Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Biopolyester Production Using Fruit Wastes as a Substrate by Soil Bacteria

Sumaira -

ssumairashah@gmail.com

Shaheed Benazir Bhutto Women University PeshawarMicrobiology

Hina Hidayath

Shaheed Benazir Bhutto Women University PeshawarMicrobiology

Research Article

Keywords: Polyhydroxyalkanoates, fruits wastes, Ralstonia eutropha MTCC1285, FTIR

Posted Date: October 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11122869/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Sumaira
ssumairashah@gmail.com
Shaheed Benazir Bhutto Women University Peshawar
Hina Hidayath
hinahidayat5@gmail.com
Shaheed Benazir Bhutto Women University Peshawar
Corresponding Author
Sumaira
ssumairashah@gmail.com
Shaheed Benazir Bhutto Women University Peshawar

Biopolyester Production Using Fruit Wastes as a Substrate by Soil Bacteria

Sumaira, Hina Hidayat

Abstract

The global dependence on petroleum-based plastics has led to severe environmental challenges due to their non-biodegradability and persistence in ecosystems. Biopolyesters, particularly polyhydroxyalkanoates (PHAs), have emerged as promising biodegradable alternatives due to their microbial origin, biocompatibility, and sustainable nature. The present study focused on the isolation and characterization of soil bacteria capable of synthesizing biopolyesters using fruit wastes—apple, orange, and banana peels—as renewable and cost-effective carbon substrates. Soil enrichment was performed through mulching with fruit and organic waste to stimulate the proliferation of biopolymer-producing bacteria, thereby enhancing the presence of naturally efficient PHA-accumulating strains in the soil environment. Primary screening for biopolyester producers was performed using Sudan Black B dye, while confirmatory screening employed Nile Blue A. Among twelve isolates, seven showed positive results for biopolymer accumulation, confirming their PHA-producing capability. Quantitative analysis using E_2 medium revealed that isolates MS-5 and MS-7 have the highest polyester production efficiencies, highlighting their superior metabolic potential. Subsequent fermentation experiments using fruit wastes as sole carbon sources demonstrated that apple peels provided the most favorable substrate for polyester yield, followed by orange and banana peels. The strain MS-7 produced quantities of polyester comparable to the positive control *Ralstonia eutropha* MTCC1285, further supporting its suitability as an efficient biopolymer-producing organism. Phylogenetic identification through 16S rRNA sequencing confirmed that MS-7 showed 99.83% sequence similarity with *Bacillus sp*., validating its genetic affiliation with this genus and supporting the accuracy of its taxonomic placement. Extraction of biopolyester was optimized through a novel sonication-assisted method employing ethylene carbonate, which achieved comparable yields to conventional chloroform extraction but with significantly reduced toxicity and cost, demonstrating a more sustainable and environmentally safe extraction approach. Fourier Transform Infrared Spectroscopy (FTIR) analysis confirmed the presence of functional groups characteristic of polyhydroxybutyrate (PHB), validating the polymer identity, although slight intensity differences indicated minor impurities in the extracted sample. Plasmid curing experiments using ethidium bromide demonstrated that the biopolyester synthesis ability of isolates was chromosomally encoded rather than plasmid-mediated, indicating stable inheritance of the trait.

Keywords: Polyhydroxyalkanoates, fruits wastes, *Ralstonia eutropha* MTCC1285, FTIR

Introduction

Plastics have become one of the most indispensable materials of the modern world, playing a central role in shaping technological progress, economic growth, and everyday convenience. Their remarkable combination of properties—lightweight structure, mechanical strength, flexibility, durability, resistance to corrosion, and affordability—has allowed them to replace traditional materials such as wood, glass, and metal in countless applications (Shah, M. *et al*., 2021).

Plastics are widely used in packaging, construction, agriculture, electronics, medical devices, transportation, and consumer goods because they offer exceptional versatility and functionality. (Luis G et al., 2024). However, the same qualities that make plastics durable and useful also make them a major environmental concern. Conventional plastics are highly resistant to natural degradation, persisting in landfills, soil, and marine ecosystems for decades or even centuries. Their accumulation has led to widespread pollution, threatening wildlife, aquatic systems, and human health (Bedade,DK., *et al.*, 2021). As plastics break down, they release microplastics and nanoplastics—minute particles that infiltrate soil, water, and even the air. These particles are now found in marine organisms, drinking water, and food, raising concerns about their long-term impact on ecosystems and the human body. Altogether, these issues reveal that while conventional plastics have contributed immensely to human development, their environmental persistence and toxicity now pose severe global challenges requiring urgent mitigation strategies(Abokalawa, F. *et al.,* 2022).

In response to these challenges, scientific and industrial efforts have increasingly turned toward the development of biodegradable and bio-based alternatives to conventional plastics. Among these, polyhydroxyalkanoates (PHAs) represent one of the most promising innovations. PHAs are natural polyesters produced by a wide variety of bacteria as intracellular carbon and energy storage compounds under nutrient-limited conditions. These polymers are completely biodegradable in soil, freshwater, and marine environments, where they are decomposed by microbial activity into water and carbon dioxide. The PHA family includes a wide range of monomeric compositions—short-chain, medium-chain, and long-chain-length monomers—that determine the resulting polymer's mechanical and thermal properties (Shah,A.A. *et al.,* 2021). Due to this structural versatility, PHAs can mimic many of the functional properties of synthetic plastics such as polypropylene while retaining biodegradability. Their biological origin and ability to decompose naturally make them ideal candidates for replacing petroleum-based polymers in numerous applications (Luis G. *et al.*, 2024).

The versatility of PHAs has led to their exploration in multiple fields. In packaging, PHAs are utilized for biodegradable containers and films that can safely decompose without harming the environment. In agriculture, PHA-based mulch films and controlled-release carriers are used to minimize soil contamination. Their biocompatibility and non-toxic degradation products make PHAs particularly suitable for medical and pharmaceutical applications, including surgical sutures, orthopedic implants, wound dressings, and targeted drug delivery systems. Additionally, advances in material science have enabled the development of PHA-based composites reinforced with natural fibers, nanomaterials, and conductive fillers, extending their use into high-performance sectors such as electronics, sensors, and biomedical devices (Elmowafy, E. et al.,

2019). The potential for PHAs to replace conventional plastics while maintaining safety and performance standards underscores their critical role in achieving a sustainable and circular economy (Tokiwa,Y. *et al.,* 2009).

Despite their advantages, the large-scale commercialization of PHAs faces several major obstacles. The foremost challenge is the high cost of production, which remains significantly greater than that of conventional plastics. Carbon sources used as feedstocks during microbial fermentation account for nearly half of the total production cost. Furthermore, the process often requires sterile fermentation conditions and controlled parameters, which add to operational expenses (Daltin, B. *et al.,* 2022). The extraction and purification of PHAs from microbial biomass typically involve the use of organic solvents such as chloroform, which are both costly and environmentally hazardous. Additionally, some PHA types, such as poly(3-hydroxybutyrate) (PHB), are brittle and have narrow thermal processing windows, limiting their industrial versatility. To address these constraints, researchers have focused on several strategies: using inexpensive and renewable feedstock's, optimizing mixed microbial cultures, employing extremophilic microorganisms that thrive in non-sterile conditions, and developing greener extraction methods using eco-friendly solvents or mechanical disruption (Ashraf, A.M. *et al.,* 2021).

One of the most effective ways to reduce the cost of PHA production is by utilizing organic wastes **i.e.** fruit wastes as carbon substrates for bacterial fermentation. Fruit wastes such as apple, orange, and banana peels are rich in carbohydrates, organic acids, and other nutrients that support microbial growth and polymer biosynthesis. Using these residues as raw materials not only decreases the cost of carbon feedstock's but also provides an environmentally friendly solution for managing food waste, which is otherwise a major contributor to landfill burden and methane emissions. This approach exemplifies the concept of waste valorization, where low-value waste materials are converted into high-value bioproducts (Mohapatra, S. *et al.*, 2017). By adopting such strategies, the process contributes simultaneously to waste reduction and sustainable materials development. Moreover, producing PHAs from renewable and locally available waste resources aligns with the principles of a circular bioeconomy, promoting economic feasibility and ecological responsibility (Kumar,SN.*et al.*, 2023).

Methodology

Soil Mulching

For preparing a soil mulch, a 4x4 feet area was selected at Shaheed Benazir Bhutto women university Peshawar. A boundary line was made to differentiate it from rest of non- experimental area. 200 ml of waste water was mixed with organic wastes (fruits waste). The mixture was layered on selected area with a thickness of about 5cm and was sprayed with water at interval of 72 hours for a period of 15 days so that soil become enriched. After 15 days the mulch layer was mixed with soil and further incubated for 3 days.

Collection of samples

150gm Soil samples were collected from 3 points of enriched soil. The soil samples were refrigerated until they are processed further. 1g of soil was serially diluted and plated on nutritional agar containing 1% glucose in order to further enhance biopolyester producers for 48 hours. The bacterial colonies were purified and cultivated on nutrient agar slants.

Rapid screening of native bacterial isolates for bioplastic production

The bacterial isolates were screened using a 0.02% concentration of Sudan Black B dye. Dye-positive isolates were verified as producers of biopolymers using 0.5% Nile blue stain added directly to nutrient agar (Crisafi, F., 2022).

Characterization of bioplastic producing bacterial isolates

Biopolymer-positive isolates were characterized macroscopically, microscopically and biochemically by tests i.e. oxidase, catalase, nitrate reduction, methyl red & vogus proskeur, H2S production, carbohydrate fermentation, triple sugar iron agar, starch, casein and gelatin hydrolysis (Holt, J.G. *et al.,* 1993).

Biopolymer production through fermentation using fruit wastes as sole carbon source

Using various organic wastes as substrate (fruit wastes) in E2 medium, polyester positive bacterial isolates were cultivated to synthesize biopolyester under liquid state fermentation condition (wong Yee and Nur Ainaa, 2022).

Extraction and identification of biopolyester

Chloroform was used to extract the biopolymer from cells in accordance with standard procedure (Musa H. *et al.,* 2016). Since chloroform is a costly and hazardous solvent, a novel technique with few amendments in already established protocol was designed that combines sonication with a less costly and hazardous solvent. Sonication was done at intervals of 1, 3, and 5 minutes, with a pulse that is maintained at 30 seconds on and 30 seconds off (Sumaira *et al.,* 2023). Ethylene carbonate was then be used for extraction. The FTIR analysis was used to characterize and identify purified biopolyester. Spectrum Two FTIR PerkinElmer USA with UATR attachment was used for FTIR analysis, which will take direct measurements of the extracted and reference samples' infrared spectra.

Plasmid curing of bacterial isolates for bio polyester producing property

Two distinct chemicals, acridine orange (50 µg/ml) and ethidium bromide (50 µg/ml) were used to cure the plasmid (Boy C. *et al.,* 2022).

Statistical Analysis

Data obtained was analyzed by using statistical analysis tool prism 8.

Molecular identification and phylogenetic analysis

16S rRNA gene sequence was determined by using traditional Sanger Sequencing method.the sequence was submitted to NCBI for accession number.

Results

Biobased plastics have the unique advantage over conventional plastics to reduce the dependency on limited fossil resources and to reduce greenhouse gas emissions or even be carbon neutral. Derived from plants and microorganisms, they can be designed to be biodegradable and compostable, enabling organic recycling and creating valuable biomass, thus promoting a circular economy and contributing to environmental protection.

In current study bacteria capable of synthesizing bio polyester were enriched by soil mulching technique. The bacterial isolates were screened by using primary dye Sudan B black and confirmatory dye Nile blue A. To reduce the production cost fruit wastes were used as a sole carbon source. Soil samples taken from enriched soil were serially diluted and bacteria were isolated. The isolated bacteria were purified.

1. Screening of Bioplyesters producing bacteria

Total of 12 bacteria were isolated. Theses bacterial isolates were screened for bioplyesters production capacity by using Sudan b black dye and Nile Blue A. ;7 seven isolates were found to be positive for biopolymers accumulation as shown in Figure 2 A, B, C, D, E & F.

After ethanol treatment the bacterial colonies retained Sudan Black B dye due to presence of polyester granules. Nile Blue A further confirmed the presence of polyester granules.

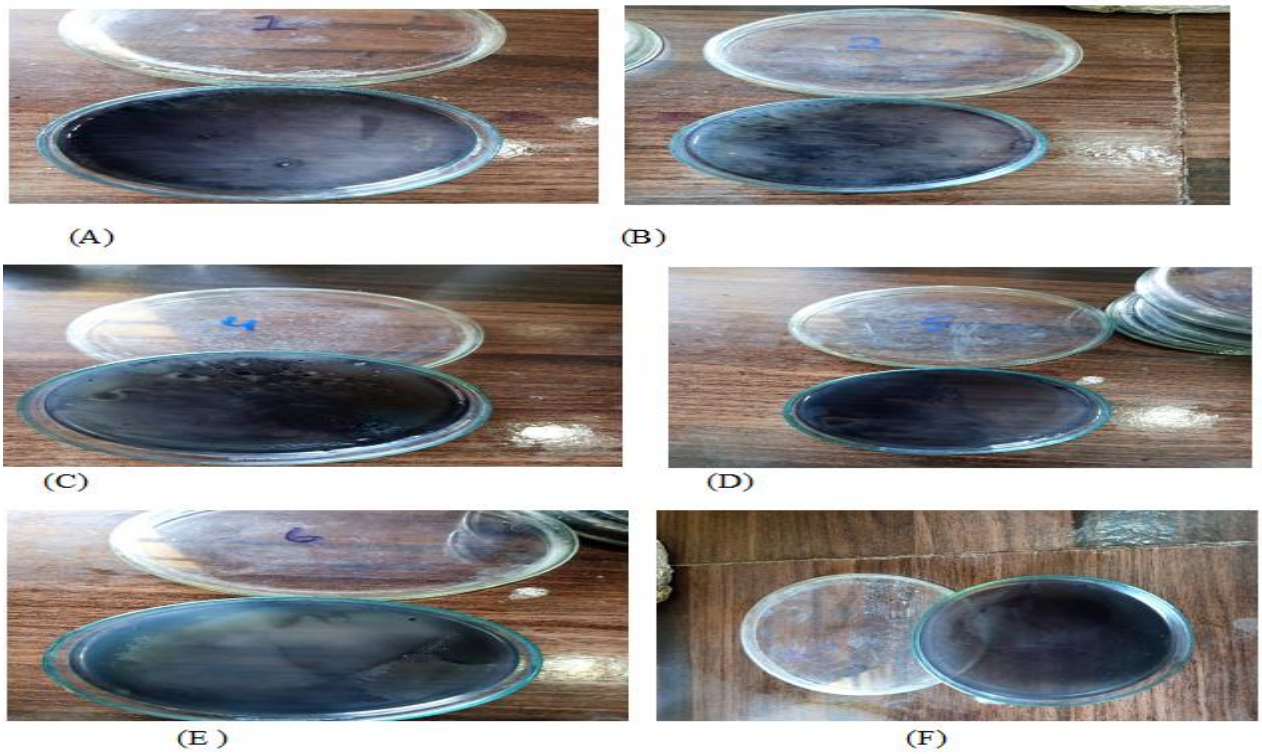

Figure: 1:Bacterial Isolates screened to be positive for biopolyester synthesis

2. Comparison of bioplyesters producing efficiency of Positive Isolates

Bio polyester positive isolates were grown on E 2 medium using pure glucose as a sole carbon source. Among 7 producers MS-5 and MS-7 isolates were having high production efficiency as described in table 1 and shown in Figure 2. Mean value and standard error of mean were calculated using prism 10.

Table 1: Quantity of biopolymer produced by positive isolates using glucose as a carbon source

	Strain	Quantity (g/l)			Mean	SEM	N
1	MS-1	0.002	0.001	0.004	0.002	0.0009	3
2	MS-2	0.003	0.0035	0.002	0.0028	0.0004	3
3	MS-3	0.01	0.01	0.01	0.01	0	3
4	MS-4	0.04	0.04	0.035	0.038	0.002	3
5	MS-5	0.5	0.55	0.52	0.523	0.015	3
6	MS-6	0.03	0.035	0.025	0.03	0.003	3

7	MS-7	0.55	0.6	0.65	0.6	0.029	3

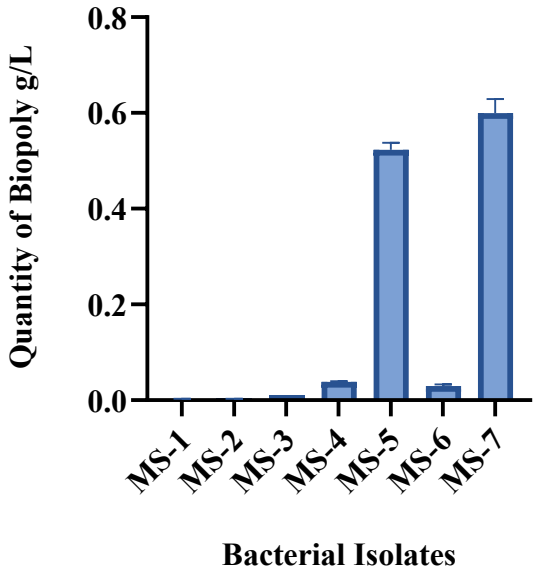

Figure 2. Quantitative determination of biopolyester produced by soil *bacterial isolates*

3. Macroscopic, microscopic and biochemical Characterization of efficient producers

These bacterial isolates were characterized macroscopically, microscopically and biochemically according to Bergey's manual of determinative Bacteriology as shown in table 2 a, b and c.

Table 2(a): Macroscopic Examination

	STRAIN	SHAPE	MARGIN	ELEVATION	TEXTURE	APPEARANCE	PIGMENTATION	OPTICAL DENSITY
1	MS-5	Round	Smooth	Convex	Rough	Shiny	Greyish-White	Opaque
2	MS-7	Round	Jagged	Convex	Rough	Shiny	Slightly-Yellow	Opaque

Table 2b: Microscopic examination of PHA produces

S.NO	**Strain**	**Gram reaction**	**Arrangement**
1	MS-5	Gram positive	Bacilli, Chains
2	MS-7	Gram positive	Bacilii, Chain

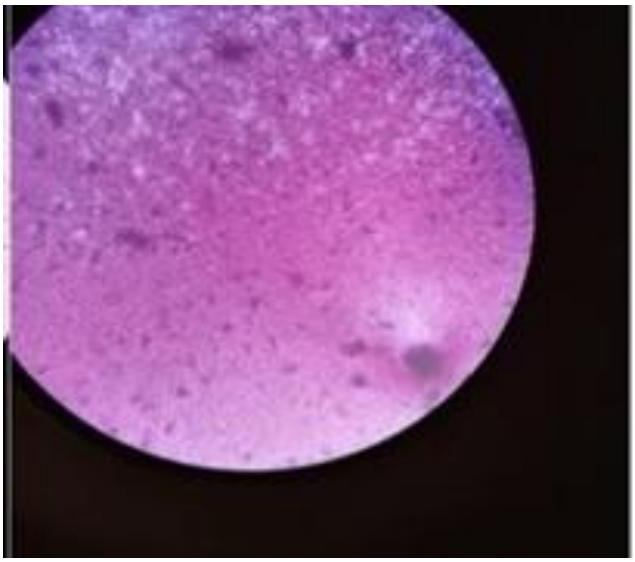

Figure 3: Gram Staining

Table 2 (c): Biochemical Characterization of bacterial isolates

S:NO	**Strain**	**Starch hydrolyss**	**Casein**	**Catalase**	**Urease**	**Nitrate Reduction**	**Gelatin**	**Citrate utilization**	**Oxidase**	**Glucose fermentation**	**Fructose fermentation**	**TSI**		
												Butt	**Slant**	**Gas**
1	MS-5	-	+	+	-	-	+	+	-	+	+	Yellow (Acid)	Yellow (Acid)	-
2	MS-7	+	+	+	+	+	+	+	+	+	+	Yellow (Acid)	Yellow (Acid)	-

4. Production of biopolymer using fruit wastes as a substrate

In the excution of this study *Ralstonia eutropha* MTCC1285 was used as a positive control. The test isolates along with positive control were grown on E 2 medium using fruit wastes i.e. orange, apple and banana peels as a substrate. It was found that MS-7 was more efficient in polyester production as compared to MS-5 and its production capacity was found to be approximately close to positive control. secondly the apple peels were more suitable for high yield followed by orange and banana peels as shown in Figure 4.

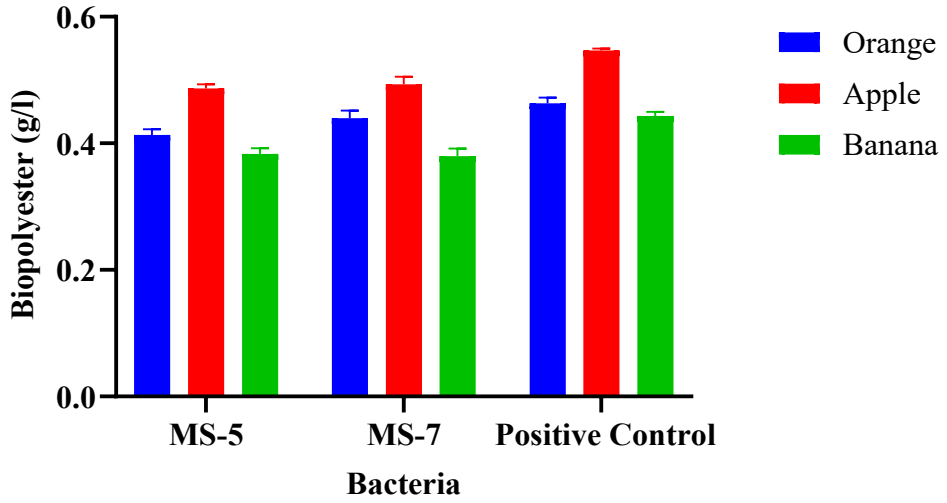

Figure 4: Biopolyester yield using fruits wastes as a substrate by test isolates and positive control

Table 3: Quantitative comparison of test isolates and positive control by using organic wastes as a substrates

Strain	Orange			Apple			Banana		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
MS-5	0.41	0.009	3	0.47	0.006	3	0.38	0.009	3
MS-7	0.44	0.01	3	0.49	0.01	3	0.38	0.01	3
Positive control	0.46	0.009	3	0.55	0.003	3	0.44	0.0071	3

5. Extraction with amended technique

Chloroform is a toxic solvent. In order to replace it with less toxic and cost effective solvent the ethylene carbonate was used in combination with sonication. It was found that efficiency of this method is approximately near to traditional chlororform extraction methd as shown in Figure 5.

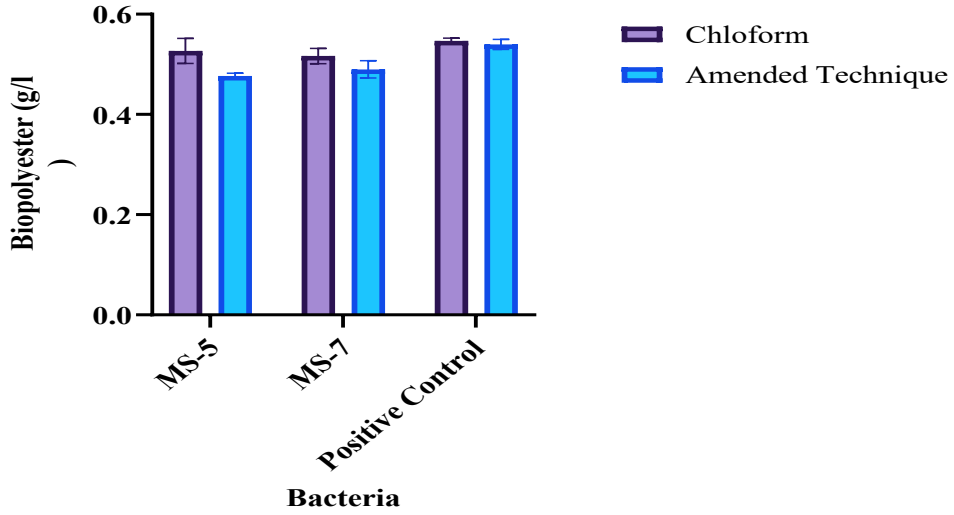

Figure 5: comparison of traditional and amended green technique for biopolester extraction

6. Qualitative Analysis of Extracted polymer

The extracted polymer was investigated qualitatively using FTIR method. It was found that intensity of referenc polyester is liitle bit higher than extarcted polymer as their might be some impurities in it as shown in Fig 6. red one indicates extrcted spectra while black one is reference polymer.

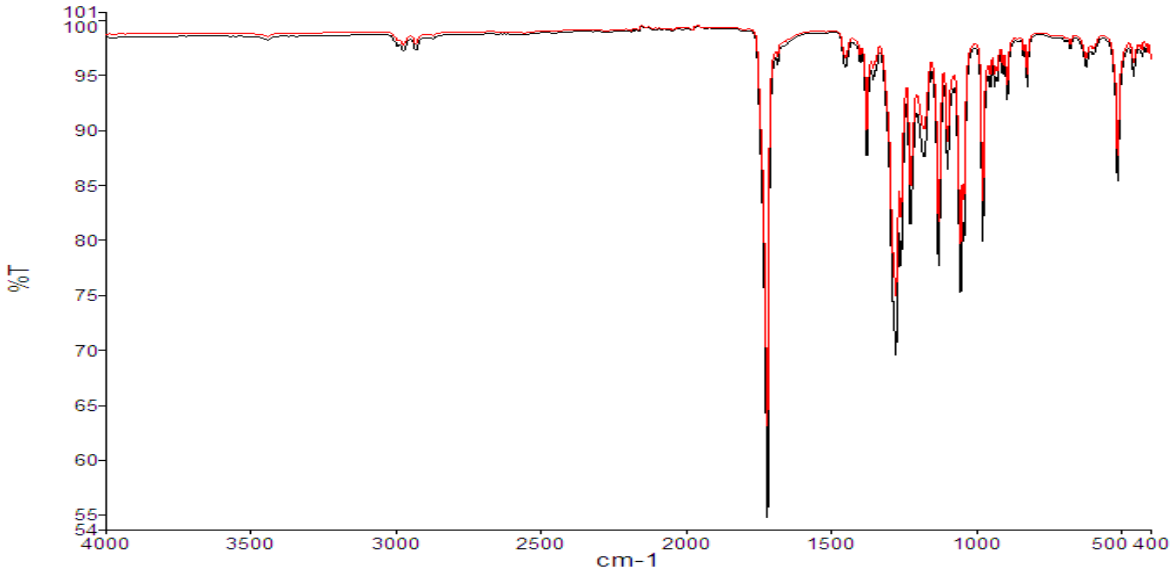

Figure 6: FTIR curves of extracted and reference biopolyesters

Figure 7: Extracted biopolyester

7. Plasmid curing

In order to determine that property of synthesis is associated with plasmid or chromosome. Plasmid inactivation was done by using ethidium bromide as curing agent. When cured isolates were grown on medium for bioplyesters synthesis using apple waste as a substrate it was found isolates were still able to produce biopolymer so it was concluded that synthesis ability is chromosome associated as shown in table 4.

Table 4: Plasmid Curing Results

Strains	Growth	Activity (Plasmid/Chromosome mediated
MS-5	Growth appeared	Chromosome mediated
MS-7	Growth Appeared	Chromosome mediated

8. Phylogenetic Analysis

As from yielding capacities it was estimated that MS-7 gave high yield so 16srRNA of this bacteria was partially sequenced by sanger method using universal primers. The sequence obtained was submitted to NCBI gene bank and accession number SUB15616023 seq_1 PX369005
was provided to it. NCBI blast was used to study sequence homology. Our strain showed maximum similarity to *Bacillus sp. (in: firmicutes)* 99.83%. MEGA soft ware was uses to built phylogenetic tree (Neighbour joining) as indicated in Figure 8 .

Table 5: Sequence similarity of MS-7 with Strains in NCBI

Descriptions | Graphic Summary | Alignments | Taxonomy

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 MA-59 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2318	2318	100%	0.0	99.68%	1267	KX426659.1
Bacillus sp. (in: Bacteria) strain LMRE23 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	2169	2169	93%	0.0	99.83%	1180	MK571661.1
Bacillus sp. (in: Bacteria) strain LMRE40 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	2043	2043	88%	0.0	99.82%	1112	MK571656.1
Bacillus subtilis strain MA-61 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2043	2043	97%	0.0	96.47%	1254	KX426661.1
Bacillus tequilensis strain SE30 16S ribosomal RNA gene, partial sequence	Bacillus tequilensis	2039	2039	97%	0.0	96.40%	1352	MH458837.1
Bacillus subtilis subsp. subtilis strain E2-03 16S ribosomal RNA gene, partial sequence	Bacillus subtilis subsp. subtilis	2039	2039	97%	0.0	96.54%	1318	OQ566942.1
Bacillus subtilis strain MA-78 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2039	2039	96%	0.0	96.61%	1239	KX426678.1
Bacillus sp. (in: Bacteria) strain IH35 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	2037	2037	98%	0.0	96.19%	1453	MT052351.1
Bacillus subtilis subsp. subtilis strain b2 16S ribosomal RNA gene, partial sequence	Bacillus subtilis subsp. subtilis	2037	2037	97%	0.0	96.54%	1244	OM213033.1
Bacillus tequilensis strain 034 16S ribosomal RNA gene, partial sequence	Bacillus tequilensis	2037	2037	97%	0.0	96.54%	1326	OR065060.1

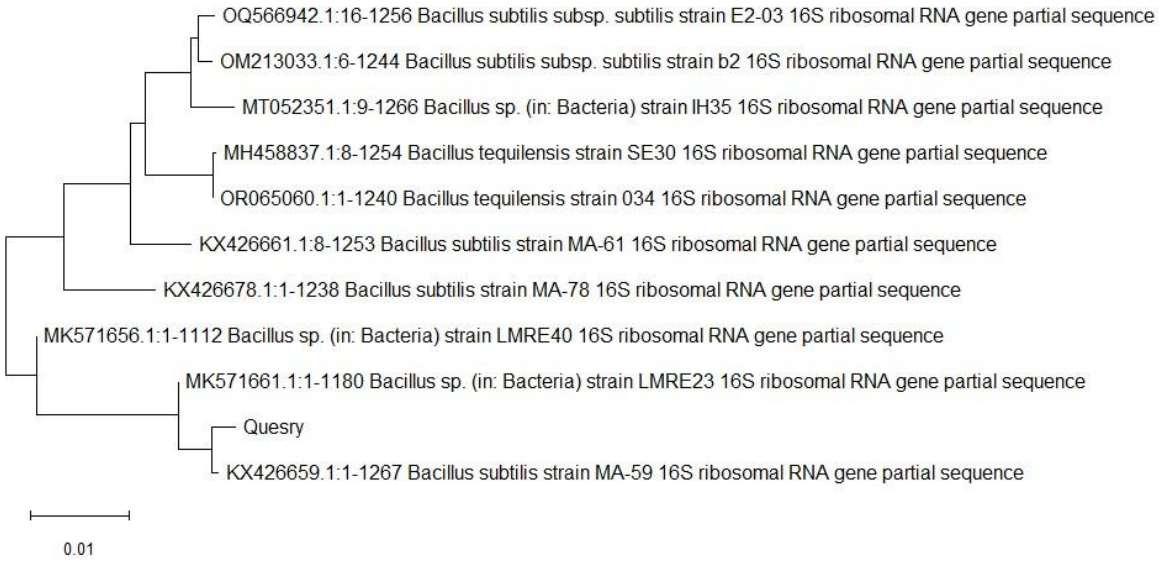

Figure 8: Phylogenetic tree 16srRNA Sequence of bacterial isolate MS-7 and other *Bacillus* spp. In NCBI database

Discussion

The current study was designed to resolve two environmental issues i.e. environmental pollution caused by synthetic plastic accumulation and disposal of organic wastes. Secondly the issue of energy crises was also addressed in this study by using organic wastes for synthesis of biopolyester as an alternate to synthetic plastic which are derived from fossil fuels. The experimental work was conducted in Micrology General and Project Laboratory of Shaheed Benazir Bhutto women University Peshawar. The extracted biopolyester was qualitatively analyzed at Centre of Physical Excellence in Peshawar University.

The findings from the current study highlighted the promising potential of biobased plastics, specifically bio polyesters synthesized by bacteria isolated from soil through mulching techniques. These biobased plastics offer significant environmental advantages over conventional fossil-based plastics by reducing dependency on nonrenewable resources and potentially lowering greenhouse gas emissions. The ability of these materials to biodegrade and compost promotes organic recycling and supports circular economy principles, which aligns with global efforts to mitigate plastic pollution and climate change. The isolation and screening of 12 bacterial strains revealed that 7 isolates were (MS-1 to MS-7) capable of accumulating biopolyesters, as demonstrated by Sudan Black B as indicated in Figure 1 and Nile Blue A staining techniques. These dyes specifically bind to polyester granules, confirming biopolymer synthesis.

Among the positive isolates, MS-5 and MS-7 demonstrated notably higher polyester production when grown on glucose, producing approximately 0.52 g/L and 0.6 g/L, respectively as described in Table 1 and Figure 2. This quantitative evaluation indicates their potential suitability for industrial biopolymer production.

Detailed characterization of the efficient producers revealed various macroscopic, microscopic and biochemical properties of both MS-5 and MS-7 as indicated in table 2 a, b and c. Both of isolates are gram positive *bacilli*. Biochemical tests, including catalase positivity and carbohydrate fermentation profiles, provide foundational insights for taxonomic classification and metabolic potential, which could facilitate future genetic and metabolic engineering approaches to enhance biopolymer yields.

As indicated in various literatures that large variety of gram positive and negative bacteria are able to produce biopolyester. These bacteria belong to various genera i.e. *Bacillus, Pseudomonas, Rhizobium, Azotobacter, Azospirillum, Rhodospirillum, Herbaspirillum, Azorhizobium, Streptomyces, Methylobacterium,* and *Nostoc* etc (Musa H. *et al.,2016: Mizunoa et al., 2010*).

An important aspect of the study was the use of fruit wastes specifically orange, apple, and banana peels as sole carbon sources for polyester production. This approach addresses a critical bottleneck in biopolymer commercialization: reducing substrate costs by valorizing agricultural residues. Among the substrates tested, apple peels supported the highest polyester yields, followed by orange and banana peels. MS-7's production capacity was nearly equivalent to the positive control strain *Ralstonia eutropha MTCC1285* as described in Figure 4 and Table 3. Such findings support the integration of waste management and bioplastic production, fostering sustainability across multiple sectors.

Several researchers have used submerged fermentation for bioplastic production. PHAs production was found to be 2.5 g/l by *Bacillus* sp. (Narayanan, A. *et al.,* 2012), 1.62 g/l by *Bacillus subtilis* KP172548 (54), 4.1 g/l by *Bacillus thuringiensis* KJ206079 (55) 1.60 g/l by *Bacillus megaterium*, 3.0 g/l by *Bacillus cereus* SPV (Liau, C. *et al.,* 2014) and 6.07 g/l by *Bacillus* sp. under submerged fermentation through sodium hypochlorite digestion multi-solvent extraction.

In order to design an environmental friendly method for extraction of biopolyester along with traditional method of extraction, an amended technique was used by combining ethylene carbonate with sonication to have efficient yield of biopolymer with less toxic solvent as described

in Figure 5. It was found that with chloroform extraction MS-5, MS-7 and positive control gave yields of 0.50 g/l, 0.53 and 0.55g/l while with amended technique 0.47g/l, 0.51g/l and 0.54g/l respectively. So by optimization of conditions of extraction yields can be further increased. Extraction of polyhydroxyalkanoates (PHA) from *Haematococcus sp* by sonication method gave 50.8 % w/v yield (Jain, S. and Kumar, A. 2021)

When FTIR spectra of biopolyester produced was compared with that of pure one, It was found that spectra of both samples were same as shown in Figure 6. The pure PHA was found to have little bit higher intensity and this was due to high purity of reference PHA.

Several chemical curing agents, including novobiocin, acriflavin, ethidium bromide and SDS has been successfully used in the curing of gram-positive bacterial plasmids (Boy C. *et al.,* 2022). In present study plasmid curing was done with ethidium bromide which showed that biopolyester synthesis capacity was associated with bacterial chromosome as indicated in table 4.

Molecular identification of MS-7 was carried out by 16srRNA sequencing. This strain showed maximum homology to Bacillus sp. (in: firmicutes) 99.83% as shown in table 5. Phylogenetic tree was constructed xxcccxcxxcxxc0063xby using MEGA as Shown in Figure 8.

Identification of bacterial isolates based upon 16S rRNA gene sequence analysis had widely been used (Liu, Y., *et al.,* 2014). It was reported in a study (Mizunoa, K. *et al.,* 2010) that PHA producing *Bacillus cereus YB-4* group was identified with 16S rDNA sequence analysis. An investigation of 16S rRNA gene sequence analysis was performed with NCBI-BLAST homology search program and isolate was characterized as *Bacillus mycoides* DFC1 strain. PHA from Gram-respondant bacteria are mostly preferred in biomedical and tissue engineering applications as these bacteria do not contain lipopolysaccharides (LPS). BLAST studies were also used for Partial sequence analysis of the 16S rRNA gene of the strain K5 which was recognized as *Bacillus shackletonii* . *Bacillus sphaericus* NII 0838 was also investigated to be efficient bioplastic producer (Sindhu, R. *et al. ,* 2011).

CONCLUSION

The present study demonstrated that fruit wastes such as apple, orange, and banana peels can serve as sustainable, low-cost carbon substrates for the microbial synthesis of biodegradable biopolyesters. Through soil enrichment technique, several promising isolates capable of intracellular polyester accumulation were obtained, with *Bacillus sp.* (isolate MS-7) emerging as the most efficient producer. A major outcome of this research was the optimization of a novel, eco-friendly extraction method combining sonication with ethylene carbonate as a safer and cost-effective alternative to chloroform. The comparable yield and reduced solvent toxicity highlight its potential for sustainable downstream processing. FTIR analysis verified the extracted biopolyester as polyhydroxybutyrate (PHB), confirming successful biosynthesis. Plasmid curing experiments indicated that biopolyester synthesis was chromosomally encoded, ensuring stability of the trait. Molecular identification through 16S rRNA sequencing revealed 99.83% similarity of the efficient isolate (MS-7) with *Bacillus sp.*, demonstrating its robustness and industrial relevance for biopolyester production. Overall, this study presents an integrated and sustainable approach to biopolyester production that unites waste valorization, cost-effective fermentation, and environmentally extraction.

Conflict of Interest

There is no conflict of interest amon g authors

References

Abokalawa, F. (2022). The effects of coronavirus disease 2019 (COVID-19) pandemic on people with epilepsy (PwE): An online survey-based study. *Acta Neurologica Belgica*, *5*(1), 59–66.

Ashraf, A. M., Imran, W., & Véchot, L. (2021). Analysis of the impact of a pandemic on the control of the process safety risk in major hazards industries using a Fault Tree Analysis approach. *Journal of Loss Prevention in the Process Industries*, *74*, Article 104649. doi.org

Bedade, D. K., Edson, C. B., & Gross, R. A. (2021). Emergent approaches to efficient and sustainable polyhydroxyalkanoate production. *Molecules*, *26*(11), Article 3463. doi.org

Boy, C., Lesage, J., Alfenore, S., Guillouet, S. E., & Gorret, N. (2022). Study of plasmid-based expression level heterogeneity under plasmid-curing like conditions in *Cupriavidus necator*. *Journal of Biotechnology*, *345*, 17–29. doi.org

Crisafi, F., Valentino, F., Micolucci, F., & Denaro, R. (2022). From organic wastes and hydrocarbons pollutants to polyhydroxyalkanoates: Bioconversion by terrestrial and marine bacteria. *Sustainability*, *14*(14), 8241–8247.

Dalton, B., Bhagabati, P., De Micco, J., Padamati, R. B., & O'Connor, K. A. (2022). A review on biological synthesis of the biodegradable polymers polyhydroxyalkanoates and the development of multiple applications. *Catalysts*, *12*(3), 319–323. doi.org

Elmowafy, E., Abdal-Hay, A., Skouras, A., Tiboni, M., & Casettari, L. (2019). Polyhydroxyalkanoate (PHA): Applications in drug delivery and tissue engineering. *Expert Review of Medical Devices*, *16*(6), 473–485. doi.org

Holt, J. G., Krieg, N. R., Sneath, P. A., Staley, J. T., & Williams, S. T. (1993). *Bergey's manual of determinative bacteriology* (9th ed.). Williams and Wilkins.

Jain, S., & Kumar, A. (2021). Sustainable extraction of polyhydroxyalkanoates using green solvents: An overview. *Bioresource Technology*, *330*, Article 120125. doi.org

Kumar, S. N., Priyam, T., & Aarti, S. (2023). Isolation and screening of Gram-positive Polyhydroxyalkanoate producing bacterial strains from Pharmaceutical Industry Effluent. *Research Journal of Pharmacy and Technology*, *16*, 373–376.

Liau, C., Ahmad, M., Shameli, K., Yunus, W., Ibrahim, A., Zainuddin, N., & Then, Y. (2014). Preparation and characterization of polyhydroxybutyrate/polycaprolactone nanocomposites. *The Scientific World Journal*, *10*, 166–178.

Liu, Y., Huang, S., Zhang, Y., & Xu, F. (2014). Isolation and characterization of a thermophilic *Bacillus shackletonii* K5 from a biotrickling filter for the production of polyhydroxybutyrate. *Journal of Environmental Sciences*, *26*, 1453–1462.

Luis, G., José, L. M., & Alejandro, C. (2024). A review of polyhydroxyalkanoates: Characterization, production, and application from waste. *Microorganisms*, *12*, 24–47.

Mizuno, K., Ohta, A., Hyakutake, M., Ichinomya, B. Y., & Tsuge, B. (2010). Isolation of polyhydroxyalkanoate producing bacteria from a polluted soil and characterization of the isolated strain *Bacillus cereus* YB4. *Polymer Degradation and Stability*, *95*, 1335–1339.

Mohapatra, S., Sarkar, B., Samantaray, D. P., Daware, A., Maity, S., Pattnaik, S., & Bhattacharjee, S. (2017). Bioconversion of fish solid waste into PHB using *Bacillus subtilis* based submerged fermentation process. *Environmental Technology*, *38*(9-10), 1058–1080.

Musa, H., Bolanle, B. B., Kasim, F. H., & Arbain, D. (2016). Screening and production of polyhydroxybutyrate (PHB) by bacterial strains isolated from rhizosphere soil of groundnut plants. *Sains Malaysiana*, *45*(10), 1469–1476.

Narayanan, A., & Ramana, K. V. (2012). Polyhydroxybutyrate production in *Bacillus mycoides* DFC1 using response surface optimization for physico-chemical process parameters. *Biotechnology Journal*, *2*, 287–296.

Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2021). Biopolymer-based sustainable alternatives to conventional plastics: A review on recent developments and future prospects. *International Journal of Biological Macromolecules*, *183*, 1579–1596. doi.org

Shah, M., Rajhans, S., Pandya, H. A., & Mankad, A. U. (2021). Bioplastic for future: A review then and now. *World Journal of Advanced Research and Reviews*, *9*(2), 56–67. doi.org

Sindhu, R., Ammu, B., Binod, P., Deepthi, S., Ramachandran, K., Soccol, C., & Pandey, A. (2011). Production and characterization of poly-3-hydroxybutyrate from crude glycerol by *Bacillus sphaericus* NII 0838 and improving its thermal properties by blending with other polymers. *Brazilian Archives of Biology and Technology*, *54*, 783–794.

Sumaira, Afzal, S., & Islam, A. (2023). Optimization of soil bacteria for bioplastic production using organic wastes as a substrate under submerged fermentation. *Iranian Journal of Science and Technology, Transactions A: Science*, *47*(2), 375–387.

Tokiwa, Y., Calabia, B. P., Ugwu, C. U., & Aiba, S. (2009). Biodegradability of plastics. *International Journal of Molecular Sciences*, *10*(9), 3722–3742. doi.org

Wong, Y. C., & Azman, N. A. N. (2022). Extraction of PHA from *Haematococcus sp.* by using sonication method for bioplastic application. *AIP Conference Proceedings*, *2454*(1), Article 089099.