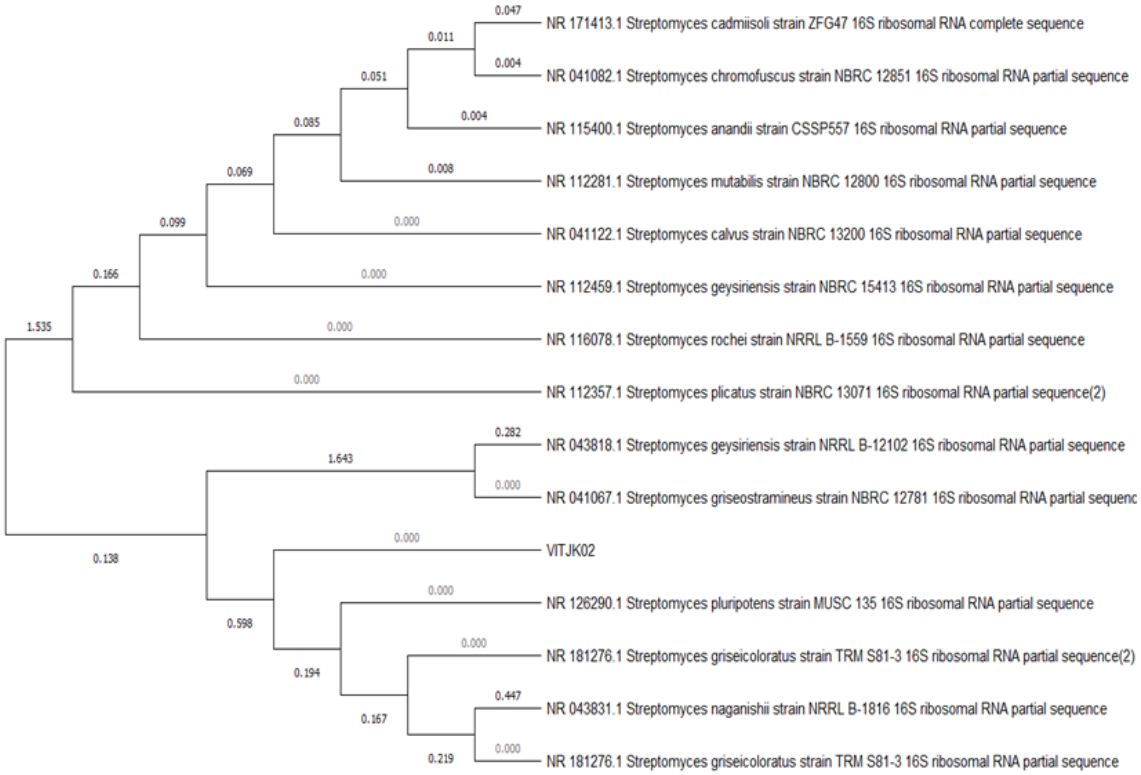

FIGURE 4: Phylogenetic relationship of Streptomyces geysiriensis sp. VITJK02 with other similar isolates based on 16S rRNA partial genome sequencing.

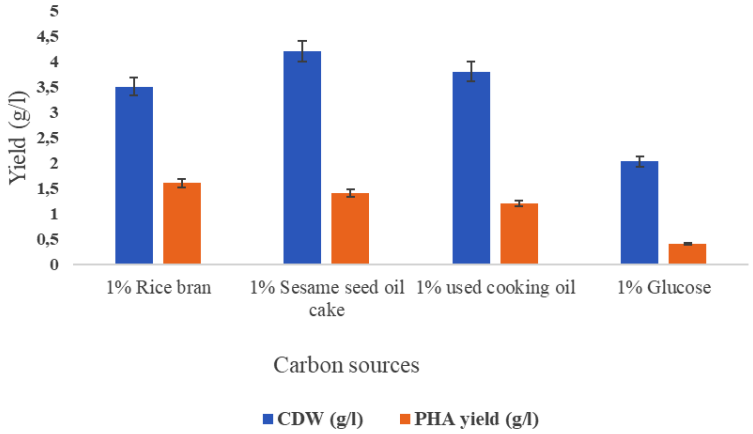

FIGURE 5: The yield of cell dry weight and extracted PHA using *Streptomyces geysiriensis* sp. VITJK02.

ic compounds observed in the GCMS spectrum have belonged to derivatives of decanoic acid and decanamide. When rice bran was used as a carbon source, the high-intensity peak of n- hexadecanoic acid (RT 18.48) and the low-intensity peaks of tetradecanoic acid (RT 16.4), tetradecanamide (RT 19.29), 9-Octadecanamide (RT 20.166), octadecanoic acid (RT 20.368) and the total area percentage of all PHA monomeric compounds were 37.55% (Figure 6 B). The retention time and area of the peak observed in the GC-MS spectrum are given in Table 1. When sesame seed oil cake was used the total area percentage of all PHA monomeric compounds was 29.28%, whereas in the case of glucose, the total area percentage of all PHA monomeric compounds was 21.92%. When compared to other carbon sources rice bran produced a maximum amount of PHA monomers. Hence, rice bran was selected as a cheap carbon source for PHA production.

When glucose was used as a carbon source which resulted in the highest peak intensity observed in octadecenoic acid (RT 20.54), moderate peak observed in pentanoic acid, 4-oxo-methyl ester (7.63), butanedioic acid, dimethyl ester (7.98), butanedioic acid, monomethyl ester (9.122), n-hexadecanoic acid (18.67), octadecenoic acid (20.58), 9,12, octadecanoic acid (20.65) whereas low-intensity peaks were observed in pentanoic acid, 4-oxo-methyl ester (7.54), pentanoic acid (8.71), pentadecanoic acid (17.15) were observed (Figure 6A).

The result analysis of the monomeric compounds was extracted from NIST libraries. The chromatogram showed the formation of monomeric compounds of many significant parent peaks, thus confirming the presence of PHA production as mentioned below. The predominant PHA monomers found was n-hexadecanoic acid, tetradecanoic acid, Phthalic acid, isobutyl octadecyl ester, Valeric

TABLE 1: GC-MS spectrum of PHA monomeric compounds produced by Streptomyces geysiriensis sp. VITJK02 in the presence of glucose and rice bran as a carbon source.

Control (glucose)	Retention time (RT) (glucose)	Area of percentage (%) (glucose)	Test (Rice bran)	Retention time (RT) (Rice bran)	Area of percentage (%) (Rice bran)
Pentanoic acid, 4-oxo-, methyl ester	7.545min	1.56	Diethyl Phthalate	14.479min	1.03
Pentanoic acid, 4-oxo-methyl ester	7.637mnin	1.08	2-propenoic acid, penta decyl ester	15.662min	1.67
Butanedioic acid, dimethyl ester	7.989min	2.82	Tetradecanoic acid	16.400min	1.88
Butanedioic acid, monomethyl ester	9.122min	2.82	Phthalic acid, isobutyl octadecyl ester	17.482min	1.02
Pentadecanoic acid	17.158min	3.24	n-hexadecanoic acid	18.48min	18.91
n-Hexadecanoic acid	18.677min	2.92	Valeric acid, 2-tetradecyl ester	20.11min	0.73
2-Ethylbutyric acid, 3-methylpent-2-yl ester	20.296min	1.17	Octadecanoic acid	20.368min	3.58
9-Octadecenoic acid, (E)-	20.547min	2.53	Butanoic acid, 2,7, dimethyl oct-7-en-5yn-4-yl ester	21.869min	0.82
Octadec-9-enoic acid	20.589min	0.84	Bis (2-ethyl hexyl) Phthalte	23.471min	7.91
9,12-Octadecadienoic acid (Z,Z)-	20.656min	0.84	-	-s	-
9-Octadecenoic acid, (E)-	25.404min	1.25	-	-	-

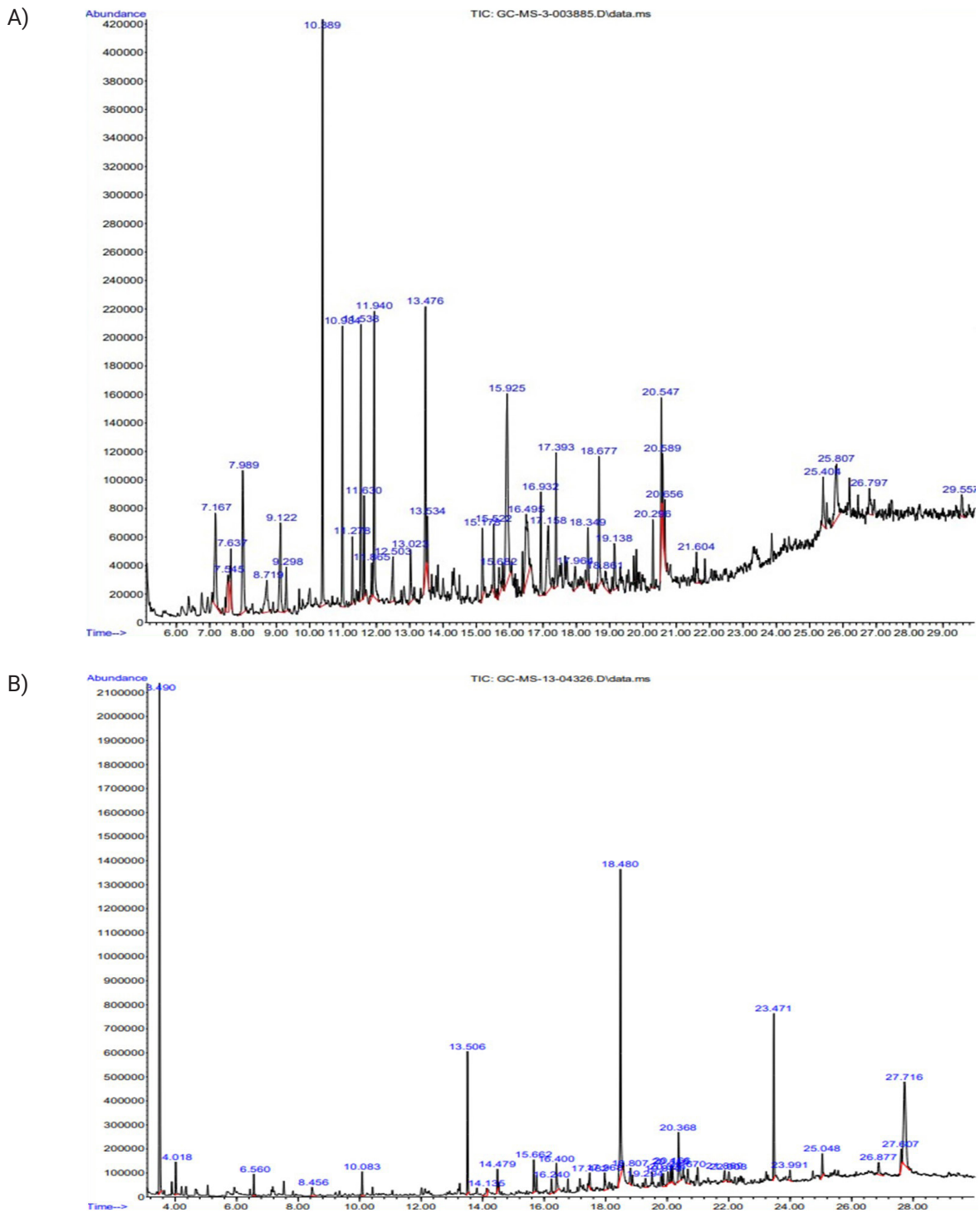

FIGURE 6: GC-MS Chromatogram of PHA monomeric compounds produced by *Streptomyces geysiriensis* sp. VITJK02 in the presence of A) glucose and B) rice bran as a carbon source.

acid, 2-tetradecyl ester, and octadecanoic acid, etc, when glucose was used as control system, predominant PHA monomers were butanedioic acid, dimethyl ester, n-hexadecanoic acid, octadecanoic acid and pentadecanoic acid.

4. DISCUSSION

Plastic exerts a profound influence on the ecosystem, engendering adverse consequences affecting the soil, water, ecosystem, and the environment. The release of greenhouse gases as a result of plastic degradation increases global warming. Increased production, widespread utilization, and improper disposal of conventional plastics lead to the accumulation of plastic waste in the soil, which remains in the soil for a prolonged period and their degradation lasts for several decades resulting in severe pollution affecting the well-being of the living systems. Multiple

strategies were followed for the control and management of plastic pollution. One such remedy is to decrease plastic production and their use and production of biodegradable plastics as an effective alternative to synthetic plastics. Bioplastics have demonstrated their superiority as a substitute for synthetic plastics, offering environmental benefits by avoiding the release of toxic compounds, plastic debris, and micro-nano plastics (Narancic et al., 2020). In order to mitigate the issue of plastic pollution, environmentalists are advocating the use of bioplastics, it is ecologically sustainable and readily degradable. The primary constituent of bioplastics is PHA/PHB. Many microbial species accumulate PHA/PHB compounds and used them as an energy source. A total of more than 150 unique hydroxyalkanoic acids have been recognized for their integration into PHAs (Castilho et al., 2009). Furthermore, the accumulation of these specific polyesters has been seen in over 90 different genera of microbial species. The degradation of PHA/PHB can occur under both aerobic and anaerobic circumstances. Under aerobic conditions, the tricarboxylic acid (TCA) cycle participates in the process of oxidation and undergoes degradation, resulting in the production of water (H2O) and carbon dioxide (CO2). Under conditions devoid of oxygen, methane (CH4) is generated. During this process, no hazardous chemicals were produced (Rai & Lee et al., 2009). The degradation process involves the participation of both biotic and abiotic factors.

In the current study, the PHA-producing Streptomyces geysiriensis sp. VITJK02 was isolated from agricultural soil samples. Streptomyces are a major group of soil bacteria belonging to the family actinomycetes are prolific producers of diversed compounds. It was reported that there are only 1-20% of culturable actinomycetes in the soil (Olanrewaju & Babalola, 2019). Streptomyces species play an essential role in agricultural soil due to their ability to create plant growth promotors, secondary metabolites, and enzymes (Vurukonda, Giovanardi & Stefani, 2018) and they also have the ability to resist both biotic and abiotic stress conditions. Streptomyces are recommended as prospective sources for the synthesis of PHAs with desirable characteristics that are acceptable for biomedical applications (Krishnan, Chinnadurai, & Perumal, 2017). Presence of the above reported monomers in PHA produced by microbes was reported by several researchers. Bacteria belonging to the actinomycetes family have been recognized as PHA-producers in recent past. Some of the PHA-producing actinomycetes are Rhodococcus species strain BSRT1 1 (Trakunjae et al., 2021), Kineosphaera limosa (Liu et al., 2002), Nocardia species, and Streptomyces species (Matias et al., 2009). PHA-producing Streptomyces species are Streptomyces toxytricini D2 (Narayanan et al., 2022), Streptomyces coelicolor A3(2) (Kalia, Lal, & Cheema, 2007), Streptomyces plumbiresistens CCNWHX 13-160T (Al-Hamdani, 2016), Streptomyces gougerotti and Micromonospora matsumotoense (Oliveira et al., 2022). PHA-producing Isoptericola variabilis species have been reported (Krishnan & Chinnadurai et al., 2021).

In this study, agro-based wastes like sesame seed oil cake, rice bran, and used cooking oil were used as a carbon source due to their easy availability, nutrient rich, and lower cost. Solid wastes like rice bran (Oh, Lee, & Jang et al., 2015), and food waste such as used cooking oil (Gómez Cardozo et al., 2016) have been reported to have high content of fatty acids and sugars for the production of PHA. Sesame seed oil cake is rich in carbohydrates, total solids, oils, and high chemical oxygen demand (Alsafadi, D., Alhesan et al., 2023). Agri waste is a low cost feed for bacteria making bioplastics (Mandal et al., 2024). Valorization of agri-food waste has been reported to contribute to the circular economy (Arriaga et al., 2025).

The monomeric compounds of PHA produced by VITJK02 were characterized using GC-MS analysis. PHA monomers such as 2-propenoic acid, penta decyl ester, tetradecanoic acid, phthalic acid, isobutyl octadecyl ester, phthalic acid, isobutyl octadecyl ester, n-hexadecanoic acid, valeric acid, 2-tetradecyl ester, octadecanoic acid, butanoic acid, 2,7, dimethyl oct-7-en-5yn-4-yl ester, bis (2-ethyl hexyl) phthalate, bis (2-ethylhexyl) ester was produced by VITJK02 when Rice bran was used as a carbon source. whereas, pentanoic acid, 4-oxo-, methyl ester, butanedioic acid, dimethyl ester, butanedioic acid, monomethyl ester, pentadecanoic acid, n-hexadecanoic acid, 2-ethylbutyric acid, 3-methylpent-2-yl ester, 9-octadecenoic acid, (E)-, octadec-9-enoic acid, 9,12-octadecadienoic acid (Z, Z)-, 9-octadecenoic acid, (E)- were produced when glucose was used. Based on GCMS analysis the total area percentage of the PHA monomeric compounds produced was 37.55% using rice bran and 23.09% for glucose. Bacillus wiedmannii AS-02 OK576278 produces octadecanoic acid, 3- hydroxy-, methyl ester, 2-butenoic acid, 1- methyl ethyl ester, 9-hexadecenoic acid when different agricultural waste was used for PHA production (Danial & Hamdy et al., 2021). Production of PHB by Bacillus cereus SH-02 (OM992297) was reported earlier (Hamdy & danial et al., 2022). For comparison, the PHAs produced by the different Streptomyces species are listed. Streptomyces griseorubiginosus DBCC 719 (9.5%) (Manna, Banerjee & Paul et al., 1999); Streptomyces toxytricini D2 (86.56%) (Narayanan et al., 2022); Streptomyces subrutilus (27.5%) (Malviya et al., 2021); Streptomyces sp.(4-8%) (Krishnan et al., 2017); Streptomyces species DG19 (100% of 3-hydroxyvalerate monomer) (Herrera et al., 2023); Streptomyces gougerotit (Oliveira et al., 2022); Streptomyces coelicolor A3(2 (Valappil et al., 2004).Production of PHA by Bacillus paramycoides strain KUMBNGBT-33 was reported recently (Nandish et al., 2023). Traina et al., 2024 have reported that the content of PHA is 0.46g/L, 0.20g/L, 0.19g/L, 1.48g/L, and 0.12g/L when sugar cane molasses, cheese whey, boiled mussel processing effluent, fruit waste and winery waste was used as a carbon source, respectively. Yoo et al., 2024 developed PHB from halophilic bacteria by improving salt tolerance. They created gamma radiation-induced mutants to improve salt tolerance. The Halo6 mutants produced 11% more PHA, which was associated with raised intracellular granule content and cell size. Petroleum-based polymers were replaced by biobased polymers in a wide range of applications like construction, biodegradable toys, automobiles, cosmetics products, medical applications like sutures, tissue engineering, heart valve engineering, organ transplantation and reconstruction, drug carrier, liver tissue

engineering, blood vessels, antibiotic delivery, etc. (Yeo et al., 2018). This study promotes a biocircular economy by utilizing waste for the production of PHA which can be used for bioplastic production.

5. CONCLUSIONS

VITJK02 was isolated from agricultural soil sample, which produced PHAs and PHBs. 16S rRNA partial genome sequencing of VITJK02 (Accession number OQ373315) showed 99.92% sequence similarity with *Streptomyces geysiriensis* (Accession number NR_112459.1). Production of PHAs and PHBs by *Streptomyces geysiriensis* sp. VITJK02 by utilizing Sesame seed oil cake, Rice bran, used cooking oil as exclusive carbon sources was not been previously reported to the best our knowledge. Optimization of culture conditions of Streptomyces geysiriensis sp. VITJK02 would yield higher production of PHAs/PHBs by utilizing household and agricultural wastes. Large scale microbial production of PHAs/PHBs using the household wastes with reduced cost is of higher commercial value.

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