Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.124

RESEARCH ARTICLE

Phenotypic and Genotypic Assortment of Polyhydroxyalkanoates Producing Bacteria from Rumen Flora of Domesticated Animals in Pakistan

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ARTICLE HISTORY (19-292)

Received: July 17, 2019
Revised: November 19, 2019
Accepted: November 21, 2019
Published online: December 23, 2019

Key words:
Pseudomonas aeruginosa
Polyhydroxyalkanoates (PHAs)
Bioplastic
FTIR
Rumen microflora

ABSTRACT

In plastic industry, a lot of attention has been gained by Polyhydroxyalkanoates (PHAs) owing to their environment friendly nature and 100% biodegradable, so they are called as "green plastics". Bacterial strains able to produce PHAs under veterinary field are reported and rumen microflora was exploited to check its potential for the PHAs production. From the rumen of domestic cattle samples were collected followed by isolation of PHA producing bacteria. Biochemical and molecular characterization of isolates was done through API 20E kit and polymerase chain reaction (PCR), respectively. In order to confirm the genetic diversity, sequencing of 16S rRNA gene, ribotyping and phylogenetic analysis was performed. A deeper insight of PHAs biopolymer by Fourier transform infrared spectroscopy (FTIR) revealed the functional groups. *Pseudomonas aeruginosa (P. aeruginosa)* was isolated and biochemically confirmed through API 20E. PCR further confirmed *P. aeruginosa* through amplification of 16S rRNA gene. Sequenced data of 16S rRNA (accession# MN625895) analyzed through ribotyping and phylogenetic linkages proved all the isolate belongs to *P. aeruginosa*. PHAs were purified from enriched nutrient broth with rumen mixture by using sodium hypochlorite confirmed through FTIR spectroscopy. Complex accumulation of bacterial biomass showed -C=O, C-H and -CH3 functional groups. Bacterial biomass (PHAs) in enriched carbon medium can be used as bioplastics for domestic purpose with less harm. It will be a better alternative to chemically synthesized plastic to reduce environmental hazards.

To Cite This Article: Rafique A, Mahmood MS, Ashraf A, Nasir S, Jabeen F, Sultana T, Sultana S, Kamran M, Jamil N, Khan JA, Luqman M and Shamim A, 2020. Phenotypic and genotypic assortment of Polyhydroxyalkanoates producing bacteria from rumen flora of domesticated animals in Pakistan. Pak Vet J, 40(1): 88-92. http://dx.doi.org/10.29261/pakvetj/2019.124

INTRODUCTION

PHAs are basically microbial polymers assembled inside the cell as a carbon and energy reserve. Bacteria synthesize PHAs in carbon enriched medium or in an environment with limited oxygen availability. PHAs can be applied as monomer for production of different biochemicals and merely a biodegradable bioplastic (Marang *et al*., 2018). PHAs granules are energy reserves in bacterial cells of 0.2-0.5 mm size produced due to excessive carbon in medium. Wide range of polymeric PHAs are potentially available and environment friendly (Thomas *et al*., 2019). Chemically synthesized plastics are present in immutable goods (like furnishings, gadgets) and nondurable products (like garbage bags, diapers, utensils and therapeutic instruments) but leading quantity of plastics reported in packaging and containers (e.g., lids, soft drink/ shampoo bottles). As plastics recycling are not done properly as a result persevere inside environment and contribute deeply to ecological contamination. Bulk amount of chemical pollutants is also generated during built-up processes of plastics (Cesario *et al*., 2014). Shift in public opinion in modern year's leads to awareness of people about side effects of chemical plastics. Synthetic

polymers are used to manufacture materials, which have miscellaneous implementation in current society. If the applications continue to nurture, greater biodegradable and compatible alternative creations are necessary (Luengo *et al*., 2003).

PHAs are important polyesters produced by bacteria. Through the aerobic fermentation, bacteria use different types of carbon sources to produce PHAs. These PHAs are biodegradable under different aerobic and anaerobic conditions with additional properties of thermo plasticity. The scientists have put their effort toward the cost-effective fermentation system to produce PHAs but still it is considerably high (5-6 $/kg), which is a major hindrance in adoption of these biopolymers as raw material. Recently, the scientists have claimed that the use of microbial mix cultures (MMCs) can be an alternative option for producing PHAs (Salehizadeh and van Loosdrecht, 2004).

The production cost of PHAs bioplastics is higher as compared to petro based plastics. So, it is the need of time to cut down the production cost of PHAs plastics. To achieve the task, the bacterial growth material has remained the focus of industrial and scientific stakeholders. Due to increasing environmental hazards it is a general approach to produce those items, which have limited shelf life and can become part of ecosystem after their usage (Boyandin *et al*., 2013). The concept to produce the alternatives with desired characters is increasing day by day (Altaee *et al*., 2016; Pais *et al*., 2016). The biodegradable polymers are also an alternative approach to reduce the plastic wastes. Among these polymers, PHAs is extensively studied series. These PHAs are produced by the bacteria from their internal reserves of the Carbon and easily degraded by soil bacteria which produce extracellular exudates like PHAs depolymerase. In addition, it is believed that mixed microbial cultures are potential candidate to produce PHAs (Moita *et al*., 2014; Samori *et al*., 2015).

PHAs are perishable thermoplastic polyesters that can be produced from the renewable energy sources. In a pilot study, a mutant strain of *P. aeruginosa* designated as EBL-8 was grown in minimum salt media enriched with soybean oil under shake flask conditions. The production of PHAs was evaluated in terms of biomass production, utilization of carbon source and PHAs yield. This study reveals commendable results (Abid *et al*., 2016).

Minimizing the production cost of PHAs is the main task for researchers working in this field. Different types of carbon sources have been used for cost effective and optimal PHAs production. A study was conducted using hexadecane (a hydrocarbon) as sole carbon source in minimal salts medium for *P. aeruginosa*. The associated factors including biomass production, carbon utilization and PHAs production were evaluated in this experiment; it produces satisfactory results to use hexadecane as carbon source (Raza *et al*., 2016).

PHAs which is aliphatic, naturally produced polyesters by microbes especially bacteria. PHAs are used to produce different materials by coupling different monomers together. For example, stuff standard of food wrappings can be increased by using the poorer oxygen permeability polymer. Bioplastics are potential candidate as alternative to petrochemical based plastics (Martinez *et al*., 2011).

Different amino acids arrangements in polymer chain leads to maximum changeability of composition and result in changed chemical characteristics. Fragile plastics are formed by thermal processing by means of compression and extrusion methods (Shishatskaya *et al*., 2016). Proteins present in higher amount in nature can be used to produce useful bio plastics. Among these whey proteins, the protein for the manufacture of cheese separated from milk are the most considered protein (Sharma and Luzinov, 2013).

In search of low-cost raw material for PHAs production, the current study was planned to exploit the rumen microflora of domesticated animals. Rumen materials were collected and processed for the bacterial isolation and identification. After that, the potential of bacterial isolates was tested for PHAs production.

MATERIALS AND METHODS

Isolation of PHAs producing bacteria: Samples were taken from rumen of domestic buffalos and stored at room temperature. One gram of rumen mixture was mixed with 99 ml of double distilled water. The whole mixture was serially diluted and dilution of 10^{-5} to 10^{-8} were cultured on the nutrient agar medium with 1% glucose and incubated for 72 hrs. After that microscopic examination was executed. For the rapid identification of PHAs producing colonies, the nutrient medium was rinsed with 0.02% alcoholic solution of Sudan black stain for 30 min and the unabsorbed stain was decanted (Juan *et al*., 1998).

Screening of PHAs producing bacteria: Sudan black stained colonies were streaked on carbon enriched nutrient medium (agar 1.5%, glucose 1%, sodium chloride 0.8%, beef extract 0.3%, peptone 0.5% and Nile blue A 0.05 ug/ml) and incubated for 72 hrs. Bacterial colonies were observed under UV light to check the accumulated PHAs (Bhuwal *et al*., 2013).

Biochemical and molecular characterization of isolates: API 20E kit (Biomerieux, USA) was used for the biochemical confirmation and confirmed isolates were shifted to nutrient broth supplemented with 1% glucose. For the molecular identification broth culture was subjected to genomic DNA isolation by GeneJET DNA purification kit (Thermo scientific). Confirmation and quantification of DNA was done through Agarose gel and Nanodrop, respectively. For the ribotyping of 16s rRNA universal primers were used 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). The PCR conditions were commenced following the Fernandez *et al*., 2005. The purified PCR product was sent for sequencing for further analysis.

Production of PHAs: 500 ul of selected broth culture and 50 ml of rumen mixture were poured into 100 ml of nutrient broth and incubated for 48 hrs at 150 rpm in shaking incubator for the production of PHAs then Broth culture was centrifuged at 10000 rpm for 15 minutes. Supernatants were discarded and pellets incubated with 50

ml of sodium hypochlorite for 1 hrs at 50°C that followed by centrifugation at 12000 rpm for 15 minutes. Purification of PHAs were done with distill water, acetone and ethanol respectively (Lee *et al*., 1995).

FTIR analysis: The chemical structure of extracted PHAs was analyzed by FTIR. The samples were subjected to infrared spectra ranging from 500 to 4000 cm^{-1} (Shamala *et al*, 2009).

Sequencing analysis: Sequenced data of isolates were analyzed for homology through Basic local alignment search tool (BLAST) and submitted to Genbank NCBI. Clustal-W software used for the ribotyping and phylogenetic analysis.

RESULTS

Isolation and screening of PHAs producing bacteria: Colonies were observed on nutrient medium enriched with glucose. The colonies were irregular, smooth, large and translucent with greenish blue diffusible pigmentation in the media. Gram negative tortillas were in abundance that confirmed the Pseudomonas. Black colonies and orange fluorescence after Sudan black and Nile blue A staining, respectively which showed the PHAs producing bacteria and their accumulation in bacterial colonies. This Screening also authenticates the presence of inclusion bodies in bacterial cells. Granules were present in all isolates.

Biochemical and molecular identification of isolates: API 20E kit confirmed all the PHAs producing isolates were belongs to *P. aeruginosa* K1 and K2 strains. For the molecular confirmation the 16 s rRNA universal primers were used that confirms the K1 strains as *P. aeruginosa.*

Analysis of PHA production and extraction: PHAs were purified from enriched nutrient broth with rumen mixture as a substrate by using sodium hypochlorite that showed the organic matter present in the rumen was good for growth of *P. aeruginosa* that were able to utilize rumen organic material and convert it into PHAs.

FTIR spectroscopy and chemical examination of PHA of bacterial strains: Lyophilized biomass of PHAs producing strains revealed the wide range of peaks in FTIR analysis, which showed complex accumulation of bacterial biomass. Non-identical peaks depict the existence of a range of functional groups showed by FTIR analysis in Fig.1. Though, ambiguous peaks in large number were also present. Functional groups shifting had also been noted.

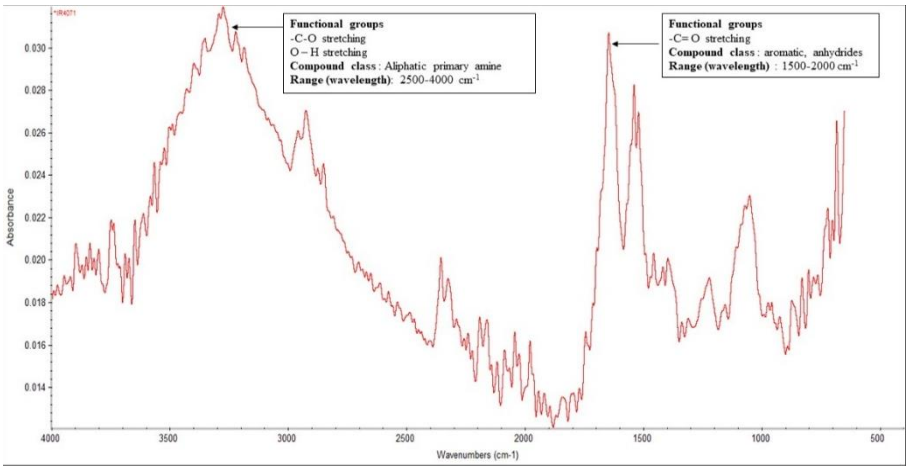

Fig. 1: FTIR analysis revealed the functional groups found in PHAs. The peaks of 1500 - 1700, 2000 - 2500, 3000- 3500 wavelength (cm^{-1}) shows the –C=O, C-H and -CH_3 functional groups respectively.

AAAAAAGTTTTAGAGTTTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACAC
ATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAA
TGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCAT
ACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTC
GGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGATCCGTAACTGGTCTGAGAGGA
TGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCG
GATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACTTTGCTGTTTTGAC
GTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGT
GCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGAT
GTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTA
GAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGT
GGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTT
GAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGG
TTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCG
AAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTG
GTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT
GTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGG
GCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATC
ATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAG
CCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCG
ACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCC
CGGGCCTTGTACACACCGCCCGTCACACCATGGGGAGTGGGGTTGCTCCAGAAGTAGCTA
GTCTAACCCGCAAGGGGGACGGTTACCACGGAAGTGATTCATGACTGGGGGTGAAGTCGT
ACAGGGGGAAACCCGAAAAATTTTTTCTT

Fig. 2: Sequence of 16SrRNA gene of *P. aeruginosa* (Accession# MN625895).

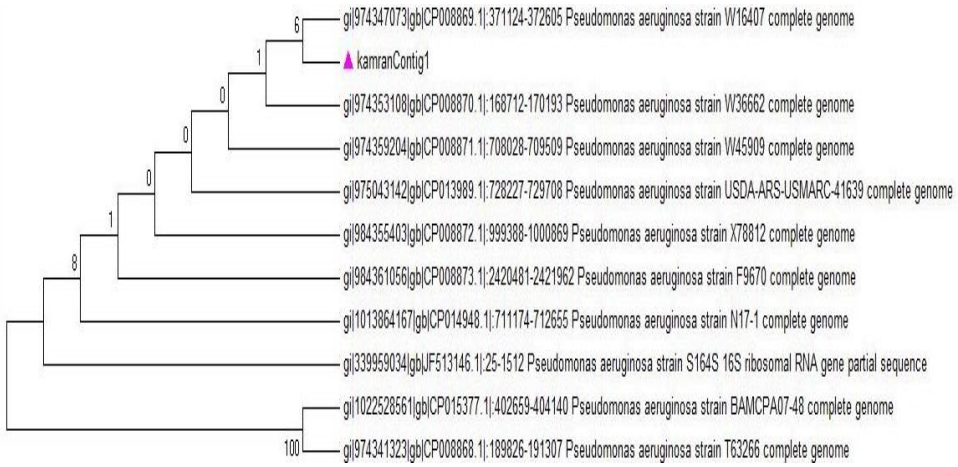

Fig. 3: Sequence placement into rooted phylogenetic tree for *P. aeruginosa* confirmation. Terminal point Kamran contig1 showed our sequence and it has similarity with the *P. aeruginosa* strain W15407. That's why both (Kamran Contig and *P. aeruginosa* w16407) has the common ancestor at internal point B.

Sequencing analysis: BLAST and clustal-W results confirmed the homology with *P. aeruginosa* strains present in gene databank. Sequence was reported in GenBank NCBI (Accession# MN625895). Sequenced data placed in rooted phylogenetic tree that revealed *P. aeruginosa* W16407 and our target sequence has common ancestors (Fig. 2, 3).

DISCUSSION

A biopolymer; PHAs are a biodegradable thermoplastic material, can be used for biocompatibility in the medical devices and waste management strategies (Zhilaa *et al.* 2015). Synthetic polymers approximately of 300 million tons are added annually in global environment and this amount is increasing day by day. PHAs were purified from enriched nutrient broth with rumen mixture as a substrate by using sodium hypochlorite that showed the organic matter present in the rumen was good for growth of *P. aeruginosa* that were able to utilize rumen organic material and convert it into PHAs. Similar work has been reported that PHAs granules are energy reserves in bacterial cells of 0.2-0.5 mm size produced due to excessive carbon in medium. Wide range of polymeric PHAs are potentially available and environment friendly (Thomas *et al*., 2019). For production of biodegradable plastics, global trades are concentrating on innovative methods or techniques. These novel procedures generate degradable plastics that will swap non-degradable ones. To be considered trustworthy and safer, PHAs are good candidates for green and environment friendly globe (Boyandin *et al*., 2013).

As the production of the PHAs is economical by the microbial mix cultures as described by (Salehizadeh and van Loosdrecht, 2004). A wide variety of bacteria have been reported to contain the PHAs accumulates. Different types of polyhydroxyalkanoics are incorporated in the polymer of PHAs and 90 bacterial genera are reported to produce these PHAs. PHAs can be applied as monomer for production of different biochemicals and merely a biodegradable bioplastic (Marang *et al*., 2018). There are few species reported from the animal origin side. In the current study microbial flora of the cattle's rumen was exploited and used as source of microbial mix culture. By the visual analysis of isolates through Sudan black staining shows the granules inside them with dark blue color while the negative sample shows light blue coloration (Carvalho *et al*., 2014; Katipoglu-Yazan *et al*., 2014). By the cultivation of samples on enriched growth media and subsequent analysis by confirmatory tests, Pseudomonas was selected for the PHA production.

For the production of PHAs, Munir *et al.* (2015) conducted experiments in the past using different carbon sources to improve the conditions. Bio-plastic manufacturing was screened by isolating 30 strains of bacteria from environmental samples (Samori *et al*., 2015). While from the results of present study it is obvious that micro flora of the rumen of animals is also a good source for the PHAs producing bacteria. Different bacteria were characterized by biochemical and molecular techniques. The interesting feature was the use of rumen mixture as source for bacterial media. The isolates successfully established on the rumen mixture and convert the ingredient into valuable products. The production conditions for PHAs in this experiment were short and simple. No other addition of ingredient except the isolates, media and substrate. With the rumen mixture these gives good quality of PHAs polymer which can be improved in future.

In past Cha *et al*. (2016) studied that from rumen micro flora bio plastic produced had eminent worth and environment friendly. Lyophilized biomass of PHAs producing strains revealed the wide range of peaks in FTIR analysis, which showed complex accumulation of bacterial biomass. By FTIR analysis it is exposed that attractive amount of PHAs produced by bacteria under examination. From FTIR analysis it is established that PHAs formed from microbial mix culture is in similarity to the configuration of poly-3-hydroxybutyric acid-co-3hydroxyvaleric acid. At wave number range 1720 cm^{-1}, carbonyl group have strong sharp peak. These findings were again an acknowledgement with previous studies. It can be utilized in bottling for soft drinks, disposable packaging and in dairy goods.

Conclusions: It will be a source of toxic free packaging. For producing the PHAs, different types of methods are employed. One of the economical procedures is by the microbial mix culture technique. Microflora of rumen can be exploited for this purpose. This study reveals about the types of microorganism and their potential to produce PHAs. By FTIR analysis it is exposed that bacteria under trial formed handsome amount of PHAs Pollution can be reduced by its production at massive scale. Production of bio-plastics at marketable level is the need of hour.

Authors contribution: AR, MSM, AA and SN conceived and designed the study. MK and NJ collected samples performed the experimental work. MSM, AR, AA and SN analyzed the data and wrote the manuscript. FJ, TS, SS, JAK, ML and AS critically reviewed the manuscript.

REFERENCES

Abid S, Raza ZA and Hussain T, 2016. Production kinetics of Polyhydroxyalkanoates by using pseudomonas aeruginosa gamma ray mutant strain EBN-8 cultured on soybean oil. Biotechol 6:142.

Altaee N, El-Hiti GA, Fahdil A, *et al*., 2016. Biodegradation of different formulations of polyhydroxybutyrate flms in soil. Springer Plus 5:762-74.

Bhuwal AK, Gulab S, Neeraj KA, *et al*., 2013. Isolation and screening of polyhydroxyalkanoates producing bacteria from pulp, paper and cardboard industry wastes. Int J Bioms 1:10.

Boyandin AN, Prudnikova SV, Filipenko ML, *et al*., 2012. Biodegradation of polyhydroxy alkanoates by soil microbial communities of different structures and detection of PHA degrading microorganisms. Appl Biochem Microbiol 48:28-36.

Boyandin NA, Prudnikova SV, Karpov V, *et al*., 2013. Microbial degradation of polyhydroxyalkanoates in tropical soils. Int Biodeter Biodeg 88:77-84.

Carvalho G, Oehmen A, Albuquerque MGE, *et al*., 2014. The relationship between mixed microbial culture composition and PHA production. Nano Biotechnol 31:257-63.

Cesario MT, Raposo RS, de Almeida MCMD, *et al*., 2014. Enhanced bioproduction of poly-3-hydroxybutyrate from wheat straw lignocellulosic hydrolysates. Nano Biotechnol 31:104-13.

Cha HS, Son JH, Jamal Y, *et al*., 2016. Characterization of polyhydroxyalkanoates extracted from wastewater sludge under different environmental conditions. Biochem Eng J 112:1-12.

Fernandez D, Rodriguez E, Bassas M, *et al.,* 2005. Agro-industrial oily wastes as substrates for PHA production by the new strain Pseudomonas aeruginosa NCIB 40045: Effect of culture conditions. Biochem Eng J 26:159-67.

Juan ML, Gonzalez LW and Walker GC, 1998. A novel screening method for isolating exo polysaccharide-deficient mutants," Applied Env Microbiol 64:4600-2.

Katipoglu-Yazan T, Pala-Ozkok I, Ubay-Cokgor E, *et al*., 2014. Acute impact of tetracycline and erythromycin on the storage mechanism of polyhydroxyalkanoates. Biochem Eng J 91:283-9.

Lee SY and Choi J, 1999. Polyhydroxyalkanoates: Biodegradable polymer. In: Arnold L, Demain J, Davis E, editor. Manual of industrial microbiology and biotechnology, American Society for Microbiology, Washington pp:616-27.

Lee Y, Chang HN and Park YH, 1995. A simple method for recovery of microbial poly-3-hydroxybutyrate by alkaline solution treatment. J Microbiol Biotechnol 5:238-40.

Luengo JM, García B, Sandoval A, *et al*., 2003. Bioplastics from microorganisms. Curr Opin Microbiol 6:251-60.

Marang L, van Loosdrecht MC and Kleerebezem R, 2018. Enrichment of PHA-producing bacteria under continuous substrate supply. New Biotechnol 41:55-61.

Martinez V, García P, García JL, *et al*., 2011. Controlled autolysis facilitates the polyhydroxyalkanoate recovery in *Pseudomonas putida* KT2440. Microbiol Biotechnol 4:533-47.

Moita R, Freches A and Lemos PC, 2014. Crude glycerol as feedstock for polyhydroxyalkanoates production by mixed microbial cultures. Water Res 58:9-20.

Munir S and Jamil N, 2015. Characterization of polyhydroxyalkanoates produced by polyhydroxyalkanoates: Preparation and biomedical application. Materials Sci Eng J 75:109-12.

Pais J, Serafim LS, Freitas F, *et al*., 2016. Conversion of cheese whey into poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by *Haloferax mediterranei*. Nano Biotechnol 33:224-30.

Raza ZA, Abid S, Rehman A, *et al*., 2016. Synthesis kinetics of poly(3-hydroxybutyrate) by using a *Pseudomonas aeruginosa* mutant strain grown on hexadecane. Int Biodeterior Biodegrad 115:171-8.

Salehizadeh H and Van Loosdrecht, MC, 2004. Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance, Biotechnol Advances 22:261-79.

Samori C, Abbondanzi F, Galletti P, *et al*., 2015. Extraction of polyhydroxyalkanoates from mixed microbial cultures: impact on polymer quality and recovery. Bioresour Technol, 189:195-202.

Shamala TR, Divyashree MS, Davis R, *et al*., 2009. Production and characterization of bacterial polyhydroxyalkanoate copolymers and evaluation of their blends by Fourier transform infrared spectroscopy and scanning electron microscopy. Indian J Microbiol 49:251-8.

Sharma I and Luzinov I, 2013. Whey based binary bioplastics. J Food Eng 119:404-10.

Shishatskaya EI, Nikolaeva ED, Vinogradova ON, *et al*., 2016. Experimental wound dressings of degradable PHA for skin defect repair. J Mater Sci Mater Med 27:165.

Thomas T, Elain A, Bazire A, *et al*., 2019. Complete genome sequence of the halophilic PHA-producing bacterium Halomonas sp. SF2003: insights into its biotechnological potential. World J Microbiol Biotechnol 35:50.

Zhilaa N, Kalacheva G and Volova T, 2015. Fatty acid composition and polyhydroxyalkanoates production by Cupriaviduseutrophus B-10646 cells grown on different carbon sources, Process Biochem 50:69-78.